Newborn screening for congenital toxoplasmosis in Ireland

Wendy Ferguson
Royal College of Surgeons in Ireland

Citation
Ferguson W. Newborn screening for congenital toxoplasmosis in Ireland [MD Thesis]. Dublin: Royal College of Surgeons in Ireland; 2016.
Creative Commons Licence:

This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 4.0 License.

This thesis is available at e-publications@RCSI: https://epubs.rcsi.ie/mdtheses/67
Newborn screening for congenital toxoplasmosis in Ireland

Dr. Wendy Ferguson MB BCH BAO MRCPI
Department of Paediatrics
The Rotunda Hospital
Dublin

A thesis submitted to the School of Postgraduate Studies,
Faculty of Medicine and Health Sciences, Royal College of
Surgeons in Ireland, in fulfilment of the degree of Doctor of
Medicine

Supervisors:  Professor M Cafferkey
            Professor K Butler

2016
CANDIDATE THESIS DECLARATION

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree (doctor of medicine), is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed ____________________________

Student Number 4228057

Date 19-08-2016
TABLE OF CONTENTS

CANDIDATE THESIS DECLARATION ............................................................................................................................ 2
TABLE OF CONTENTS .................................................................................................................................................. 3
ABBREVIATIONS ......................................................................................................................................................... 13
LIST OF FIGURES ....................................................................................................................................................... 16
LIST OF TABLES ........................................................................................................................................................ 18
SUMMARY ................................................................................................................................................................. 19
ACKNOWLEDGEMENTS ............................................................................................................................................... 21
CHAPTER 1 - TOXOPLASMOsis .................................................................................................................................. 25
  1.1 INTRODUCTION .................................................................................................................................................. 25
  1.2 OCULAR MANIFESTATIONS OF TOXOPLASMOsis ............................................................................................ 26
  1.3 CONGENITAL TOXOPLASMOsis ........................................................................................................................ 28
  1.4 GLOBAL SEROPREVALENCE OF TOXOPLASMA ANTIBODY AND INCIDENCE OF CONGENITAL
  TOXOPLASMOsis ...................................................................................................................................................... 31
CHAPTER 2 - DISCOVERY, HISTORICAL SIGNIFICANCE AND NOMENCLATURE ....................................................... 37
  2.1 DISCOVERY OF THE PARASITE .......................................................................................................................... 37
  2.2 DISCOVERY OF THE CLINICAL SPECTRUM OF T. GONDII INFECTION IN HUMANS ............................... 38
  2.3 HISTORY OF OCULAR TOXOPLASMOsis: CONGENITAL, ACQUIRED AND REACTIVATED .................. 42
  2.4 CLASSIFICATION AND IDENTIFICATION OF THE DEFINITIVE HOST ................................................................ 45
CHAPTER 3 - T. GONDII FORMS, LIFE CYCLE AND SOURCES OF INFECTION ......................................................... 46
  3.1 PARASITE FORMS ............................................................................................................................................... 46
    3.1.1 OOCYSTS ....................................................................................................................................................... 46
    3.1.2 SPOROZOITES ............................................................................................................................................. 46
    3.1.3 TACHYZOITES ............................................................................................................................................ 46
    3.1.4 BRADYZOITES ........................................................................................................................................... 47
  3.2 LIFE CYCLE ......................................................................................................................................................... 47
  3.3 SOURCES OF TOXOPLASMA INFECTION ........................................................................................................... 47
CHAPTER 4 - LITERATURE REVIEW: STRATEGIES TO PREVENT CONGENITAL TOXOPLASMOsis .................... 52
  4.1 SUMMARY OF PREVENTIVE MEASURES ........................................................................................................... 52
  4.2 PRIMARY PREVENTION ..................................................................................................................................... 52
  4.3 SECONDARY PREVENTION ................................................................................................................................ 53
  4.4 TERTIARY PREVENTION .................................................................................................................................... 54
  4.5 ARGUMENTS FOR OR AGAINST SCREENING FOR CT .......................................................................................... 54
CHAPTER 5 - LITERATURE REVIEW: PRENATAL SCREENING AND TREATMENT FOR CONGENITAL
  TOXOPLASMOsis ...................................................................................................................................................... 56
  5.1 INTRODUCTION .................................................................................................................................................... 56
  5.2 REVIEW OF METHODS FOR DIAGNOSIS OF CT IN PREGNANCY .................................................................... 58
  5.3 ANTENATAL SCREENING AND TREATMENT FOR CT IN FRANCE .................................................................. 60
  5.4 VARIATION IN TREATMENT REGIMENS EMPLOYED FOR CT TREATMENT IN-UTERO ............................... 63
  5.5 THE EUROPEAN MULTICENTRE STUDY ON CONGENITAL TOXOPLASMOsis (EMSCOT): PRENATAL
    SCREENING AND INFANT OUTCOME .................................................................................................................. 64
    5.5.1 EMSCOT SUMMARY AND CONCLUSIONS .............................................................................................. 64

3
CHAPTER 9 - METHODS EMPLOYED FOR IMPLEMENTATION OF A NEWBORN SCREENING PROGRAMME FOR CONGENITAL TOXOPLASMOsis IN IRELAND

9.1 INTRODUCTION

9.2 OBJECTIVES

9.3 METHODS

9.3.1 THE STEERING COMMITTEE FOR IMPLEMENTATION OF THE CT SCREENING PROGRAMME

9.3.2 DEFINITION OF CONGENITAL TOXOPLASMOsis

9.3.3 STUDY METHODOLOGY AND INCLUSION AND EXCLUSION CRITERIA

9.3.4 METHODS FOR COMMUNITY AND HOSPITAL MEDICAL STAFF EDUCATION AND PROGRAMME IMPLEMENTATION

9.3.5 LABORATORY METHODOLOGY: SCREENING OF DRIED BLOOD SPOTS FOR TOXOPLASMA IgM

9.3.6 ARRANGEMENTS FOR MOTHER-INFANT RECALL FOR CONFIRMATORY TESTING

9.3.7 CONFIRMATORY SEROLOGY

9.3.8 FOLLOW-UP OF CONFIRMATORY SEROLOGY RESULTS

9.4 RESULTS

9.5 DISCUSSION

9.5.1 DELAY WITH INITIATION OF SCREENING

9.5.2 TIMING OF INFORMED CONSENT

9.5.3 TWO-STEP SCREENING PROTOCOL FOR RECOVERY OF TOXOPLASMA IgM

9.5.4 RECALL FOR REPEAT FILTER PAPER CARDS WHERE NECESSARY

9.5.5 MOTHER AND INFANT RECALL FOR CONFIRMATORY VENOUS SAMPLING

9.5.6 DISPATCH OF CONFIRMATORY SAMPLES TO THE TOXOPLASMA REFERENCE LABORATORY SWANSEA UK

9.6 CONCLUSIONS

CHAPTER 10 - RESULTS FROM 24 MONTHS OF NATIONAL NEWBORN SCREENING FOR CONGENITAL TOXOPLASMOsis AND INCIDENCE DERIVED FROM THESE RESULTS

10.1 INTRODUCTION
11.2 OBJECTIVE ........................................................................................................... 147

11.3 METHODS .............................................................................................................. 147

11.3.1 POPULATION STUDIED ..................................................................................... 147

11.3.2 METHODS FOR CONFIRMATION OF NEGATIVE STATUS ................................ 147

11.4 RESULTS .................................................................................................................. 148

11.4.1 SUMMARY OF CONFIRMATION OF INFANT STATUS ......................................... 148

11.4.2 MATERNAL DEMOGRAPHICS ............................................................................. 148

11.4.3 SUMMARY OF PAIRED MOTHER-INFANT CONFIRMATORY SEROLOGY .............. 148

11.4.4 ELEVEN SCREEN POSITIVE INFANTS CONFIRMED NEGATIVE: MATERNAL SEROCONVERSION PRIOR TO PREGNANCY WITH PASSIVE TRANSFER OF ANTIBODY TO INFANT ......................................................... 149

11.4.5 FOUR SCREEN POSITIVE INFANTS LIKELY UNINFECTED: MATERNAL SEROCONVERSION DURING PREGNANCY WITH PASSIVE TRANSFER OF ANTIBODY TO INFANT ................................................................. 150

11.5 DISCUSSION ............................................................................................................. 151

11.5.1 DEMOGRAPHICS OF WOMEN WITH FALSE POSITIVE INFANTS ......................... 151

11.5.2 PAIRED MOTHER-INFANT CONFIRMATORY SEROLOGY .................................... 151

11.5.3 SEROLOGICAL EXCLUSION OF CT IN 11 INFANTS BORN TO WOMEN WITH SEROCONVERSION PRIOR TO PREGNANCY: ROLE OF THE AVIDITY TEST IN CONFIRMING TIMING OF SEROCONVERSION ............................................. 153

11.5.4 UNUSUAL MATERNAL SEROLOGICAL PROFILE IN ONE CASE OF SEROCONVERSION PRIOR TO PREGNANCY ................................................................. 154

11.5.5 SEROLOGICAL EXCLUSION OF CT IN FOUR SCREEN POSITIVE INFANTS BORN TO WOMEN WITH ESTIMATED SEROCONVERSION IN PREGNANCY ......................................................................................... 155
CHAPTER 14 - TREATMENT OF CONGENITAL TOXOPLASMOSIS WITH ANTIPROTOZOALS AND ADVERSE EFFECTS ENCOUNTERED

14.1 INTRODUCTION ........................................................................................................... 220
14.2 OBJECTIVES ............................................................................................................... 220
14.3 METHODS ................................................................................................................... 220
  14.3.1 POPULATION STUDIED ......................................................................................... 220
  14.3.2 TREATMENT REGIMEN ......................................................................................... 221
  14.3.3 TREATMENT MONITORING AND INTERVENTIONS FOR ADVERSE EVENTS ................................................................. 221
14.4 RESULTS .................................................................................................................... 222
  14.4.1 SUMMARY OF INFANT TREATMENT ..................................................................... 222
  14.4.2 GASTROINTESTINAL SIDE EFFECTS ...................................................................... 223
  14.4.3 HAEMATOLOGIC TOXICITY: NEUTROPENIC EVENTS ............................................. 223
    14.4.3.1 Grades 1 and 2 neutropenic events (mild and moderate neutropenia) ................ 223
    14.4.3.2 Grades 3 and 4 neutropenic events (severe and life threatening neutropenia) .... 224
  14.4.4 ADDITIONAL INTERVENTIONS NECESSARY FOR NEUTROPENIC EVENTS ........ 226
  14.4.5 COMPLIANCE WITH THE RECOMMENDED TREATMENT REGIMEN ..................... 226
  14.4.6 SUMMARY OF INTERVENTIONS DURING INFANT TREATMENT AND PERCENTAGE OF TREATMENT COMPLETED ............. 227
  14.4.7 SUMMARY OF RESOLUTION OF NEUTROPENIA DURING TREATMENT ............. 227
  14.4.8 LIVER AND RENAL FUNCTION .............................................................................. 228
14.5 DISCUSSION ................................................................................................................. 228
  14.5.1 SUMMARY OF RATIONALE FOR TREATMENT OF CT DURING INFANCY .............. 228
  14.5.2 TREATMENT INITIATION IN THE COHORT OF 14 INFANTS .................................... 229
  14.5.3 TREATMENT ADVERSE EFFECTS ........................................................................ 230
  14.5.4 COMPLIANCE WITH THE TREATMENT REGIMEN .............................................. 231
  14.5.5 COMPARISON OF TOXICITY ENCOUNTERED IN THE COHORT WITH OTHER STUDIES ..................................................... 231
  14.5.6 FUTURE RESEARCH ............................................................................................ 232
14.6 CONCLUSIONS ............................................................................................................. 232

CHAPTER 15 - EFFECT OF TREATMENT ON INTRACRANIAL SIGNS IN A COHORT OF CHILDREN WITH CONGENITAL TOXOPLASMOSIS

15.1 INTRODUCTION ......................................................................................................... 237
15.2 OBJECTIVE ............................................................................................................... 237
15.3 PATIENTS AND METHODS ....................................................................................... 238
  15.3.1 COHORT STUDIED ............................................................................................... 238
  15.3.2 METHODS ......................................................................................................... 238
15.4 RESULTS .................................................................................................................... 238
  15.4.1 INTRACRANIAL IMAGING AT INITIAL ASSESSMENT AND FOLLOW-UP .................. 238
  15.4.2 CSF ANALYSIS .................................................................................................... 239
15.5 DISCUSSION .............................................................................................................. 239
15.6 CONCLUSIONS .......................................................................................................... 242

CHAPTER 16 - OPHTHALMIC OUTCOME IN A COHORT OF CHILDREN WITH CT ................. 248
16.1 INTRODUCTION ......................................................................................................... 248
16.2 OBJECTIVE ............................................................................................................... 248
16.3 PATIENTS AND METHODS ....................................................................................... 249
  16.3.1 COHORT STUDIED ............................................................................................... 249
  16.3.2 METHODS ......................................................................................................... 249
16.4 RESULTS .................................................................................................................... 249
16.5 DISCUSSION .............................................................................................................. 251
16.6 CONCLUSIONS .................................................................................................................. 256

CHAPTER 17 - SEROLOGICAL PROFILE DURING AND AFTER TREATMENT FOR CONGENITAL TOXOPLASMOsis ................................................................. 259

17.1 INTRODUCTION ........................................................................................................... 259

17.2 OBJECTIVES ............................................................................................................... 259

17.3 PATIENTS AND METHODS ....................................................................................... 259

17.3.1 COHORT STUDIED ............................................................................................... 259

17.3.2 METHODS ............................................................................................................ 259

17.4 RESULTS ................................................................................................................... 260

17.4.1 TREATMENT PERIOD ......................................................................................... 260

17.4.2 SEROLOGY PROFILE DURING TREATMENT .................................................. 260

17.4.3 DURATION OF SEROLOGY MONITORING BEYOND THE FIRST YEAR OF LIFE .................................................................................................................. 260

17.4.4 REBOUND OF THE DYE TEST IN TREATED CHILDREN ................................... 261

17.4.5 REAPPEARANCE OF IGM AND IGA FOLLOWING TREATMENT COMPLETION ...... 261

17.4.6 SEROLOGICAL REBOUND AND CLINICAL CORRELATION ......................... 262

17.4.7 REACTIVATION AND THE SEROLOGICAL PROFILE ...................................... 262

17.5 DISCUSSION ............................................................................................................. 262

17.6 CONCLUSIONS ......................................................................................................... 265

CHAPTER 18 - CLINICAL, NEURODEVELOPMENTAL AND EDUCATIONAL PROGRESS SUMMARY IN A COHORT OF INFANTS WITH CONGENITAL TOXOPLASMOsis IDENTIFIED BY NEWBORN SCREENING ...................................................... 267

18.1 INTRODUCTION ......................................................................................................... 267

18.2 OBJECTIVES ............................................................................................................. 267

18.3 PATIENTS AND METHODS ....................................................................................... 267

18.3.1 COHORT STUDIED ............................................................................................... 267

18.3.2 METHODS ............................................................................................................ 267

18.3.2.1 Clinical follow-up .......................................................................................... 267

18.3.2.2 Developmental follow-up during the first decade ....................................... 268

18.3.2.3 Educational and cognitive assessment during the first decade .................. 268

18.4 RESULTS ................................................................................................................... 269

18.4.1 CLINICAL OUTCOME ......................................................................................... 269

18.4.2 DEVELOPMENTAL PROGRESS ......................................................................... 270

18.4.3 EDUCATIONAL PROGRESS ............................................................................ 271

18.5 DISCUSSION ............................................................................................................. 272

18.5.1 CLINICAL OUTCOME ......................................................................................... 272

18.5.2 NEURODEVELOPMENTAL AND EDUCATIONAL OUTCOME TO DATE .......... 272

18.6 CONCLUSION ........................................................................................................... 274

CHAPTER 19 - CONGENITAL TOXOPLASMOsis: 15 INDIVIDUAL CASE HISTORIES ................................................................. 278

19.1 CASE NUMBER 1 ....................................................................................................... 278

19.1.1 SCREENING AND CONFIRMATION ............................................................... 278

19.1.2 INFANT EVALUATION AND TREATMENT .................................................. 278

19.1.3 COMPLIANCE WITH THE MANAGEMENT PROTOCOL AND CHALLENGES ENCOUNTERED ............................................................... 278

19.1.4 CLINICAL FOLLOW UP AND OUTCOME TO DATE ................................... 279

19.2 CASE NUMBER 2 ....................................................................................................... 280

19.2.1 SCREENING AND CONFIRMATION ............................................................... 280

19.2.2 INFANT EVALUATION AND TREATMENT .................................................. 280

19.2.3 COMPLIANCE WITH THE MANAGEMENT PROTOCOL AND CHALLENGES ENCOUNTERED ............................................................... 280

19.2.4 CLINICAL FOLLOW UP AND OUTCOME TO DATE ................................... 281

19.3 CASE NUMBER 3 ....................................................................................................... 282
APPENDIX 1: THE CT SCREENING PROGRAMME CONSENT FORM AND INFORMATION LEAFLET FOR PARENTS
APPENDIX 2: THE CT SCREENING PROGRAMME INFORMATION LEAFLET FOR HEALTH CARE PROFESSIONALS ................................................................. 385
APPENDIX 3: AUTODELFIA® IGM SCREEN .................................................................................................................. 387
APPENDIX 4: TOXOPLASMA ISAGA IGM ASSAY: (MANUFACTURER’S LEAFLET) ..................................................... 407
APPENDIX 5: TOXOPLASMA ISAGA IGA ASSAY: (MANUFACTURER’S LEAFLET) .......................................................... 411
APPENDIX 6: PUBLICATIONS AND PRESENTATIONS ASSOCIATED WITH THE THESIS ................................. 416
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>negative</td>
</tr>
<tr>
<td>+</td>
<td>positive</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ANC</td>
<td>absolute neutrophil count</td>
</tr>
<tr>
<td>ART</td>
<td>antiretroviral therapy</td>
</tr>
<tr>
<td>B/L</td>
<td>bilateral</td>
</tr>
<tr>
<td>Bayley-III</td>
<td>Bayley scales for infant and toddler development third edition</td>
</tr>
<tr>
<td>BD</td>
<td>borderline</td>
</tr>
<tr>
<td>CCT</td>
<td>cranial computed tomography</td>
</tr>
<tr>
<td>CDC</td>
<td>Centres for Disease Control and Prevention</td>
</tr>
<tr>
<td>CEO</td>
<td>Chief Executive Officer</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>cm</td>
<td>centimetres</td>
</tr>
<tr>
<td>CMO</td>
<td>Chief Medical Officer</td>
</tr>
<tr>
<td>CMV</td>
<td>cytomegalovirus</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CSO</td>
<td>Central Statistics Office</td>
</tr>
<tr>
<td>CT</td>
<td>congenital toxoplasmosis</td>
</tr>
<tr>
<td>CTYI</td>
<td>Centre for Talented Youth of Ireland</td>
</tr>
<tr>
<td>CWIUH</td>
<td>The Coombe Women and Infant’s University Hospital</td>
</tr>
<tr>
<td>DBS</td>
<td>dried blood spots</td>
</tr>
<tr>
<td>dL</td>
<td>decilitre</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DoHC</td>
<td>Department of Health and Children</td>
</tr>
<tr>
<td>DT</td>
<td>dye test</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immuno Sorbent Assay</td>
</tr>
<tr>
<td>EMCS</td>
<td>emergency caesarean section</td>
</tr>
<tr>
<td>EMSCOT</td>
<td>European Multicenter Study on CONgenital Toxoplasmosis</td>
</tr>
<tr>
<td>Est</td>
<td>estimated</td>
</tr>
<tr>
<td>EU/EEA</td>
<td>European Union (EU) and European Economic Area (EEA)</td>
</tr>
<tr>
<td>EUROTOXO</td>
<td>The European TOXOplasmosis prevention project</td>
</tr>
<tr>
<td>FBC</td>
<td>full blood count</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GCSF</td>
<td>granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPSC</td>
<td>Health Protection Surveillance Centre</td>
</tr>
<tr>
<td>HSE</td>
<td>Health Service Executive</td>
</tr>
<tr>
<td>HSV</td>
<td>herpes simplex virus</td>
</tr>
<tr>
<td>I</td>
<td>one</td>
</tr>
<tr>
<td>ID</td>
<td>infectious diseases</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>II</td>
<td>two</td>
</tr>
<tr>
<td>III</td>
<td>three</td>
</tr>
<tr>
<td>IND</td>
<td>indeterminate</td>
</tr>
<tr>
<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>ISAGA</td>
<td>Immuno Sorbent Agglutination Assay</td>
</tr>
<tr>
<td>IU/mL</td>
<td>international units per millilitre</td>
</tr>
<tr>
<td>IUGR</td>
<td>intrauterine growth retardation</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LT</td>
<td>left</td>
</tr>
<tr>
<td>Mat</td>
<td>maternal</td>
</tr>
<tr>
<td>mg</td>
<td>milligrams</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>N</td>
<td>normal</td>
</tr>
<tr>
<td>NA</td>
<td>not available or no answer</td>
</tr>
<tr>
<td>NCBI</td>
<td>The National Council for the Blind in Ireland</td>
</tr>
<tr>
<td>NCCA</td>
<td>National Council for Curriculum and Assessment</td>
</tr>
<tr>
<td>NCCCTS</td>
<td>National Collaborative Chicago-based Congenital Toxoplasmosis Study</td>
</tr>
<tr>
<td>ND</td>
<td>not detected</td>
</tr>
<tr>
<td>Neg</td>
<td>negative</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>NNBSL</td>
<td>National Newborn Bloodspot Screening Laboratory</td>
</tr>
<tr>
<td>NP</td>
<td>not performed</td>
</tr>
<tr>
<td>NSC</td>
<td>National Screening Committee</td>
</tr>
<tr>
<td>NT</td>
<td>not tested or not treated</td>
</tr>
<tr>
<td>OLCH</td>
<td>Our Lady’s Children’s Hospital</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PHN</td>
<td>public health nurse</td>
</tr>
<tr>
<td>PLCS</td>
<td>planned caesarean section</td>
</tr>
</tbody>
</table>
Pos | positive
---|---
PVL | periventricular leucomalacia
RCTs | randomised controlled trials
RT | right
SFDT | Sabin Feldman dye test
SNSD | severe neurological sequelae or disease
SP | strongly positive
STEN | standardized academic testing out of ten
SVD | spontaneous vaginal delivery
SWGT | Swiss Working Group on Toxoplasmosis
SYROCOT | Systematic Review Of COngenital Toxoplasmosis
*T. gondii* | Toxoplasma gondii
TCUH | The Children’s University Hospital
TgERP | *T. gondii* embryogenesis-related protein
TLRs | toll-like receptors
TRL | Toxoplasma Reference Laboratory
U.S | United States
U.S.A. | United States of America
UK | United Kingdom
VHQ | ventricular head quotient
VL | very low
VP | ventriculoperitoneal
WB | Western blot
WHO | World Health Organization
WISC-III | Wechsler Intelligence Scale for Children–Third Edition
yrs | years
LIST OF FIGURES

Figure 1.1: Eye fundus aspects of ocular toxoplasmosis .................................................33
Figure 1.2a: Risk estimates of mother-to-child transmission of toxoplasmosis: risk of congenital infection by duration of gestation at maternal seroconversion based on a cohort of 554 women by Dunn et al .................................................................34
Figure 1.2b: Risk estimates of mother-to-child transmission of toxoplasmosis: risk of developing clinical signs before age 3 years according to gestational age at maternal seroconversion when congenital infection is known .......................................................35
Figure 1.2c: Risk estimates of mother-to-child transmission of toxoplasmosis: risk of developing clinical signs before age 3 years according to gestational age at maternal seroconversion when infection status of the fetus is not known ........................................36
Figure 3.1 a, b, c and d: toxoplasma parasite forms ..........................................................50
Figure 3.2: Life cycle of T. gondii ......................................................................................51
Figure 6.1: McLeod 2006; Outcome of Treatment for Congenital Toxoplasmosis, 1981-2004:NCCCTS ...............................................................................................92
Figure 6.2: comparison of subgroups from the NCCCTS treated (blue box) and untreated (purple box) for CT .........................................................................................93
Figure 7.1: Map of Surveillance systems for toxoplasmosis in Europe .................................101
Figure 7.2: Geographical representation of nationwide and regional policies for prevention of CT in Europe ............................................................................................102
Figure 8.1: Counties in Ireland with toxoplasma seroprevalence above or below national average ..................................................................................................................116
Figure 9.1: Summary of the CT screening protocol ................................................................138
Figure 9.2: The second adaptation of the AutoDELFIA assay for the CT screening protocol ..............................................................139
Figure 10.1: Blood IgM concentration (AutoDELFIA) in 299 infants with a detectable initial screen ............................................................................................................151
Figure 10.2: Outcome of the population screened for congenital toxoplasmosis ..........152
Figure 10.3: Infants screened per six month period over 24 months (n=34) ......................153
Figure 10.4: Geographical comparison of maternal seroprevalence of Toxoplasma antibody (map a) and number of screen positive cases by county (map b) .................154
Figure 10.5: Maternal ethnicity for 34 screen positive infants ...........................................155
Figure 11.1: Geographical comparison of maternal seroprevalence (map a) and number of false positive cases by county (map b) ...................................................176
Figure 11.2: Maternal ethnicity for 19 screen positive infants confirmed serologically negative ................................................................................................................177
Figure 11.3: Summary of 19 screen positive infants with serological exclusion of CT ....178
Figure 13.1: Geographical comparison of maternal seroprevalence of toxoplasma antibody (map a) and number of congenital toxoplasmosis cases by county (map b)....217
Figure 15.1: Cranial imaging for case number 3 ...............................................................243
Figure 15.2: Cranial imaging for case number 5 ...............................................................244
Figure 15.3: Intra-cranial imaging for case number 15 at age three weeks ...................245
Figure 15.4: Follow-up imaging for case number 15 .......................................................246
Figure 16.1: Eye fundus photographs of case number 7 ..................................................257
Figure 18.1: A continuum of primary school assessment methods .....................................275
Figure 18.2: Outcome of a cohort screened for CT in Ireland at median 9-year follow-up ..............................................................276
Figure 19.1: Cranial imaging for congenitally infected case number 3 .........................327
Figure 19.2: CT case number 5: cranial imaging ............................................................328
Figure 19.3: CT case number 7: eye fundus photographs ......................................................... 329
Figure 19.4: Intra-cranial imaging for CT case number 15 at age three weeks ................. 330
Figure 19.5: Case number 15; imaging at 3 years ................................................................. 331
LIST OF TABLES

Table 7.1: European correspondents contacted to participate in the survey on epidemiological surveillance of toxoplasmosis .................................................................103
Table 7.2: Characteristics of European epidemiological surveillance programmes for toxoplasmosis (Eurotoxo 2007) ........................................................................................................104
Table 7.3: National public health policies and local routine practices to prevent CT in Europe 2005 ..................................................................................................................105
Table 8.1: Number of births registered per county, number screened and percentage positive, with 95% confidence intervals in each county ..................................................117
Table 8.2. Percentage positive in each county & mean maternal age ........................................118
Table 10.1: 34 screen positive results and confirmatory serology for 34 mother-infant pairs ......156
Table 10.2: Summary of infant and maternal results in those with a positive screen ..............160
Table 10.3: Positive predictive value for the AutoDELFIA IgM screen ..................................161
Table 10.4: Positive predictive value for the ISAGA IgM screen .............................................162
Table 11.1: Mother-infant toxoplasma antibody profiles for 19 screen positive infants with serological exclusion of CT ......................................................................................179
Table 12.1: 15 infants with confirmed congenital toxoplasmosis; screening results and confirmatory serology (15 mother-infant pairs) ......................................................................203
Table 12.2: Initial evaluation summary for 15 congenitally infected infants ..........................204
Table 12.3: Serological profile for congenitally infected infant case number 4 .......................205
Table 12.4: Signs on initial evaluation of cohorts screened for CT: Ireland compared with Denmark ..................................................206
Table 13.1: Maternal data (n=15) ............................................................................................218
Table 13.2: Infected infants and corresponding maternal risk factors for pregnancy seroconversion ...................................................................................................................219
Table 14.1: Classification of Paediatric Neutropenia* ................................................................234
Table 14.2: Summary of infant treatment ...............................................................................235
Table 14.3: Summary of interventions applied during antiprotozoal treatment for CT in 14 infants ....................................................................................................................236
Table 15.1: Intracranial imaging and effect of treatment on intracranial signs in a cohort with CT .......................................................................................................................247
Table 16.1: Clinical findings and ophthalmic outcome of 15 children with CT ......................258
Table 17.1: Serological profile during treatment and rebound post treatment in a cohort of infants with CT ...........................................................................................................266
Table 18.1: Clinical, developmental and educational progress summary for 15 congenitally infected infants ........................................................................................................277
Table 19.1: Congenital toxoplasmosis; screening results and confirmatory serology for 15 mother-infant pairs ..................................................................................................332
Table 19.2: Serological profile for congenitally infected infant case number 4 .......................333
SUMMARY

Introduction: The majority of infants with congenital toxoplasmosis (CT) in Europe are asymptomatic and remain undetected during routine postnatal care. Postnatal screening for CT facilitates early diagnosis, targeted therapy and intervention to optimise neurodevelopmental outcome. A previous study in Ireland demonstrated that maternal toxoplasma seroprevalence was 25%, which implied that 75% remained susceptible to seroconversion in pregnancy. Thus funding was sought for a study to determine 1) the feasibility of postnatal screening for CT, 2) the incidence of CT and 3) outcome during the first decade of life.

Methods: A two-year pilot newborn screening programme was initiated on July 1st 2005. A two-step IgM screening protocol was employed (AutoDELFIA and ISAGA assays) to test dried heel blood spots obtained 72-120 hours after birth. Diagnosis was confirmed using paired mother/infant serology. Detailed clinical evaluation, one-year anti-protozoal therapy and clinical follow-up was offered to all diagnosed infants.

Results: Toxoplasma IgM was easily recovered from dried heel blood. The screening assays were not highly predictive. Thirty-four screened positive, 19 were false positives. A diversity of confirmatory serology was encountered that necessitated serial testing in a minority. Congenital toxoplasmosis was confirmed in 15 children giving an incidence of 1 per 10,000 births. Two of 15 (13%) were symptomatic. Thirteen of 15 (87%) were asymptomatic, four (31%) of whom had detectable clinical signs: all four had inactive chorioretinal scars and two had intracranial calcification. Most women seroconverted in trimester 3 and reported food sources as risk factors with lack of awareness. Antiprotozoal treatment was prescribed for 14 infants; reversible neutropenia was common during treatment. One asymptomatic child was not treated due to non-confirmatory serology up to nine months.

Median follow-up was 9 years (range 2 to 10 years). Fourteen have normal neurodevelopmental outcome and visual acuity including one asymptomatic infant who had a unilateral inactive retinal lesion at birth that reactivated at age two years. Regression of intracranial calcification and ventriculomegaly was noted following treatment completion. One child with hydrocephalus and ventriculoperitoneal shunt has minor co-ordination deficits but normal cognition and attends main stream school.
Discussion
The low incidence of CT in Ireland was comparable with rates previously reported for other parts of Western Europe. The rate of false positive screening results (1.3 per 10,000) demonstrates that if CT were to be considered for inclusion in the routine postnatal screening programme, alternative screening assays would be more desirable than those chosen for this study.

The predominance of asymptomatic cases of CT was likely due to the avirulent type II strain of *T. gondii* in Europe plus predominance of trimester 3 seroconversion in the cohort of women with infected infants. However, lesions in the central nervous system were detected only because of screening in one third of asymptomatic infants.

The maternal dietary and lifestyle risk factors identified in the study can be potentially targeted as future primary prevention strategies for CT in Ireland. The frequency of adverse events encountered during antiprotozoal treatment demonstrated the need for alternative, less toxic treatment regimens for CT and research in this area.

Reactivation in an asymptomatic child with subclinical disease on evaluation demonstrates the importance of treatment and ophthalmological surveillance for all cases of CT and not just those with symptoms or severe signs at initial assessment.

Overall, better than anticipated visual and neurodevelopmental outcomes were demonstrated in children with CT where early treatment was facilitated by the postnatal screening program. Limitations to this study were the small sample size of 15 infected infants and lack of an untreated control group.
ACKNOWLEDGEMENTS

The study presented in this thesis was a nationwide screening programme which took place between 2005 and 2007 with clinical follow-up offered for a decade to those recruited.

I would like to first acknowledge the programme director and the 2 programme supervisors listed below. Their immense background work became the evolution of this study, supported the screening proposal and enabled funding and execution of a 2-year national pilot programme of newborn screening for congenital toxoplasmosis in Ireland. Many thanks to you all for your support to me in my role as the screening programme co-ordinator throughout.

To the programme supervisors; Professor Mary Cafferkey, Consultant Microbiologist RCSI, and Professor Karina Butler Consultant Paediatrician & Paediatric Infectious Diseases Specialist at OLCHC. Thank you both for your unending and reliable support during and after the programme, for assistance with all laboratory and clinical issues and for provision of guidance throughout with the various aspects of patient management and follow-up over the last decade. It was always reassuring to know that despite our individual roles for the study, challenges encountered as the programme co-ordinator could be shared. A sincere thanks also for your supervisory input with the preparation and completion of this thesis over the years. Your time, advice, encouragement, help and patience were greatly valued to say the very least.

To the programme director; Professor Philip Mayne, Consultant Chemical Pathologist and Director of the National Newborn Bloodspot Screening Laboratory at TCUH Dublin. Thank you for facilitation of newborn screening; provision, interpretation and collation of screening results, and for management on my behalf of all the issues that arose in and out of the screening laboratory upon screening implementation and during the 2 years. Thank you also for your advice and input into the relevant areas of the thesis.

I would like to acknowledge and thank The Department of Health and Children for provision of funding for this programme.
Many thanks to Dr Edward Guy, Director and Consultant Clinical Scientist at the Toxoplasma Reference Laboratory Singleton Hospital Swansea UK, for facilitation of prompt serological confirmation of screen positive cases, adjunctive confirmatory methods when necessary, 5-year serological follow-up of recruited children, and for assistance with interpretation of all serology results. Many thanks also to Ms Janet Francis (Deputy head of unit) and colleagues at the laboratory for prompt delivery of results during the programme.

A sincere thanks to the parents who consented for their children to participate in the screening study, and in particular to the affected children and their families who continued with follow-up over the decade.

The following were directly involved in the screening programme or follow-up of cases identified. Many thanks for your valued contribution and cooperation.

- Directors of midwifery and public health, screening liaison midwives at all maternity and paediatric units, hospital and community staff responsible for routine collection of newborn dried blood spots on filter paper cards
- Ms Nicola Finnegan (clinical scientist) and colleagues at the NNBSL Dublin who tested filter paper cards for toxoplasma IgM during the 2 years of screening
- Microbiology teams nationwide who cooperated and facilitated transport of confirmatory samples to The Dispatch Laboratory at TCUH
- Mr Pat Healy and colleagues at The Dispatch Laboratory, TCUH Dublin who prepared and arranged transport of confirmatory samples to the UK Reference Laboratory in a timely manner
- Staff at the National Virus Reference Laboratory who provided stored maternal samples for testing at the Toxoplasma Reference Laboratory
- Obstetric teams nationwide who provided antenatal data where necessary
- The ID team at OLCH and TCUH Dublin who were involved with management of affected children
- Dr Brendan Murphy (consultant paediatrician, Cork) and all other paediatric teams nationwide involved in the management and follow-up of cases
- Ms Kathryn Mc Creery (consultant ophthalmologist) and colleagues at OLCH Dublin, and ophthalmologists nationwide who provided follow-up surveillance
• Drs Stephanie Ryan, Ailbhe Tarrant, Ethna Phelan (consultant radiologists) and colleagues, in addition to radiology teams at Cork University Hospital
• Audiology services nationwide
• Public health staff who provided a link with families during treatment and follow-up of children

Special thanks to: Dr Valerie Jackson, clinical surveillance scientist at The Rotunda Hospital for her technical assistance with the many tables and figures of the thesis and for document formatting; and to Anne O'Byrne, librarian at The Rotunda Hospital, for her management of the Reference Manager database used for this thesis. Many thanks to you both for your time, input and constant patience especially during the many periods of technical difficulty with its associated trials and tribulations. Special thanks also to Drs Richard Drew and Joanna Griffin for their valued input.

I would like to thank my parents, in addition to family members, friends and colleagues, too many to name, but who all provided invaluable moral support during my writing of the thesis. Thank you all for your interest, encouragement and good wishes, they did not go unnoticed.

Finally, to my husband Alan and children Dean, Julie and Lisa. An enormous thank you for being so patient, tolerant, supportive and understanding during the many times I chose a computer screen instead of your company in order to complete this thesis, which would have been a greater task without you four wonderful people in my life.
Dedicated to Dean, Julie and Lisa
CHAPTER 1 - Toxoplasmosis

1.1 Introduction
Toxoplasmosis, an infection with global distribution, is caused by an intracellular obligate protozoan parasite, *Toxoplasma gondii* (*T. gondii*). The parasite has the capacity to infect most species of warm-blooded animals and inhabit various regions worldwide.

Toxoplasma infection can be acquired at any stage in life. Toxoplasmosis in an immunocompetent individual generally poses little threat to health. Infection can be entirely asymptomatic or mimic a ‘flu’ or mononucleosis-like syndrome. However there are some literature reports of severe disease and grave consequences in healthy subjects and this possibly relates to variations in parasite virulence and host genetic factors (Snopkova et al., 2013; Undseth et al., 2014).

*T. gondii* is classified into different serotypes or strains (Ajzenberg et al., 2002). The most prevalent serotypes found worldwide are I, II and III. Studies have demonstrated that serotypes I and III are common in South America, serotypes I and II predominate in North America and serotype II is predominant in Europe (Shobab et al., 2013). Serotype I infection is associated with severe clinical manifestations and serotype II correlates with a milder or benign course of disease (Peyron et al., 2006; Khan et al., 2006; Gilbert et al., 2008). In addition, atypical and non-typeable recombinant strains of *T. gondii* exist and are associated with aggressive disease even in immunocompetent individuals (Bossi et al., 2004; Leal et al., 2007); such serotypes are also commonly reported in South American countries and less frequently in North America (Vaudaux et al., 2010; Wujcicka et al., 2014).

The highest risk for adverse outcomes associated with toxoplasmosis occurs with infection of an immunocompromised host, which includes the developing fetus (Moncada et al., 2012). Toxoplasma infection occurring *in-utero* or during fetal life is known as congenital toxoplasmosis (CT), infection that occurs for the first time beyond fetal life is referred to as acquired toxoplasmosis in order to distinguish from congenital infection.
The most common clinical manifestations of both congenital and acquired toxoplasma infection are ocular lesions featuring as inflammation of the retina and choroid known as chorioretinitis or retinochoroiditis (Commodaro et al., 2009).

1.2 Ocular manifestations of toxoplasmosis

Manifestations of ocular toxoplasmosis vary from severe to mild reflecting variation in parasite characteristics and the immune response of the host (Holland, 2004; Garweg et al., 2009). Lesions of acute ocular toxoplasmosis have indistinct borders and appear as yellow/white cotton wool patches which represent inflammatory exudates that are cast off from acute lesions (figures 1.1 a and c). They may be the same size as the optic disk or larger. Older lesions appear sharply outlined and are speckled with dark choroidal pigment (figures 1.1 b and d).

Retinal oedema and oedema of the macula can occur during the acute and sub-acute phases of ocular toxoplasmosis and temporarily blur vision but the primary cause of this symptom is the inflammatory response within the vitreous fluid. On clinical examination, this results in the so called pathology “headlamp in the fog”. Macular oedema is usually temporary but if long standing can cause cystic changes in the fovea and permanently impair central visual acuity even in the absence of central lesions or involvement of the optic nerve. Optic nerve damage can be primary from destruction of the macula or retina when T. gondii lesions are present in the optic nerve; or secondary due to damage from papilloedema. In severely affected eyes, focal necrotizing retinitis can occur (Melamed et al., 2010). Pupillary discrepancy may represent severe intraocular inflammation. Peripheral lesions can occur in one or more quadrants of the retina and choroid.

Irreversible visual loss or impairment may occur if the macula and structures in the optic pathway are affected (Bosch-Driessen et al., 2002). Macular lesions can cause atrophy of the optic nerve, which is associated with a poor prognosis. Normal visual acuity may occur with large macular scars sparing or involving the fovea.
Severe intraocular changes occur in immunocompromised individuals and have also been described in immunocompetent adults infected with atypical or virulent strains of the parasite (Switaj et al., 2006; Alvarez et al., 2014).

Permanent scarring occurs following resolution of a focus of chorioretinitis and these are referred to as inactive chorioretinal scars which contain *T. gondii* cysts (Holland, 2004). Retinal scars can be central, peripheral, unilateral or bilateral and are associated with a risk of partial or complete retinal detachment. Inactive retinal scars can cause permanent visual field defects depending on location (Scherrer et al., 2007).

Congenitally infected infants may initially have a normal ocular exam and present with chorioretinal lesions or scarring at any stage following diagnosis. A histopathological study of congenital ocular toxoplasmosis in mice found toxoplasma-containing cysts within the retina and optic nerve irrespective of disease severity (Remington, 2011).

The risk of chorioretinitis in a child with CT is highest in the first two years of life. Thereafter, existing ocular lesions can remain dormant and reactivate and progress after many years, primarily in the first decade of life up to adolescence but also up to the second decade of life. Chorioretinal lesions can also appear in children who had no intraocular abnormalities in the newborn period (Kodjikian et al., 2006; Kieffer et al., 2008). Ocular toxoplasmosis is the most common cause of posterior uveitis worldwide and in some instances may represent a late manifestation of congenital toxoplasmosis (Holland, 2003; Guex-Crosier, 2009).

In addition to retinochoroiditis, other less common ocular manifestations of congenital infection are described such as microphthalmia, optic nerve atrophy, cataract, strabismus, nystagmus, anisometropia and iris abnormalities (Wallon et al., 2004). The differential diagnosis of congenital ocular toxoplasmosis includes: lymphohoriomeningitis, syphilis, cytomegalovirus (CMV), herpes simplex virus (HSV), rubella, birth injury, retinoblastoma and congenital anomalies such as congenital aneurysm or telangiectasia (Miller et al., 1969; Silveira et al., 2002).
Toxoplasma infection can also manifest as extra-ocular systemic disease, e.g., pneumonitis, encephalitis or disseminated disease, particularly in the immunocompromised host (Schmidt et al., 2013).

### 1.3 Congenital toxoplasmosis

Congenital toxoplasmosis is the consequence of maternal primary infection and seroconversion during pregnancy or the periconceptional period (Binquet et al., 2004; Robert-Gangneux et al., 2009). Women who acquire toxoplasma infection prior to pregnancy are generally immune and not susceptible. Rarely, congenital infection has been reported following maternal parasitaemia up to six months prior to conception, or re-infection with an atypical strain in a previously exposed immunocompetent individual (Vogel et al., 1996; Elbez-Rubinstein et al., 2009). Primary infection in an immunocompetent woman is asymptomatic in 60% of cases. Symptoms, if any during pregnancy are often mild and non-specific such as fatigue, malaise, low-grade pyrexia, lymphadenopathy and myalgias (Kravetz et al., 2005). In immunocompromised women, latent toxoplasma infection with reactivation during pregnancy may lead to infant congenital infection (Fernandes et al., 2012).

Fetal infection can occur in-utero or peripartum. Transmission by breastfeeding has not been reported. During the acute phase of maternal infection, rapidly multiplying parasites can invade the placenta and enter the fetal circulation causing inflammation and damage to developing organs. Studies have demonstrated that parasitaemia may occur at an early stage during acute infection in pregnancy, prior to the onset of host clinical signs or demonstrable serum antibodies, or there may be a delay between maternal seroconversion and contamination of the placenta (Marx-Chemla et al., 1990). The exact timing between initial maternal infection and development of parasitaemia and subsequent antibodies is unknown. More importantly, precise data is not available on duration of parasitaemia after acute maternal infection. Persistent parasitaemia has rarely been reported in immunocompetent individuals but recurrent parasitaemia has been demonstrated in patients with acquired immune deficiency syndrome (AIDS) (Jongert et al., 2009).

The diagnosis of CT can be confirmed by:
1) The presence of *T. gondii* in tissues or fluids by polymerase chain reaction (PCR), cell culture, immunocytochemistry or animal inoculation;
2) The presence of specific immunoglobulin (Ig) M or IgA antibodies;
3) Persistent IgG positivity up to and beyond one year of age
4) *T. gondii* cysts visualised in placental or fetal and newborn tissues at post mortem.

*T. gondii* organisms have been isolated from human placentas with congenitally infected infants. Histology of placental tissue with acute toxoplasma infection demonstrates inflammation around tissue cysts and necrotizing lesions associated with the presence of the parasite. Sarrut et al historically reported a correlation between the clinical severity of neonatal manifestations and the presence of parasites in the placenta. Parasites were not demonstrated in the placentas of infants who did not have clinical signs at birth but developed signs weeks later (Sarrut, 1967).

The risk of mother-to-child transmission of toxoplasmosis increases with gestational age at maternal seroconversion (Dunn et al., 1999). Maternal infection acquired during the first trimester is associated with a relatively low, i.e., < 10% risk of transmission to the fetus but a higher incidence of stillbirth and long-term neurological sequelae in infants who become infected. This contrasts with later infection during the third trimester where there is a > 60% risk of transmission to the fetus but a more favourable infant outcome (figure 1.2 a, b and c). Rarely, signs of severe congenital infection may occur in infants of immunocompetent women who seroconvert during the third trimester (Armstrong et al., 2004).

Transmission of infection during weeks 10 to 24 is associated with the highest risk of severe or clinically apparent disease, whereas transmission in the period of 26 to 40 weeks (trimester 3) may result in subclinical or unapparent disease, which can manifest later in life. The risk of having an infant with symptoms at birth or later in childhood is 60% with maternal seroconversion at 12 weeks gestation and decreases to 5% or less with seroconversion after 36 weeks gestation (Figure 1.2 a and b).

Congenital *T. gondii* infection may present in one of four clinical scenarios:
1) Symptomatic neonatal infection;
2) Asymptomatic neonatal infection with or without signs consistent with CT on evaluation, also referred to as subclinical infection;
3) Disease manifesting in the first months of life or infancy;
4) Sequelae or relapse of previously undiagnosed infection, usually within the first two decades of life.

Spontaneous abortions, prematurity or stillbirth occur in approximately 1% of infected infants (Freeman et al., 2005; Havelaar et al., 2007). In countries north of the equator, approximately 10% of congenitally infected infants are symptomatic at birth, 2% of whom will have severe neurological impairment (McLeod et al., 2012). Common neurological signs of CT include intracranial calcifications and chorioretinal lesions.

In Europe, up to 85% of all cases of CT are asymptomatic at birth, some of who will have clinical signs of disease (Foulon et al., 1999). Overall, in Europe approximately one in six infants with CT will have chorioretinal lesions at birth and 9% will have intracranial calcifications (Gilbert et al., 2008; Peyron et al., 2011). Chorioretinal lesions at birth may be active or inactive. Intracranial calcification and inactive chorioretinal lesions both indicate that infection was active in fetal life. Other central nervous system (CNS) signs of CT include: hydrocephalus, microcephaly, destructive lesions such as porencephalic cysts and cystic encephalomalacia; seizures, encephalitis, and to a lesser degree sensorineural hearing loss. The classic triad associated with CT; hydrocephalus, chorioretinal lesions and intracranial calcification is found in only a minority i.e., less than 2% of symptomatic infants (Loewer-Sieger et al., 1985; Remington, 2011).

Clinical manifestations at birth may also be non-specific such as prematurity, intrauterine growth retardation (IUGR), rash, hepatosplenomegaly, anaemia, thrombocytopenia, pancytopenia, prolonged jaundice, abnormal liver function, and pneumonitis (Freeman et al., 2005).

The most common clinical sequel of CT, chorioretinitis, occurs as a result of reactivation of dormant toxoplasma cysts in the retina with associated inflammation. Chorioretinitis eventually manifests during the first two decades of
life in approximately 75% of those congenitally infected (Wallon et al., 2004; McLeod et al., 2006).

The frequency and characteristics of ocular recurrence vary internationally and may be explained by differences in parasite strain and host genetic factors (Jamieson et al., 2008; Rico-Torres et al., 2012). In Europe the overall prognosis of treated CT is good with a low risk of late ocular manifestation (Peyron et al., 2006).

1.4 Global seroprevalence of toxoplasma antibody and incidence of congenital toxoplasmosis

Toxoplasmosis and toxoplasma antibody seroprevalence varies worldwide and is affected by migration trends. The distribution of human infection is highly variable even within a country due to environmental, socioeconomic and cultural factors, parasite potency and host genetics.

Foci of high prevalence (50% to 80%) have been reported in South America, parts of the Middle East, south-east Asia, Africa, Eastern and Central Europe, (Pappas et al., 2009). Lower seroprevalence (20% to 40%) is observed in many European countries and in the United States of America (U.S.A).

Worldwide the incidence of congenital toxoplasmosis varies from 1 in 1,000 to less than 1 per 10,000 births (Lopez et al., 2000). The overall incidence of CT in Europe is estimated at 1 to 5 cases per 10,000 live births (Benard et al., 2008).

In the U.S.A the seroprevalence of toxoplasma antibody in the pregnant population is estimated at 20% (Jones et al., 2007). The actual disease burden or incidence of CT in the U.S.A is not known as there are no national toxoplasma screening programmes and CT is not a notifiable disease. The incidence of CT is estimated to be 1 to 5 cases per 10,000 live births, yielding 500 to 5,000 cases of congenital toxoplasmosis per year with a birthrate of 4,000,000 infants per year (Kim, 2006; Olariu et al., 2011).

In the states of Massachusetts and New Hampshire a newborn screening programme was implemented in 1986 and 1988 respectively. Newborn IgM screening in Massachusetts revealed a CT incidence of approximately 1 case per
10,000 live births which, based on seroprevalence rates in pregnancy of 20%, was consistent with a transmission rate of 10% (Guerina et al., 1994).

In South America, high *T. gondii* antibody seroprevalence has been reported, up to 70% in some parts of Brazil. Limited newborn screening programmes have estimated that the incidence of CT in South America ranges from 1 in 5,000 to as high as 1 in 1,000 in some provinces. Significant regional variance in the prevalence of CT in Brazil has been demonstrated (Neto et al., 2004; Gomez-Marín et al., 2011).

More aggressive clinical disease has been reported in South America and to a lesser extent North America compared with Europe (Peyron et al., 2006; Olariu et al., 2011). This is explained by geographic variations in parasite strain and virulence interacting with host factors (Ajzenberg et al., 2002; Khan et al., 2006).
Figure 1.1: Eye fundus aspects of ocular toxoplasmosis

a, active lesion; b and d, scar; c, active lesion and scars

(Source: Toxoplasmosis – Recent Advances, Djurković Djaković 2012; chapter 7 Risk Factors, Pathogenesis and Diagnosis of Ocular Toxoplasmosis, pp 129-144)
Figure 1.2a: Risk estimates of mother-to child transmission of toxoplasmosis: risk of congenital infection by duration of gestation at maternal seroconversion based on a cohort of 554 women by Dunn et al
Figure 1.2b: Risk estimates of mother-to-child transmission of toxoplasmosis: risk of developing clinical signs before age 3 years according to gestational age at maternal seroconversion when congenital infection is known.
Figure 1.2c: Risk estimates of mother-to child transmission of toxoplasmosis: risk of developing clinical signs before age 3 years according to gestational age at maternal seroconversion when infection status of the fetus is not known

(Source: Dunn et al, The Lancet 1999; 353: p1829-1833)
CHAPTER 2 - Discovery, historical significance and nomenclature

2.1 Discovery of the parasite

*T. gondii* was discovered in the early 20th century by two French scientists based in Tunisia; Charles Henry Nicolle and Louis Herbert Manceaux who in 1908 observed the parasites in blood, spleen and liver of a North African rodent. In that year Alfonso Splendore, an Italian scientist who spent most of his career in Brazil also observed the parasite in rabbit tissues. Splendore assisted in defining the parasite in 1908 and was the first to acknowledge that the parasite existed in different forms and specific classification would only be possible by elucidation of the whole life cycle (Morrissette et al., 2009).

The organism was initially named *Leishmania gondii* because of its close resemblance with *Leishmania*. Nicolle and Manceaux later decided on the basis of morphologic criteria the organism was not *Leishmania* and in 1909 proposed the name toxoplasma for its arc-like shape (Ajioka et al., 2009). The name derives from the Greek word *toxikon* (poisoned arrow, from toxon [bow]) and plasma (something moulded). The specific name *gondii* was derived from the type of host, the *gundi*, a small North African rodent that was used as a laboratory animal at the institute in Tunisia.

Historically two other physicians, Alphonse Laveran and Samuel Darling, have been credited with the discovery of toxoplasma in animals and humans. In 1900, Alphonse Laveran, a French physician who identified Plasmodium as the agent of malaria, was the first to observe what may have been the toxoplasma parasite in nucleated red blood cells of Java sparrows. However, for many years after, toxoplasma was confused with either Sarcosporidia or Encephalitozoon until Nicolle and Manceaux formally labeled the parasite in 1909. After 1909, the name *T. gondii* was applied to avian parasites described before or after 1909. However it is unlikely that the avian parasite detailed by Laveran in 1900 was *T. gondii* as toxoplasma invades the organelle-free red cells of mammals. Bird red cells are nucleated and lack organelles; hence the parasite observed by Laveran was likely to be a different species (Dubey, 2008).
Samuel Taylor Darling, an American pathologist working in Panama during the decade of the Panama Canal construction, first described toxoplasmosis in humans in 1908 (Chaves-Carballo, 1970). Darling observed the parasite in post mortem examination of human muscle biopsies but at the time identified it as Sarcosporidia. However retrospective examination of the biopsy illustrations strongly suggested that the parasite was *T. gondii* and hence Darling was credited with the discovery of toxoplasmosis in humans.

### 2.2 Discovery of the clinical spectrum of *T. gondii* infection in humans

Almost 20 years lapsed between reports of possible toxoplasmosis in humans in 1908 and its confirmation as a human pathogen and even then initial observations could not identify the parasite.

The first case of congenital toxoplasma infection was described in 1923 by a Czech ophthalmologist Josef Janku who evaluated an 11-month-old child suffering from progressive hydrocephalus, seizures and unilateral microphthalmia. The child died and Janku presented the clinical and autopsy findings. Prior to death the child’s eyes contained striking retinal lesions of chorioretinitis. Histology of the eyes demonstrated numerous pigmented lesions, drop-like and ring-like small shapes with absent retinal vessels in the area of the lesion. Janku’s illustrations to accompany the case retrospectively demonstrated classic fundoscopic changes of toxoplasmic chorioretinitis. Photomicrographs identified sporocysts surrounding the entire pathological region. Janku referred to the organism as Sporozoa and did not diagnose toxoplasmosis or identify the observed cysts as those of *T. gondii* (Weiss et al., 2009). The documents from this case were presumably destroyed during World War II bombings.

In 1927, Magarinos Torres, a Brazilian pathologist, described an infant in Rio de Janeiro deceased 29 days after birth with microorganisms in histological sections of brain, myocardium and skeletal muscle. However he failed to recognise the causative organism as *T. gondii* and labeled it as *Encephalitozoon chagasi* (Vaz, 2011).

In 1928 Constantin Levaditi, a Romanian bacteriologist in Paris, recognised the parasite as *T. gondii* and suggested that both aforementioned cases of infant demise were due to *T. gondii* and that there was a possible connection between...
the infection and congenital hydrocephalus. Subsequently a French pathologist, Coulon in 1929 observed similar parasites, which were presumed to be *Encephalitozoon brumpti* in the spinal fluid of a 17-year-old male from Corsica who died of meningitis.

Thereafter similar clinical findings were reported in patients from other countries and hence the medical history and implications of toxoplasma to human health began 20 years after its discovery as an infection in rabbits and rodents. Many years elapsed before toxoplasma was established as a causative agent for neurologic disorders in children.

It was not until 1937 that toxoplasmosis was recognised and confirmed as a disease entity in humans by two American scientists, Abner Wolf and David Cowen who described a parasite in the nervous system and retina of an infant with hydrocephalus, encephalitis and chorioretinitis. In that year, Albert Sabin, a Polish American virologist performed the first detailed scientific analysis of *T. gondii* using techniques previously developed for analyzing viruses and discovered that this parasite was indistinguishable from *T. gondii*. Sabin and colleague Olitsky showed that *T. gondii* was an obligate intracellular parasite and that mice fed *T. gondii*-contaminated tissue also contracted the infection. Thus Sabin and Olitsky demonstrated *T. gondii* was a pathogen transmissible between animals. However there was slight confusion as the appearance of the organism fixed in a tissue histology specimen differed slightly compared with culture smears or peritoneal fluid from infected animals and humans (Sabin et al., 1937).

In 1939 Wolf, Cowen and colleague Beryl Paige at Columbia University in the U.S.A were the first to conclusively identify *T. gondii* as a disease entity in humans. The confirmatory case was a female infant born by caesarean section on May 23rd 1938 in New York. On day three of life the infant developed seizures and bilateral chorioretinitis was observed. The infant died aged one month and post mortem demonstrated free and intracellular *T. gondii* in the brain and retina. Infant samples from the brain and spinal cord were homogenised in saline and inoculated intra-cerebrally into rabbits and mice. The animals developed encephalitis from which *T. gondii* was isolated and further inoculated into other experimental mice. Sabin concluded that the human strain of *T. gondii* was no
different from previous isolates from pigs. Wolf Cowen and Paige concluded that human infection with *T. gondii* caused a distinct syndrome of clinical signs in infants.

Wolf and Cowen subsequently performed an extensive review, which established beyond question that the infantile form of toxoplasmosis was prenatal in origin. Wolf, Cowen, and Paige determined these findings represented the syndrome of severe congenital *T. gondii* infection. Wolf and collaborators retrospectively re-examined and re-classified literature reports of infants with neurologic abnormalities as cases of congenital toxoplasmosis and concluded that clinical signs of chorioretinitis, intracranial calcification and hydrocephalus or microcephaly defined congenital toxoplasmosis. The contributions of Wolf Cowen and Paige provided the foundation for existing knowledge on congenital toxoplasmosis (Wolf et al., 1940; Paige et al., 1942).

In 1939, Sabin isolated *T. gondii* from two children aged six and eight years with fatal encephalitis (Sabin et al., 1949). He noted that the six-year-old child presented with neurological signs; however there were also signs outside the nervous system, mainly generalised lymphadenopathy and splenomegaly. The child died on day 13 of the illness. Following inoculation of CNS tissue from the child into mice, one of the mice developed a swollen abdomen with peritoneal fluid, which contained *T. gondii* organisms extracellularly and intracellularly. Sub-inoculation into other mice produced fatal toxoplasmosis in each case, and cats that fed on the dead mice produced toxoplasma oocysts in their faeces. After prolonged passage in mice in many laboratories its pathogenicity stabilised and the parasite lost the capacity to produce oocysts in cats. This laboratory strain was labeled as the type I strain of *T. gondii* and that name has remained to this day (Frenkel et al., 1976).

In 1940, Henry Pinkerton, a pathologist at the school of medicine Wisconsin and David Weinman, a microbiologist at Yale University school of medicine, were the first to report acute toxoplasmosis in an adult without neurological signs. The patient was a 22-year-old Peruvian man immune-suppressed and recovering from a preceding Bartonella infection. Lymphadenopathy was found at autopsy and *T. gondii* was observed in tissue sections. Lymphadenopathy was later
recognised as a characteristic sign of toxoplasmosis when it was described during pregnancy in 1951 (Gard et al., 1951).

Subsequently Pinkerton reported on two adults aged 40 and 50 years who died in Missouri with a spotted fever like syndrome and atypical pneumonia. *T. gondii* was found in tissue at post mortem and by serial passage into animals (PINKERTON, 1961).

In 1948 Albert Sabin collaborated with Harry Feldman, a senior fellow in virology to develop a serologic test for detecting toxoplasma infection, the dye test (DT). This was one of the most major advances in the study of toxoplasmosis. The dye test was sensitive and specific with no evidence for false results in humans (Sabin et al., 1948). The test facilitated the study of various aspects of toxoplasmosis and demonstrated that *T. gondii* caused a spectrum of disease in humans. The dye test assisted in differentiating congenital toxoplasmosis from other congenital infections.

Albert Sabin also contributed to the discovery of effective treatment against toxoplasma infection. In 1942 Sabin and Warren reported the effectiveness of sulfonamides against murine toxoplasmosis (Sabin, 1950).

The pioneering work of Wolf, Cowen and Sabin allowed retrospective identification of the earliest case of CT on record in the U.S.A. In 1948 Jacob Karl Frenkel a senior assistant pathologist based at the San Francisco medical centre studied preserved pathological material from 1923 from a fatal case of hydrocephalus with microphthalmia and the report was published in 1950 (Frenkel et al., 1950). The infant was born prematurely in San Francisco in 1923 and died on day five. Slides, paraffin blocks and the eyes of the infant had been preserved and stored. Examination of the specimens in 1948 demonstrated that both eyes contained foci of retinal degeneration with *T. gondii* infiltrates. Microscopic sections of other organs demonstrated myocarditis, pneumonitis and hepatitis. Twenty-five years after giving birth to the infant the mother was tested for *T. gondii* antibody using the dye test and a titre of 1:64 was obtained.
In 1951 Frenkel and colleague Saul Friedlander published a report of five fatal cases of congenital hydrocephalus associated with toxoplasmosis; the parasite was isolated from two of the cases. Frenkel and Friedlander explained that hydrocephalus in these infants was secondary to a *T. gondii* antigen-antibody reaction which caused ventriculitis and blockage of the aqueduct of Sylvius. This was the first detailed description of the pathogenesis of lesions of congenital toxoplasmosis in the CNS (Frenkel, 1988).

In 1956 Jorgen Siim, a virologist at The Statens Serum Institut Copenhagen, reported on lymphadenopathy as the only presentation associated with acute toxoplasmosis in adults and these findings were confirmed in 1958 in a study of 30 adult patients (BEVERLEY et al., 1958). However, the spectrum of clinical manifestations increased with reports of encephalitis caused by *T. gondii* during the 1960s and 1970s, albeit in immunosuppressed patients with Hodgkin’s disease (Frenkel et al., 1978).

CNS toxoplasmosis was rarely reported in adults until the 1980’s. In 1983, the first case series of CNS toxoplasmosis, noted to be fatal if untreated, complicating human immunodeficiency virus (HIV) was reported (Luft et al., 1983). Remington recognised that reactivation and dissemination of toxoplasma infection was related to defective T-Cell immunity in HIV infected patients, in whom toxoplasmosis was often the first indicator of immune compromise (Remington, 2011). CNS toxoplasmosis was one of the most common HIV associated opportunistic infections prior to the development of effective antiretroviral therapy (ART) regimens (Bertoli et al., 1995).

CNS toxoplasmosis remains a problem in people living with HIV where antiretroviral therapy is not available. Even in resource rich settings e.g., Ireland, CNS toxoplasmosis remains a regular diagnosis in people living with HIV, as approximately half of these individuals present with low CD4 counts, and many also present with an AIDS defining illness due to late presentation.

2.3 History of ocular toxoplasmosis: congenital, acquired and reactivated

In 1951 Michael Hogan, an ophthalmologist at The University of California school of medicine was the first physician to describe in detail the pathogenesis of
ocular toxoplasmosis (Hogan, 1958). Hogan fully characterised ocular manifestations of congenital and postnatally acquired acute infections. He noted that symptoms of acute ocular toxoplasmosis in adults occurred mainly with other systemic symptoms and that chorioretinal lesions of congenital toxoplasmosis were indistinguishable from ocular lesions in patients who presented with acute toxoplasmosis later in life. Hence, at the time, all cases of ocular toxoplasmosis were thought to be congenitally acquired, with some cases only manifesting later in life (Kimura et al., 1964).

In 1951 a German ophthalmologist, Horst Rieger, introduced the theories that ocular toxoplasmosis could occur in the setting of either CT, CT with late reactivation, adult acquired disease or reactivation of intraocular infection in the immunocompromised.

Treatment of ocular toxoplasmosis with antimicrobials began in the early 1950s. In 1953 parasitologist Don Eyles and colleague Miss Nell Coleman stationed at the laboratory of tropical medicine National Institute of Health, Memphis Tennessee discovered the synergistic effect of combined therapy with sulfonamides and pyrimethamine which became the standard therapy for toxoplasmosis in humans (EYLES et al., 1952). Subsequently Hogan demonstrated that this combination of antiprotozoals effectively resolved chorioretinitis in adults.

Studies of *T. gondii* by electron microscopy began in the 1950s. In 1952, Helenor Campbell Wilder Foerster, a technician in ophthalmic pathology at the Armed Forces Institute of Pathology Washington, was the first to histologically demonstrate *T. gondii* as a cause of chorioretinitis in otherwise healthy adults. Wilder reported on a series of 53 adult eyes enucleated because of pain and blindness secondary to severe intraocular inflammation and identified microbes in what was up to then assumed to be tuberculosis. Patient age ranged from 14 to 83 years. All 53 eyes had granulomatous lesions with central necrotic areas containing *T. gondii*. On further collaboration with medical colleagues, Wilder found that most of the 53 patients had low-level dye test antibody titres, and in one patient antibody was only demonstrable in undiluted serum. This study demonstrated that toxoplasmosis, not tuberculosis, was the cause of these
chorioretinal lesions. As a result of this study cohort with low-level toxoplasma antibody it became accepted that ocular toxoplasmosis resulting from congenital infection was the leading cause of posterior uveitis in adults (WILDER, 1952; Holland et al., 2002).

Further studies by Edward Perkins professor of ophthalmology in the UK supported Wilder’s proposal that almost all toxoplasmic chorioretinitis in children and adults was a consequence of congenital infection that went unrecognised at birth (Perkins, 1961).

However, later studies disproved the earlier assumptions that ocular toxoplasmosis could only result from congenital infection and it subsequently became accepted that ocular disease was often the only manifestation of toxoplasmosis acquired later in life (Holland et al., 1999; Jones et al., 2015). Ophthalmologists from southern Brazil discovered ocular toxoplasmosis in siblings and proved that toxoplasmosis could be acquired at any time in life and not just in-utero. In addition, those patients with acquired toxoplasmosis who did not initially have chorioretinal scars subsequently developed retinal lesions at follow up sometimes long after initial infection (Silveira et al., 2001). The possibility of T. gondii transmission via consumption of undercooked meat was first proposed by Weinman et al in 1954, which provided investigators at the time with an explanation for clustering of T. gondii infection in families (Dubey, 2008).

In 1954, senior scientist Leon Jacobs and colleagues at The National Institute of Allergy and Infectious Diseases Maryland were the first to isolate T.gondii from an eye of a 30-year-old male in the U.S.A who required enucleation for elevated intraocular pressure (JACOBS et al., 1954).

A more complete appreciation of the spectrum of ocular and systemic symptoms associated with acute acquired toxoplasmosis was subsequently achieved with reports of outbreaks in adults in the U.S.A and Canada (Teutsch et al., 1979; Bowie et al., 1997). In 1995, there was a waterborne outbreak of toxoplasmosis in Canada, which resulted in 95 cases of acute toxoplasmosis, 20 of whom developed ocular lesions whilst others displayed other systemic symptoms without ocular involvement (Burnett et al., 1998). It has been
suggested that in some geographic areas, acquired infection with aggressive strains of the parasite may account for the majority of cases of ocular toxoplasmosis (Holland, 2003; Gilbert et al., 2008).

2.4 Classification and identification of the definitive host

The initial classification of *T. gondii* was based on comparison with other well-known protozoa. Despite the intensive work that followed the discovery of the parasite, its classification and life cycle was not elucidated for more than half a century. It was not until 1969, some 60 years after the discovery of the parasite, that *T. gondii* was found to be a coccidian with a sexual cycle and the definitive host was the cat (Frenkel et al., 1970). Several independent working groups worldwide accomplished elucidation of the sexual cycle of the parasite. Scientists were able to pass the parasite through animals and grow the organism in cell culture, which facilitated biologic and genetic studies.

The classification of *T. gondii* was proposed by Levine et al in 1977. According to Levine, *T. gondii* was a protozoan parasite of the: phylum Protozoa; subphylum Apicomplexa; class Sporozoa; family Sarcocystidae; subfamily Toxoplasmatinae; genus Toxoplasma; species *T. gondii* (Levine, 1977). The classification has since been changed and the parasite is now classified in phylum *Apicomplexa*, class Conoidasida. Deoxyribonucleic acid (DNA) sequence homology with depiction of a phylogenetic tree can illustrate its connectedness to other species (McAuley, 2014).
CHAPTER 3 - *T. gondii* forms, life cycle and sources of infection

3.1 Parasite forms

*T. gondii* is an intracellular protozoan with an asexual and sexual cycle. The parasite exists in three forms: oocysts from which active sporozoites are formed; tachyzoites and bradyzoites.

3.1.1 Oocysts

Following ingestion of toxoplasma parasite, oocysts are formed by the sexual cycle of *T. gondii*, which occurs in the intestines of the cat family (figure 3.1a). Oocysts are then shed in the faeces of infected cats for approximately seven to 20 days.

3.1.2 Sporozoites

Infective sporozoites are contained within oocysts and when oocysts are shed from the cat intestine into the environment, sporulation of oocysts with release of sporozoites must first occur for oocysts to become infectious. Oocysts are spherical at first but after sporulation become more oval shaped (figure 3.1b). The time taken for sporulation to occur varies between one and 21 days and depends on temperature and oxygen levels, e.g., sporulation takes three days in temperate climates and longer in cold climates. Oocysts cannot sporulate below four degrees Celsius (°C) and above 37°C (Sagel et al., 2010). Oocyst survival is strongly influenced by seasons in rural areas but remain stable in urban areas with moderate peaks. Sporozoites can remain viable in soil for at least one year. They do not survive in arid cool climates. Contact with contaminated soil and ingestion of sporozoites are sources of toxoplasma infection for humans and soil grazing animals (Cook et al., 2000; Munoz-Zanzi et al., 2010).

3.1.3 Tachyzoites

Following ingestion of sporozoites by animals or humans, the parasite travels through blood and lymphatics in the form of tachyzoites, which multiply rapidly during acute infection and can cause tissue destruction. Tachyzoites are crescentic in shape and exist within vacuoles in host cells (figure 3.1c). Tachyzoites represent parasitaemia or actively replicating parasites and systemic infection. Gastric enzymes can destroy tachyzoites; however, a small proportion of
parasite may survive digestion. Parasite invasiveness and rate of multiplication varies with strain virulence (Jamieson et al., 2009; Brenier-Pinchart et al., 2010). Tachyzoites continue to multiply within the host cells until pseudocysts are formed which ultimately sequester in muscle and other tissue. These cysts are known as latent tissue cysts or bradyzoites, which contain slowly multiplying organisms.

### 3.1.4 Bradyzoites

Bradyzoites remain dormant and persist in host tissue for life (figure 3.1d). This period of infection is often referred to as latent infection. When animal muscle tissue containing bradyzoites is ingested by humans in the form of rare or undercooked meat, gastric enzymes degrade the cyst wall and bradyzoites which can remain viable for three to six hours, replicate and re-enter blood and lymphatics in the tachyzoite form. Thereafter tachyzoites re-form bradyzoites or cysts. In humans cyst formation primarily occurs in the brain and eye but other organs may also contain latent cysts. The degree of organ involvement in humans who become infected depends on host and parasite factors (Peyron et al., 2006; Vallochi et al., 2008).

### 3.2 Life cycle

Cats excrete infective oocysts and serve as the definitive host supporting the sexual stage of the life cycle of *T. gondii* in their intestine (Boyer et al., 2011). Young kittens not previously exposed to the parasite acquire infection by scavenging infected birds and rodents (figure 3.2). Cats that prey on wild mice are at increased risk of infection. Older cats are more likely to have toxoplasma immunity from previous exposure and hence pose a lesser risk of toxoplasma infection to humans. Cats with antibodies experience decreased repetition of oocyst discharge and are safer pets.

The asexual phase requires warm blooded animals (extra-intestinal cycle) to serve as intermediate hosts; these include many species of mammals and birds as well as humans (Vesco et al., 2007).

### 3.3 Sources of toxoplasma infection

In humans, primary toxoplasma infection in a seronegative individual can occur following ingestion of active sporozoites or inactive cysts (bradyzoites) (Petersen
et al., 2010). Contaminated blood products and transplanted organs are also a risk exposure (Derouin et al., 2008; Gajurel et al., 2015).

Risks for acquiring primary toxoplasmosa infection vary worldwide and are related to socio-demographic, biological, environmental and lifestyle factors. Socio-demographic factors include: race, education, place of residence and prevalence of toxoplasma antibody in a given population. The prevalence of toxoplasma antibody increases with age (Jones et al., 2009).

Biological risks in women of child bearing age refer to the number of pregnancies and births; the prevalence of toxoplasma antibody increases with number of pregnancies (Villena et al., 2010). Lifestyle risk factors encompass exposure to oocysts or bradyzoites in contaminated food, unsanitary food preparation techniques and improper hand hygiene. Bradyzoite ingestion may also occur through occupational exposure e.g., abattoir workers, veterinarians and restaurant workers who handle and taste raw meat (Schluter et al., 2014).

The cat is the only animal to produce oocyst in faeces, which can then contaminate the soil and vegetation acting as the main environmental reservoir. Oocyst and sporozoite exposure typically occurs following contact with cat litter, ingestion of unwashed soil grown fruits and vegetables or gardening without gloves followed by improper hand hygiene (Lass et al., 2012).

Acute toxoplasmosis in cats can be avoided by feeding cats dried or canned food. Gloves should be used when handling cat litter and boxes. Oocysts in cat litter boxes can be destroyed by contact with boiling water for five minutes or by contact with 7% ammonium nitrate for at least three hours. Veterinarians should wear gloves when handling cat litter and care should be taken to avoid contamination of surrounding surfaces.

In countries which lack water purification technologies, water contamination with oocysts is a major source of human infection (de-Moura L. et al., 2006; Heukelbach et al., 2007). Heavy rain and surface water migration can cause contamination of adjacent land, wells and spring water. Undercooked eggs and unpasteurised milk can also contain oocysts. Arthropods can also transport oocysts to food and water.
Sources of bradyzoites include raw or undercooked meat and unwashed cooking surfaces and utensils contaminated with raw meat. Raw, cured or undercooked pork meats such as salami and prosciutto ham pose the highest risks. The seroprevalence of toxoplasma antibodies in animals bred for slaughter is as follows: sheep, 9%-23%; pigs, 12%-15%; cattle, 5%-10%; poultry and game 0.3%-5% (Vesco et al., 2007; Villari et al., 2009). Quoted prevalence rates vary geographically with farming practices and meat consumption of the population.

Bradyzoites can be rendered nonviable by temperatures greater than 67 °C or less than minus 12°C (Bader et al., 1997). Studies have demonstrated that freezing meat at minus 20°C for 18 to 24 hours followed by thawing could destroy tissue cysts (Dubey, 1974).

European multicentre studies have demonstrated that ingestion of rare or undercooked meat strongly predicted infection in pregnant women. Approximately 30% to 63% of infections in different centres were attributed to consumption of undercooked or raw cured meat products, whilst soil contact accounted for 6% to 17% of infection in pregnant women. Exposure to cats or kittens was not identified as a major risk factor for toxoplasma infection in Europe (Cook et al., 2000).

In the U.S.A exposure to raw or undercooked meat was identified as the main risk factor for primary toxoplasma infection but environmental exposure to oocysts from cat faeces was also found to pose a significant risk (Jones et al., 2009; Boyer et al., 2011). In South American countries both contaminated meat and environmental sources such as water supply are risks for infection (Demar et al., 2007; Munoz-Zanzi et al., 2010).
Figure 3.1 a, b, c and d: toxoplasma parasite forms

a, unsporulated oocyst; b, sporulating oocysts; c, tachyzoites on blood film tissue; d; bradyzoites in animal muscle

(Source: Infectious Diseases of the Foetus and Newborn Infant, Remington et al 2006; p947-1091)
Figure 3.2: Life cycle of T. gondii

(Source: Textbook of Diagnostic Microbiology Mahon and Manuselis 2000; chapter 24)
CHAPTER 4 - Literature review: strategies to prevent congenital toxoplasmosis

4.1 Summary of preventive measures

*T. gondii* is a parasite that replicates in cells and tissues, particularly the brain and eye of immunocompromised hosts, which includes the developing fetus. CT is a disease which if untreated or inadequately treated has adverse outcomes (Torgerson et al., 2013). The majority of congenitally infected infants are asymptomatic at birth, some of who will demonstrate signs if specifically sought. The disease spectrum of CT ranges from mild to severe (Kieffer et al., 2002; Kodikjian et al., 2006; Havelaar et al., 2007). Manifestations at birth and or later in life are influenced mainly by gestation at maternal infection and host and parasite genetics (Vallochi et al., 2008; Jamieson et al., 2009).

Studies have proven that combination anti-parasitic agents are effective in tissue culture and animal models by eliminating actively replicating parasites (Meneceur et al., 2008). In addition, antiprotozoal treatment of immune-compromised individuals with active CNS and chorioretinal lesions results in resolution of signs of disease (Bertoli et al., 1995).

Available data, both historic and recent, suggests that early detection and treatment of infected infants may improve long term outcome, given that approximately 70% to 80% of untreated CT will develop eye disease at some stage in the first two decades of life (Garweg et al., 2008). Some studies have demonstrated that treatment during gestation and infancy reduces parasite burden and can thereby result in favorable outcomes and prevent adverse sequelae from CT (Gomez-Marín et al., 2007; Wallon et al., 2013). Based on these data, a variety of approaches to the prevention of CT have been used encompassing primary, secondary and tertiary prevention.

4.2 Primary prevention

Primary prevention is based on maternal pre- and post-conception education to avoid risks for seroconversion in pregnancy. Consistent with the earlier work of Foulon (Foulon et al., 1994) in Belgium, Breugelman and colleagues found that simple preventative measures, such as prenatal education on food hygiene, risk
factor awareness and measures to avoid contact with oocysts, were effective in preventing pregnancy seroconversion (Breugelmans et al., 2004). They noted that guidelines should be reinforced verbally and with written informative documents to pregnant women by all medical personnel throughout pregnancy in order to be effective. Others analysing the effect of health education on primary prevention have concluded that whilst prenatal education was a simple, inexpensive and desirable intervention, its effectiveness has not been adequately evaluated in randomised controlled trials (RCTs) (Gollub et al., 2008; Di Mario S. et al., 2009).

### 4.3 Secondary prevention

Secondary prevention involves screening of pregnant women to detect seroconversion, treatment of acute maternal infection in pregnancy to block transmission to the fetus, and treatment of infected fetuses to reduce morbidity. In some countries, pregnancy termination is also offered for severely affected fetuses.

The value of antenatal screening to prevent CT remains controversial. Experts against screening argue that clinical evidence suggests that only symptomatic infants will benefit from treatment, the majority of whom will be identified without screening (Gras et al., 2005).

Alternatively beneficial effects of maternal treatment in pregnancy to reduce transmission have been reported in cases of maternal seroconversion detected and treated early (Douche et al., 1996; Prusa et al., 2015). In addition, early identification and treatment of infected infants in-utero can potentially improve outcome. Identification of subclinically infected infants permits a full diagnostic assessment and regular monitoring to facilitate early detection of chorioretinitis. While some countries, e.g. France, have adopted routine antenatal screening others e.g. the United Kingdom (UK) has rejected it as lacking in proven efficacy (Gilbert et al., 2002). As yet however, there is no international consensus on the value of antenatal screening strategies or on the best treatment for infected mothers.
4.4 Tertiary prevention
Tertiary prevention focuses on postnatal screening of all infants for early detection and treatment of CT to minimise infant sequelae and maximise outcome. There remains however a deficit in randomised placebo-controlled studies of CT treatment in infancy.

4.5 Arguments for or against screening for CT
Many of the same arguments pertaining to the benefits or lack thereof of antenatal screening also pertain to postnatal screening. It is argued that in the absence of large RCTs, definitive conclusions cannot be made with regards to what extent prenatal or postnatal treatment following screening for toxoplasmosis reduces the risk of congenital infection or severe sequelae.

The cost efficacy of such interventions has also been subjected to scrutiny (Bader et al., 1997; Thiebaut et al., 2006). Evaluation of cost effectiveness leads to different strategies in different countries. The general argument against screening for CT is provided by studies which demonstrate that antenatal and or postnatal screening does not prevent infant sequelae long term (Ricci et al., 2003; Khoshnood et al., 2007).

However, what has been established beyond doubt is that the diagnosis of CT needs to be confirmed in infected infants (Montoya et al., 2005; McLeod et al., 2006). Early diagnosis of infection in the infant can facilitate full diagnostic evaluation, early treatment and recognition of ocular reactivation, should it occur.

Before CT preventive strategies can be employed in a given population, related topics need to be clarified, in particular: seroprevalence in the general population or in women of child bearing age, incidence of primary infection in pregnancy, population risk factors for acquiring infection, clinical severity of CT, optimal prenatal treatment and postnatal treatment efficacy for prevention of recurrence or reactivation in affected infants.

Thus the utility of screening programs for diagnosing toxoplasmosis, either during pregnancy or postnatally remains controversial due to absence of standardised management protocols for women with pregnancy seroconversion and limited long
term monitoring studies of congenitally infected infants, particularly asymptomatic infants. Therefore the CT prevention programmes that have been employed regionally or nationally in resource rich countries are based on consensus amongst the expert community in the particular country in the absence of evidence from RCTs.
CHAPTER 5 - Literature review: prenatal screening and treatment for congenital toxoplasmosis

5.1 Introduction

Prenatal serologic screening and treatment has been investigated by a number of researchers (Peyron et al., 2000). However, in the absence of randomised trials, the questions that remain are whether systematic maternal screening and antenatal treatment can reduce vertical transmission of *T. gondii*, reduce the frequency of CT and improve outcomes for those infected compared with those whose mothers received no prenatal treatment.

Prenatal screening involves monthly or 3-monthly testing of women who are seronegative at the first antenatal visit. The rationale for prenatal screening is based on evidence that suggests that earlier treatment is associated with a lower risk of mother-to-child transmission and less severe infant sequelae (Hotop et al., 2012).

Transmission rates are the lowest in trimester 1 and there is speculation that in order for transmission to occur there must first be a functioning placenta. In addition, the exact timing of fetal infection following maternal infection is unknown. Antenatal studies have shown that this may vary, i.e., fetal infection can occur promptly during maternal parasitaemia, or the placenta may not become infected until weeks later. Thus there may be a window of opportunity to treat a mother before the infant becomes infected. This concept has been supported by the results from some investigators who report a decrease in transmission rates in treated women (Henri et al., 1992; Douche et al., 1996). Some studies quote a 40% reduction in infant transmission but with no direct comparative evidence for such an effect (Wallon et al., 2013). Other studies have failed to show a dramatic effect or that intensive antenatal treatment protocols are beneficial to infant outcome (Gras et al., 2001).

Animal studies demonstrate that fetal infection occurs during maternal parasitaemia which ceases as the maternal antibody response develops to control infection (Remington, 2006). Once serum antibodies are demonstrable in animals, parasitaemia is no longer present. In addition, in animal models, parasite
transformation from free tachyzoites to bradyzoite cysts, which are not susceptible to antiprotozoals, occurs within days of infection (Roberts et al., 1999; Luder et al., 1999). If extrapolated to pregnancy in humans, there may not be a latent phase between maternal infection and fetal infection during which treatment may be beneficial to prevent or reduce fetal tissue damage. Hence interventions following maternal seroconversion may be ineffective as transmission may occur prior to maternal treatment, and this would account for results of studies that fail to demonstrate a beneficial effect of maternal on transmission (Foulon et al., 1999).

On the other hand, the opposing argument is provided by studies that demonstrate that maternal infection in pregnancy does not always result in congenital infection, even in the absence of maternal treatment, and for those who do transmit infection to the fetus, timing of transmission following maternal seroconversion is varied and can be delayed. Large French cohort studies have shown that in some instances of proven maternal seroconversion in pregnancy, amniotic fluid PCR can be negative, with infant CT identified at birth. This suggests that there can be a delay in transmission following seroconversion during which treatment may benefit the fetus. An alternative explanation for these findings could be a false negative PCR result. Hence, the scenario described supports the proposal that maternal-fetal transmission of infection can be delayed but it does not prove the hypothesis. Transmission rates vary in populations and depend on host and parasite factors (Hohlfeld et al., 1989; Kieffer et al., 2011).

Some researchers have concluded that whilst antenatal screening and treatment did not absolutely prevent transmission to the fetus, it reduced parasite burden, the severity of fetal infection and number of fetal deaths (Cortina-Borja et al., 2010). Hence maternal treatment allows the progression of pregnancies that may have otherwise been lost. Consequently, it has been considered unethical to undertake placebo controlled trials of maternal antenatal treatment and thus it is difficult to accurately determine the true effect of antenatal screening on the rate of congenital infection and infant outcome.

Recommendations to commence or continue prenatal screening in a given country or region are usually based on the following: seroprevalence in the antenatal population, availability of accurate testing services, the ability to make a precise
fetal diagnosis, provision of effective or curative treatment and availability of resources.

5.2 Review of methods for diagnosis of CT in pregnancy

Prenatal screening requires the inconvenience of repeat testing throughout pregnancy and may generate unacceptable levels of anxiety in pregnant women. Antenatal screening ideally needs to be initiated as early as possible with prompt accurate interpretation of results available by 10 to 12 weeks gestation and as such relies on early antenatal booking. In addition, the success of an antenatal screening programme depends on patient compliance throughout pregnancy and standardised methods for testing and follow up of results (Hartup et al., 1997; Binquet et al., 2004).

The literature lacks robust evidence on the reliability of serological tests for diagnosis in pregnancy. However many studies have demonstrated that a combination of serological tests can reliably diagnose or rule out pregnancy seroconversion (Sickinger et al., 2008; Gay-Andrieu et al., 2009).

The ultimate usefulness of serology tests depends on:

1) Quality control of commercial kits;
2) Reliability of the laboratory performing the test;
3) Skill and accuracy of personnel interpreting the test.

The IgM test is usually the first-line choice for diagnosis of acute toxoplasmosis or seroconversion in pregnancy. Whilst most IgM tests are highly sensitive, almost 100% with the Immuno Sorbent Agglutination Assay (ISAGA) method, there is also a high rate of false positivity, up to 12% in some instances, as the IgM response can vary in pregnancy (Gras et al., 2004). One of the main issues with the use of IgM for the diagnosis of pregnancy seroconversion is that IgM specific antibodies can persist for many months following primary infection and may be detected for up to two years following infection (Fricker-Hidalgo et al., 2013). Thus the presence of \( T. gondii \)-specific IgM antibodies in pregnancy does not necessarily indicate an acute infection.

The toxoplasma specific IgG avidity assay used for testing of IgM positive samples collected in the first half of pregnancy has improved diagnostics in early pregnancy
The avidity test is useful in ruling out seroconversion in pregnancy for women with a positive IgM and high dye test titres who would otherwise have been diagnosed with recent infection. Low IgG avidity indicates recent infection, whilst high avidity indicates infection that was not recent. However persistence of low avidity is a recognised feature for many pregnant women and in these cases the test will not confirm or rule out recent infection (Lefevre-Pettazzoni et al., 2007). In addition, standard IgM and avidity tests are not useful in distinguishing between acute infection and reactivation in pregnant immunocompromised women (Fernandes et al., 2012). Thus serological determination of acute or recent toxoplasma infection remains imperfect.

Confirmation of fetal infection requires amniocentesis. Introduction of PCR technique in the 1990s increased the accuracy of fetal diagnosis (Bhalla et al., 1999). The specificity is almost 100% in reference laboratories but its sensitivity remains below 83% (Olariu et al., 2014). However, sensitivity and specificity of PCR diagnosis in amniotic fluid may differ widely between laboratories and it is therefore recommended that toxoplasma PCR analysis should only be undertaken in a reference laboratory where the test can be performed and interpreted by those experienced in doing so (Hohlfeld et al., 1994; Pratlong et al., 1996). A negative PCR in amniotic fluid cannot definitively exclude fetal infection. Thus, it is recommended that in cases of highly probable CT albeit with negative amniotic fluid PCR, maternal treatment with antiprotozoal medication is indicated and should continue until delivery (Romand et al., 2001). Parasite isolation from amniotic fluid is diagnostic but this can only be attained in reference laboratories and results can take up to six weeks.

The value of amniocentesis has to be counterbalanced against the risks associated with the procedure. While in general amniocentesis is considered a relatively safe procedure, evidence from available controlled studies suggest that fetal loss as a consequence of mid-trimester amniocentesis with ultrasound guidance is 0.6% to 1% and may exceed the number of prevented cases of CT (Bader et al., 1997; Khoshnood et al., 2007). In addition there are other complications associated with amniocentesis including leakage of amniotic fluid, vaginal bleeding, infection, fetal injury and last but not least maternal stress and anxiety.
5.3 Antenatal screening and treatment for CT in France

The French antenatal screening programme for CT implemented in 1978 provided data to guide antenatal programmes in other European countries. The purpose of the programme was to prevent infection in seronegative pregnant women, to diagnose infection early in pregnancy and to treat women who seroconverted to minimise infant sequelae. In the absence of directly comparative control groups, rigorous longitudinal studies conducted in France over many decades have concluded that antenatal treatment reduces placental infection, and treatment of an infected fetus in-utero reduces infant manifestations and CNS sequelae (Binquet et al., 2004; Faucher et al., 2012).

Historically this approach developed from studies as follows. Desmonts and Couvrreur demonstrated that without treatment; children infected in early gestation had poor outcomes, outcomes were of moderate severity in mid-gestational infections, and more than 50% of infants born to mothers infected in late gestation had subclinical disease evident on evaluation at birth. In a subsequent decade maternal treatment with spiramycin reduced rates of placental infection (Couvreur et al., 1993). Without treatment, parasites were isolated from placentas in 95% of those infected. With spiramycin treatment parasite isolation was reduced to 80%. With the introduction of spiramycin the incidence of infected infants was 50% less in each trimester in the decade during which spiramycin treatment was used compared to the preceding decade when there was no treatment.

Following the work by Hohlfeld and Foulon on diagnosis of fetal infection by fetal blood sampling and PCR testing of amniotic fluid, subsequent studies demonstrated that more severe infant disease correlated with higher parasite burden in the amniotic fluid. The investigators also found that when infected fetuses were treated in-utero with pyrimethamine and sulfadiazine, it was only possible to isolate parasites from the placentas of 50% of mothers treated for confirmed infection which contrasted with 95% historic isolation rates of untreated women. In addition, treatment of mothers of infected infants during gestation markedly reduced disease severity and manifestations of congenital infection at birth. Hohfield et al demonstrated that *T. gondii* was present in fewer infants born to treated (17%) vs untreated (69%) mothers and infants treated in-utero had less
cerebrospinal fluid (CSF) abnormalities consistent with encephalitis (Hohlfeld et al., 1989; Foulon et al., 1999).

Prenatal screening has been a mandatory component of antenatal care in France for many decades. In the initial stages of the screening programme roll-out in 1976, women were offered a serology test preconception, usually prior to marriage. This was amended in the 1980’s and from 1985 antenatal testing was offered at the first antenatal visit or on just one occasion during pregnancy. Experts believed that this was not sufficient, as late pregnancy seroconverters would not be identified. Hence by the late 1980’s antenatal testing was performed once during each trimester. Recommendations were further revised, and from 1992 testing was offered monthly for all women who were seronegative at the first antenatal visit; this policy currently remains in practice (Cornu et al., 2009). For confirmed maternal seroconversion at any gestation, treatment is offered with spiramycin three grams (g) daily until delivery.

With the advent of PCR testing in the early 1990s, treatment guidelines were amended. Currently, when maternal seroconversion is diagnosed in the first or second trimester, spiramycin is prescribed until amniocentesis is performed to determine the fetal infection status. If amniotic fluid PCR is negative, spiramycin is continued to delivery. If fetal infection is proven by amniocentesis, spiramycin is replaced with pyrimethamine and sulfadiazine until birth. Fetal ultrasound is performed monthly for signs of CT such as IUGR, macrocephaly, microcephaly, intracranial abnormalities and evolving ascites. Termination is offered for cases with intracranial signs. Maternal blood samples are also monitored for evidence of antiprotozoal toxicity, namely bone marrow suppression. If maternal infection is diagnosed after 32 weeks gestation, treatment is with pyrimethamine 50 milligrams (mg) daily plus sulfadiazine 3 g daily with folinic acid 50 mg weekly, alternating three-weekly with spiramycin until delivery.

All infants born to women who demonstrate pregnancy seroconversion in France are tested following delivery and if CT is excluded in the newborn period, infants are monitored serologically for one year to demonstrate decline in antibody status until negativity is confirmed. All confirmed infected infants are evaluated and treated with combination antiprotozoal therapy for one year and followed up long
term. In a collaborative U.S and French study of 18 infants infected before 25 weeks gestation; antenatal and postnatal treatment yielded favourable outcomes for all but one infant (Brezin et al., 2003). Observational studies and analyses performed on the French programme for systematic screening of pregnant women and treatment of infected infants have described favourable infant outcomes with a low incidence of recurrent or new eye disease in childhood (Patel et al., 1996; Wallon et al., 2004). Severe cognitive and neurological disability was also not observed (Foulon et al., 1999; Faucher et al., 2012).

Based on the French experience, maternal diagnosis and treatment in pregnancy is associated with reduced transmission to infants and severity of disease manifestations at birth and thereafter. Whilst new chorioretinal lesions were demonstrated to occur up to late childhood, the frequency of recurrence was low and lesions were mainly peripheral without associated visual impairment (Wallon et al., 2004). The improved outcomes for those infected infants, however, cannot solely be attributed to maternal treatment in pregnancy as all of those infants had the additional benefit of early postnatal antiprotozoal treatment.

The French programme also associated antenatal screening with a decline in the incidence of CT and severe disease at birth. Seroprevalence among pregnant women fell from 84% in the 1960s to 44% in 2003 (Garcia-Meric et al., 2010). However, whether this was attributable to the programme or the decrease in Europe of seropositivity rates is uncertain. In addition termination is offered to women if fetal ultrasound demonstrates intracranial signs or a severely affected fetus. In France there are approximately 300 to 400 cases of CT per year of which 40 to 50 are trimester 1 infections. There are between five and 13 terminations of pregnancies due to CT each year. Thus the small percentage of terminations might influence results only slightly and cannot account for marked improvement in outcomes (McLeod et al., 2009).

The incidence of CT in France for the year 2007 was estimated based on laboratory surveillance of confirmed positive infant samples. The overall prevalence for that year was 3.3 per 10,000 live births and the incidence rate of
the disease at birth was 2.9 per 10,000 live births. The estimated incidence of symptomatic CT was 0.34 cases per 10,000 live births (Villena et al., 2010).

An open labeled trial is currently taking place in France to determine the safety and efficacy of treatments used for pregnancy seroconversion. The TOXOGEST study is a multicentre, randomised clinical trial to compare the efficacy and tolerance of prenatal therapy with pyrimethamine and sulfadiazine vs spiramycin to reduce vertical transmission of *T. gondii* following primary infection in pregnancy (available at: [http://clinicaltrials.gov/ct2/show/NCT01189448](http://clinicaltrials.gov/ct2/show/NCT01189448)).

Outcome measures will include:

1) Rate of mother-to-child transmission of *T. gondii*, determined by PCR on amniocentesis and/or synthesis of specific antibodies by the neonate;

2) Antiprotozoal tolerance in mothers and neonates (toxicities);

3) Severity of infection at birth in cases of congenital toxoplasmosis (parasite load in amniotic fluid, clinical and biological signs).

The study commenced in September 2010 and data collection was proposed to end in March 2015 (Wallon et al., 2011).

### 5.4 Variation in treatment regimens employed for CT treatment *in-utero*

In general, therapeutic interventions for *T. gondii* infection in pregnancy lack standardization. Treatment protocols differ between European countries and studies are on-going to determine safer, more effective and less toxic maternal regimens without compromising infant outcome.

A recent non-randomised study in Germany demonstrated a reduction in fetal transmission and infant clinical signs with a prenatal treatment regimen different to that employed in France. Hotop et al performed a retrospective analysis of 685 women in Germany with pregnancy seroconversion to determine the effectiveness of rapid treatment initiation following infection in pregnancy. In Germany spiramycin is offered to all first trimester seroconverters until the 16th week of gestation followed by a minimum of four weeks of pyrimethamine, sulfadiazine and folinic acid. If fetal infection is confirmed by PCR or fetal ultrasound, antiprotozoal treatment is continued until delivery.
In France spiramycin alone is given for early gestation seroconversion unless fetal infection is proven. The German cohort of 685 women demonstrated that overall transmission rate (4.8%) and infant clinical manifestations (1.6%) were lower compared with France. However there may be factors other than treatment that influence difference in rates, such as population characteristics and adherence to the screening programme. The conclusions were that for first trimester diagnosis, use of spiramycin to week 16 followed by at least four weeks antiprotozoals in combination with standard follow up was efficient in reducing transmission to the fetus and disease burden in the newborn (Hotop et al., 2012).

5.5 The European Multicentre Study on Congenital Toxoplasmosis (EMSCOT): prenatal screening and infant outcome

5.5.1 EMSCOT summary and conclusions
EMSCOT was a prospective cohort study funded by the European Commission that examined the effect of prenatal treatment on infant outcome and sequelae. The study was an attempt at obtaining consensual guidelines for managing pregnancy seroconversion in Europe. The cohort study was based on more than 1200 women with toxoplasma infection in pregnancy. Women were recruited between 1997 and 2000. The study aim was to determine the risks of mother-to-child transmission, signs in the infant and to demonstrate if risks were modified by prenatal treatment. Children with CT were followed up for a median of four years for information on the risk of impaired development, vision or behavior and whether prenatal treatment affected these outcomes. Data collection for the cohort ended in 2003. The study involved 15 centres in seven countries and included France (seven centres), Italy and Brazil (two centres each), Austria, Denmark, Poland and Sweden (one centre each).

Conclusions were:
1) Tests for screening and diagnosis in pregnant women lacked standardization and reliability (Romand et al., 2001; Gras et al., 2004; Thalib et al., 2005);
2) There were no pharmacokinetic or pharmacodynamic studies in treated pregnant women. Data on serum concentrations of spiramycin suggested
that levels attained may be below the minimal inhibitory concentration (MIC) for *T. gondii*;

3) There were no randomised controlled trials of prenatal treatment. Existing observational studies and their conclusions potentially incorporated bias (Thiebaut et al., 2006);

4) There was no clear evidence that prenatal treatment had an effect on transmission or infant outcome;

5) Prenatal screening and treatment had a small effect on intracranial calcification but no effect on ocular sequelae (Gilbert et al., 2003; Gras et al., 2005);

6) Congenital infection remained a problem despite antenatal screening and interventions (Gras et al., 2001).

An EMSCOT related project was SYROCOT, a Systematic Review Of COngenital Toxoplasmosis. This project was a systematic review (meta-analysis) from individual data of observational cohorts to assess the effect of prenatal treatment on mother-to-child transmission and occurrence of CT clinical manifestations.

SYROCOT found weak evidence that prenatal treatment initiated within three weeks of seroconversion could reduce risk of transmission to the infant, and no evidence that prenatal treatment significantly reduced the risk of clinical signs in infected children (Thiebaut et al., 2007).

In 2007, SYROCOT concluded that despite three decades of prenatal screening for CT in some European countries, there was still uncertainty about the effectiveness of prenatal treatment. They concluded only a large RCT could provide valid evidence of the potential benefit of prenatal treatment. However, this raised the ethical issue associated with how to manage a placebo group; clinicians were reluctant to assign pregnant women with primary infection into a non-treatment group.

**5.5.2 EMSCOT critical review**

Despite the issues put forward by both the EMSCOT and SYROCOT studies, experts in the field continue to argue that whilst some observational studies have associated prenatal treatment with no significant benefit, there is also no evidence
which demonstrates that prenatal screening is entirely ineffective, and in the absence of comparative trials to determine outcome with and without treatment, prenatal screening and treatment should be established in high incidence areas and continue in established areas. Recommendations from Europe are not applicable elsewhere due to differences in incidence, parasite strain and disease burden.

Philippe Thulliez a microbiologist from The Laboratoire de la Toxoplasmose, Institut de Puériculture Paris, France, analysed the flaws and weaknesses from the EMSCOT study and its associated publications by Gilbert and Gras et al which found no beneficial effect of antenatal screening on transmission to the infant and intracranial and ocular lesions in congenitally infected infants.

In response to the retrospective cohort study by Gilbert et al of 554 mother-child pairs in Lyon which showed no significant effect of prenatal treatment on the risk of vertical transmission of toxoplasmosis (Gilbert et al., 2001), Thulliez in his analysis of the data in 2001 observed that (Thulliez, 2001):

1) The study group contained very few untreated women hence comparison analysis of untreated women vs those treated with pyrimethamine and sulfadiazine was restricted to half the group who did not undergo amniocentesis;
2) The confidence interval was very wide for untreated vs treated women and could include a doubling in risk for transmission in untreated women;
3) Most of the untreated women were infected in trimester 3 when the risk for infant transmission was highest;
4) Only three women infected before 28 weeks gestation were not treated and 28 untreated women were infected after 28 weeks;
5) The effect of treatment in trimester 3 cannot be generalised to the whole of pregnancy;
6) The authors concluded by suggesting that transmission occurred soon after infection during parasitaemia, but this was not supported by any scientific study in humans.

In response to the publications by Gras et al which found no evidence that antenatal treatment with pyrimethamine and sulfadiazine was more effective than
spiramycin in reducing intracranial and intraocular lesions in congenitally infected infants (Gras et al., 2001; Gras et al., 2005), Thulliez analysed the data and noted that:

1) Women who transmitted toxoplasmosis to the fetus soon after infection confirmed by positive amniocentesis were more likely to be treated with antiprotozoals than those infected at the same gestation but with a negative initial amniotic fluid PCR, in whom transmission was delayed until later in pregnancy;

2) Women infected before 32 weeks gestation were treated with antiprotozoals pyrimethamine plus sulfadiazine when fetal infection was confirmed by amniocentesis. Other women infected before 32 weeks were treated only with spiramycin until delivery either because prenatal diagnosis was negative or not performed. Hence in the latter group where infant transmission occurred after amniocentesis or later in gestation, those infected infants received sub-optimal antiprotozoal treatment in-utero;

3) Treated and non-treated groups may not be comparable as fetuses infected earlier in pregnancy have a higher risk of clinical signs;

4) There were delays of three to six weeks in starting treatment with antiprotozoals. The study was done when mouse inoculation was the standard fetal diagnostic test. Today PCR is widely used with results available in one day and infected fetuses are treated much earlier;

5) Women with infected fetuses received pyrimethamine and sulfadiazine alternating with spiramycin treatment. Periods of spiramycin treatment may have resulted in parasitic relapses. The current treatment recommendation for women with positive amniocentesis is continuous pyrimethamine and sulfadiazine;

6) The location of new eye lesions was not specified for children who experienced recurrences, hence new lesions may have represented previously undetected lesions;

7) The methods for detection of intracranial lesions may have been suboptimal in some cases; i.e., skull x-ray and cranial ultrasound rather that computerised or magnetic resonance imaging (MRI).
**5.5.3 EMSCOT related publications**

The original EMSCOT study cohort formed the basis for a number of publications. In addition to the results collated from the larger 15-centre cohort (Freeman et al., 2008) data from three of the centres that participated in the original cohort, where the methods of diagnosis and approach to treatment were considered more uniform were analysed and described separately (Kieffer et al., 2008). The coalesced results from three of the centres, differed from the results of the main 15-centre report and led to different conclusions. This illustrated that variations in approaches to diagnosis and treatment in the large grouped EMSCOT cohort in which outcomes for centres were combined and meta-analyses performed, potentially obscured significant findings identified from rigorous separate studies that were part of these larger combined analyses.

Freeman et al, in their analysis of 281 patients in the EMSCOT cohort that included the three centres also analysed separately, concluded that prenatal treatment was ineffective in decreasing the incidence of retinochoroiditis. This was from a prospectively studied cohort of 281 children with CT identified by prenatal and neonatal screening in six European countries to determine the effects of prenatal treatment and prognostic markers soon after birth on the age at first detection of retinochoroiditis.

Of 281 children with CT, 34 (12%) had intracranial calcification at birth, 50 (18%) developed ocular disease, 17 of which had recurrent retinochoroiditis during a median follow-up of 4.1 years. Prenatal treatment had no significant effect on the age at first or subsequent lesions and delayed start of postnatal treatment did not increase retinochoroiditis, but the analysis lacked power. Older gestational age at maternal seroconversion was weakly associated with a reduced risk of retinochoroiditis. The presence of non-ocular clinical manifestations of CT at birth strongly predicted retinochoroiditis. For 92% (230 of 249) of children with no retinochoroiditis detected before four months of age, the probability of retinochoroiditis by four years was low, whether clinical manifestations were present or not. Conclusions were: prenatal treatment did not significantly reduce the risk of retinochoroiditis in this European cohort; children with no retinochoroiditis in early infancy were at low risk of subsequent ocular disease which may not justify postnatal treatment and repeated ophthalmic assessments.
during childhood and controlled trials were needed to address the lack of evidence for the effectiveness of postnatal treatment (Freeman et al., 2008).

However when data from 129 of 281 of the patients included in the Freeman analysis were analysed separately from three French centres that provided care, the conclusion was that a delay of more than eight weeks between maternal seroconversion and the initiation of prenatal treatment was a risk factor for retinochoroiditis in the first two years of life in infants treated for CT in-utero (Kieffer et al., 2008).

Although both studies were published in 2008, the cohorts were studied over different time periods. The Freeman et al cohort analysis was from infants born between 1996 to 2000. The Kieffer et al data was from infants born between 1996 to 2002, the data included additional children and was based on fewer centres. Thus the two studies, which resulted in different conclusions, were from non-identical data with regards to the children included. In the Freeman analysis, some children only had one ophthalmologic examination. In the Kieffer study, children had repeat ocular examinations.

The differing conclusions reported by Freeman et al and Kieffer et al supported Thulliez’s conclusion in 2001 that the design of the EMSCOT study was flawed and suboptimal. The EMSCOT data analysed by Gras et al using coalesced cohorts concluded that there was insufficient evidence to prove that prenatal treatment improved infant outcomes and called for additional placebo controlled trials.

In contrast, Thulliez and Remington concluded that there were sufficient carefully performed studies supporting prenatal treatment efficacy and thus randomised double blind placebo controlled studies to further prove this would be harmful to those affected.

The final EMSCOT related publication in 2010 was based on an observational prospective study of the cohort to determine the effect of prenatal treatment on serious neurological sequelae or disease (SNSD) of CT defined by one or more of
functional neurological abnormalities, severe bilateral visual impairment, or pregnancy termination for confirmed CT (Cortina-Borja et al., 2010).

The researchers monitored 293 children in whom congenital toxoplasmosis had been identified by prenatal screening (in France, Austria and Italy) or by neonatal screening (in Denmark, Sweden and Poland) for an average of four years. Two-thirds of the children received prenatal treatment for toxoplasmosis and 23 (8%) fetuses developed SNSD; nine of these cases of SNSD were terminated during pregnancy. By comparing the number of cases of SNSD among children who received prenatal treatment with the number among children who did not receive prenatal treatment, the researchers estimated that prenatal treatment reduced the risk of SNSD by three-quarters. They also estimated that to prevent one case of SNSD after maternal infection at 10 weeks of pregnancy, it would be necessary to treat three fetuses with confirmed infection. To prevent one case of SNSD after maternal infection at 30 weeks of pregnancy, 18 fetuses would need to be treated. The researchers reported that the effectiveness of pyrimethamine-sulfonamide was similar to spiramycin, which was less toxic, and that a third of live-born infants with brain damage detected after birth subsequently developed SNSD.

The findings suggested that prenatal treatment of CT could substantially reduce the proportion of infected fetuses that develop SNSD and would be particularly effective in fetuses whose mothers acquired T. gondii during the first trimester of pregnancy. However the researchers advised that the findings should be interpreted with caution, because of the small number of fetuses in the study with SNSD and because of uncertainty about the timing of maternal infection. Furthermore, the investigators pointed out that these findings only related to the relatively benign strain of T. gondii that predominates in Europe and further studies were needed to determine whether prenatal treatment was effective against more virulent strains of the parasite found in South America. Because this study was an observational study, its findings might reflect differences between the study participants other than whether or not they received prenatal treatment.

The final conclusion of the EMSCOT study group was that findings needed to be confirmed in randomised controlled trials of prenatal screening before any policy
decisions were made regarding routine prenatal screening and treatment for congenital toxoplasmosis (Cortina-Borja et al., 2010).

5.6 Situation with maternal screening in the U.S.A.
CT in the U.S.A often presents with substantial signs and symptoms, causing considerable morbidity and mortality (Ajzenberg, 2012). Maternal screening for toxoplasma infection in pregnancy is only rarely approached in a standardised manner. In order to evaluate the benefits of screening for all pregnant women, Bader et al used decision analysis to compare three strategies for prenatal management of CT: 1) no testing for CT; 2) current practice which is to perform targeted screening only in cases of incidental abnormalities noted on ultrasound; and 3) universal serologic screening of pregnant women followed by amniocentesis to diagnose fetal infection in cases of maternal seroconversion.

For each of the three strategies, two available treatment options were considered; intrauterine antiprotozoal treatment or pregnancy termination. Findings were that universal screening reduced the total number of CT cases compared with no testing or targeted screening. However, compared with no testing, universal screening with interventions resulted in 18.5 additional pregnancy losses for each case of toxoplasmosis avoided. If infected pregnancies underwent termination, universal screening resulted in 12.1 additional pregnancy losses for each case avoided. He concluded that maternal screening reduced the number of cases of CT but at a substantial clinical cost, the rarity of the disease and limitations in diagnostics and treatment options limited the effectiveness of screening strategies and the risks associated with amniocentesis were particularly important (Bader et al., 1997). Hence serologic screening of pregnant women is not routine in the U.S.A and at present is dependent on patient and obstetrician preference (Mittendorf et al., 1999).

Opinions on antenatal screening for CT differ amongst the expert community in the U.S.A and some continue to campaign for introduction of antenatal screening (Montoya et al., 2008;Boyer et al., 2011).
5.7 Summary of issues and controversies associated with prenatal screening

There is a lack of economic analyses to address the values of antenatal screening programmes in countries where they exist. Existing economic meta-analyses have concluded that universal prenatal screening for CT may be too costly or unwarranted, particularly in areas with low disease burden (Capretti et al., 2014), and that cost-effectiveness should determine appropriate public health decision-making.

It has been suggested in a French report that in countries with no screening programme and where interventions to reduce the risk of congenital infection are not routine, antenatal screening should not be introduced outside a carefully controlled trial (Gollub et al., 2008).

Almost two decades ago Wallon et al performed an extensive Medline search for evidence that prenatal treatment of CT reduced transmission to the infant and improved outcome. Various studies were reviewed dating from 1966 up to 1995 that compared at least two concurrent groups of pregnant women with proven or likely acute toxoplasma infection, in which treatments were compared with no treatment and infant outcomes at age one year reported.

Out of 2591 papers identified, only nine met the inclusion criteria. Congenital infection was common in treated groups. Five studies showed that treatment was effective and four that it was not. Wallon et al concluded that screening was expensive, the effects of prenatal treatment and impact of screening programmes required further evaluation, in countries where prenatal screening or treatment was not routine, screening should not be introduced outside carefully controlled trials, and it was unclear whether treatment of mothers who seroconverted during pregnancy prevented fetal infection and improved infant outcome (Wallon et al., 1999b). However, in Wallon's review there were no RCTs and control groups were generally not directly comparable with the treatment groups. Subsequent improvements in diagnostic techniques and management of neurological disease have influenced the course of CT in current patients and thus these conclusions may not pertain to the current situation.
A more recent analysis by Wallon et al supports the benefits of monthly prenatal screening. Data from 2084 mother-infant pairs with toxoplasma infection in Lyon, France from 1987-2008 was analysed to determine the relation between gestational age at maternal infection and infant clinical signs at age three years. The analyses concluded that introduction of both monthly antenatal screening in 1992 and PCR testing of amniotic fluid in 1995 significantly reduced the rate of CT and improved outcome at three years of age in children infected with *T. gondii* genotype II (Wallon et al., 2013).

The controversies surrounding prenatal screening for CT can be summarised as follows:

1) There are concerns about the reliability of screening tests to accurately diagnose acute or recent infection in pregnancy. There is an associated false positive rate for seronegative women who undergo repeat testing;

2) There is no general consensus on prevention of infection in pregnancy and on the optimal management of the risk of transplacental infection in women who seroconvert during pregnancy;

3) There is a paucity of evidence for a latent phase when treatment might have an effect, between maternal infection and fetal transmission, and between fetal infection and organ damage in fetal or postnatal life;

4) Comparison with historic data suggests that antenatal screening can result in reduction in transmission of up to 60 to 70% and reduction of severe infant sequelae. Both these conclusions were made in the absence of RCTs.
CHAPTER 6 - Literature review: postnatal screening and treatment for congenital toxoplasmosis and infant outcome

6.1 Introduction
Newborn screening for CT identifies congenitally infected infants, in particular asymptomatic cases that would not be detected by routine paediatric surveillance. The potential benefits of newborn screening are early diagnosis, implementation of antiprotozoal treatment and early referral of infants with visual or neurological impairment who require remedial intervention. In addition the asymptomatic infants who are vulnerable to ocular sequelae are provided with essential surveillance. Antiprotozoal treatment with pyrimethamine and sulfadiazine in the first year of life can achieve parasite control whilst the infant's immune system is maturing. The theory supporting early infant treatment is that with less circulating parasite in a vulnerable immune system, the risks of sequelae such as new retinochoroidal lesions and neurological impairment are reduced (Patel et al., 1996;McLeod et al., 2006).

During pregnancy, serology results can be inconclusive and difficult to interpret (Gras et al., 2004;Signorell et al., 2006). Experts in the field of CT have argued that newborn screening is a more cost effective alternative to antenatal screening (Lebech et al., 1999), particularly in countries where the incidence of CT is low or in resource constrained settings with a high incidence of CT such as South America (Gomez-Marin et al., 2011). Screening at birth may identify more infected infants than screening in pregnancy, which may not identify late trimester 3 seroconverters.

6.2 Methods for postnatal screening and confirmation of CT

6.2.1 Newborn screening methods
Neonatal screening of large populations is usually based on detection of toxoplasma-specific IgM eluted from newborn filter paper dried blood spots (DBS). This has been reported as a feasible method for newborn screening in regions without antenatal screening and relies on detection of IgM in infant heel blood (Patel et al., 1996;Paul et al., 2000).
Some centres have employed cord blood screening for CT, the disadvantage of which can be a high yield of falsely positive infants from maternal contamination (Berger et al., 1992; Signorell et al., 2006). All screen positive infants require serological confirmation.

6.2.2 Newborn confirmatory serological tests for the diagnosis of CT
Confirmatory serological tests vary globally but are usually performed using a combination of methods, namely toxoplasma-specific IgM and IgA. The sensitivity of serology tests for IgM or IgA used on their own in early infancy is limited, ranging from 52% to 66% and hence it is recommended that newborn confirmatory testing should always incorporate more than one assay (Pinon et al., 2001).

IgM and IgA tests are relatively low cost compared to more sophisticated methods and remain the gold standard for diagnosis of CT in most centres. However it is estimated that approximately 25% of infected infants can potentially be missed using these serological tests due to paucity of infant antibody production for those infected late in trimester 3. Hence confirmation may require serial serological testing in some newborns, or demonstration of increasing concentrations of toxoplasma-specific IgG antibody in infancy or its persistence beyond one year.

The EMSCOT study was used to assess the accuracy of postnatal testing for toxoplasma-specific IgM and IgA. Data was analysed from 10 centres in three European countries. Results of the first postnatal IgM or IgA test from infants born to infected mothers identified by prenatal screening were compared with the reference standard for congenital infection defined by IgG status at one year of age. The specificity and sensitivity for each IgM and IgA test was individually analysed.

Overall, IgM or IgA testing detected only 52% to 55% of infected infants. Sensitivity was highest up to week two of life but declined thereafter. Specificity was highest from four weeks after birth. For IgM, sensitivity was statistically significantly lower if the mother seroconverted in the first and second trimesters of pregnancy (29% and 34%, respectively) compared with the third trimester (71%). Prenatal treatment with pyrimethamine-sulfonamide was not found to significantly reduce IgM or IgA sensitivity in the newborn.
Sensitivity was low for the enzyme-linked immunosorbent assay (ELISA) IgM test (29%), but similar for the ISAGA IgM (54%), ISAGA IgA (58%) and ELISA IgA (52%) tests. Specificity was lower for the ISAGA IgM test (91%) than for the ISAGA IgM (96%) and ELISA IgA tests (98%). Gilbert et al concluded that poor performance of IgM and IgA tests in the newborn, particularly if the mother seroconverted in early pregnancy, casts doubt on the value of neonatal screening in industrialised countries where the risk of clinical manifestations during childhood is low, and more accurate diagnostic tests were needed for newborns identified by prenatal screening (Gilbert et al., 2007).

However these results were primarily based on serology from newborns treated antenatally with antiprotozoals and could not be used to draw conclusions on postnatal screening in populations where antenatal screening is not performed. Historic observations that only 25% to 50% of infected infants are IgM positive at birth are not widely applicable as these studies were all performed in France and other countries where prenatal treatment is applied (Couvreur et al., 1993).

To date only two studies conducted in Denmark and Switzerland have assessed serologic tests used for confirming CT in newborns who were not diagnosed or treated in-utero. The best performances were observed using a combination of ISAGA and ELISA tests for IgM and IgA which obtained the most accurate results with sensitivity of 94% and specificity of 99.9% (Lebech et al., 1999; Signorell et al., 2006).

Two dimensional immunoblot analysis (Western Blot) for detection of toxoplasma-specific IgG antibodies is performed in conjunction with standard serological tests in cases where the diagnosis is difficult to confirm in the newborn. Conventional tests may not be useful in detecting infant antibody present in low concentrations which can yield equivocal or borderline results. Western blot (WB) has been found to be a useful serological tool to reliably determine the presence or absence of toxoplasma antibodies. The test employs comparison of maternal and infant bands of electrophoresis to differentiate between maternal and infant specific IgG responses to *T. gondii*. Immunoblot is a sensitive technique that allows early differentiation between passively transferred maternal *T. gondii*-specific IgG
antibodies and antibodies synthesised by the newborn child (Ho-Yen et al., 2000; Nielsen et al., 2005; Franck et al., 2008).

PCR testing of infant blood and CSF is usually performed in addition to confirmatory immunoglobulin tests. However most studies on the use of PCR for diagnosis of CT have been performed on amniotic fluid from screened antenatal cohorts (Wallon et al., 2010).

In a recent study from a Romanian cohort of 58 congenitally infected and 103 uninfected infants all born to untreated mothers, CSF-PCR was positive in 27 of the 58 (46.5%) congenitally infected infants and negative in all 103 uninfected infants. CSF-PCR was positive in 70.9%, 53.3% and 50.9% of those with hydrocephalus, cerebral calcifications and/or eye disease, respectively. Three of six infants who were negative for both IgM and IgA antibodies, had a positive CSF-PCR. The authors concluded that in infants with clinical and serologic findings suggestive of CT and born to untreated mothers, CSF-PCR combined with IgM and IgA antibodies had the potential to increase the frequency of cases confirmed (Olariu et al., 2014)

The use of recombinant antigens with immunoglobulin tests to distinguishing *T. gondii*-infected from uninfected infants has been studied in centres in Italy and Poland. Assays based on recombinant antigens have been shown to be effective in facilitating early diagnosis of newborns with congenital toxoplasmosis. However this method is not routinely used as it is costly and requires larger volumes of infant sera (Buffolano et al., 2005; Pietkiewicz et al., 2007). The performance of gamma interferon release assays on whole blood for the diagnosis of CT has been evaluated in centres in Lyon France. Whilst the assay was simple to perform and was suitable for early diagnosis or exclusion of CT particularly in cases with equivocal serology, the authors concluded that further studies with larger cohorts was necessary before this method replaced serological testing in newborns (Chapey et al., 2015).
6.3 Postnatal treatment regimens for CT

A confounding issue with postnatal treatment for CT is the lack of international consensus on the efficacy of antiprotozoal treatment for infected infants, both symptomatic and asymptomatic, and whether treatment is effective against latent bradyzoite cysts and prevention of recurrences.

The majority of children with untreated symptomatic congenital toxoplasmosis develop long-term sequelae, including intellectual disability, seizures, motor deficits, impaired vision and hearing loss. Sequelea can also occur in children with subclinical infection, but it is not possible to accurately predict their likelihood (Alford, Jr. et al., 1974; McLeod et al., 2006).

Given the risk of late sequelae in untreated CT, anti-parasitic treatment is also recommended for asymptomatic infants. This has been supported by remote and recent studies which demonstrate that the majority of congenitally infected infants are asymptomatic at birth and more than 70% of untreated congenitally infected infants develop new chorioretinal lesions during the first and to a lesser extent the second decade of life (Koppe et al., 1986; Phan et al., 2008b). These studies reinforce the importance of establishing the diagnosis in the newborn period and subsequent long-term ophthalmology follow up. Currently, in most countries all children diagnosed with CT are treated postnatally and followed up through childhood.

Ocular and systemic toxoplasmosis have traditionally been treated with pyrimethamine and sulfadiazine since the 1950’s. Neutropenia is a common side effect of these drugs; hence folinic acid is administered thrice weekly to support blood counts for the duration of therapy. In the early 1980’s, CT was treated with spiramycin alternating with four cycles of pyrimethamine-sulfadiazine every three weeks for one year. The treatment duration of one year was used initially for AIDS patients with toxoplasma encephalitis in the 1980’s (Remington, 2006). Spiramycin was found to have insufficient treatment efficacy in AIDS patients and in 1986 a new protocol for children with CT was developed which recommended at least one year of treatment with pyrimethamine and sulfadiazine, especially for severely affected infants.
Bone marrow suppression, rash and gastrointestinal symptoms are reported side effects in 10% to 50% of children treated for CT. These medications are otherwise generally well tolerated in infancy.

The population pharmacokinetics of pyrimethamine and sulfadoxine, an alternative to sulfadiazine, in 32 children in France with congenital toxoplasmosis was investigated by nonparametric modelling analysis. Wide variability was observed amongst patients. The estimated minimum and maximum concentrations of pyrimethamine in serum differed eight- and 25-fold among patients respectively and those of sulfadoxine differed four- and five-fold respectively. Increases in the concentration of pyrimethamine were observed for eight children, and increases in the sulfadoxine concentration were observed for seven children. Serum concentrations for both drugs were unpredictable even when the dose was standardised for body weight. The investigators concluded that the most efficacious concentrations of combination antiprotozoals have not yet been established and should be further investigated with long-term follow-up to determine the correlation between exposure to antiprotozoals and clinical outcome in congenital toxoplasmosis (Corvaisier et al., 2004).

A similar study performed in Denmark to determine plasma concentrations of pyrimethamine and sulfadiazine in children treated for CT concluded that drugs given in the recommended doses led to concentrations within expected therapeutic limits. Neutropenia was the main toxicity observed but treatment was well tolerated in the majority of children (Schmidt et al., 2006b).

There are many studies which report on alternative treatment options for patients with acquired toxoplasmosis and recurrent chorioretinitis (Guex-Crosier, 2009; dela-Torre et al., 2011). However, there are limited studies on the use of the standard pyrimethamine-sulfadiazine combination and optimal treatment duration for congenital infection (McLeod et al., 1992; Petersen et al., 2003).

In 1988 the World Health Organisation (WHO) recommended three months duration for children identified with CT by screening, but this was not evidence based (Torgerson et al., 2013). Treatment of CT with a shortened course of three months of antiprotozoals has not been widely evaluated or proven to be effective.
Eichenwald et al demonstrated that untreated CT yielded poor outcomes and a shorter treatment duration of one month was ineffective (Eichenwald H F, 1959).

The absence of RCT’s to provide evidence for optimum treatment duration has led to lack of consensus on treatment duration for infants identified by newborn screening. Thus current regimens vary worldwide from three months e.g., Denmark, to two years in some centres in France and the U.S.A (McLeod et al., 2006; Roser et al., 2010).

Furthermore, the biggest debate is generated by the asymptomatic group of infants who lack demonstrable clinical signs and who also comprise the majority of CT cases in Europe. In 2009 Swiss health authorities revised their CT treatment policy so that only infants with symptomatic infection are treated; previously asymptomatic infants were also treated. The change of paradigm was due to the low incidence and even lower morbidity of CT in Switzerland, combined with the lack of comparative evidence to demonstrate a beneficial impact of treatment on asymptomatic CT (Swiss Working Group on Congenital Toxoplasmosis, 2008; Stricker et al., 2009).

Other infant treatment regimes for CT employ clindamycin or azithromycin as alternatives to sulfadiazine when sulfadiazine is contraindicated, e.g., in glucose-6-phosphate-dehydrogenase (G6PD) deficiency, renal insufficiency and sulfadiazine allergy (Yazici et al., 2009). Quinolone drugs such as atovaquone have been found to be effective against bradyzoites but more studies are needed to determine their efficacy for treatment of congenital infection (Anquetin et al., 2006).

A CT treatment trial is currently underway in France. The TOSCANE study is an interventional multicentre randomised strategy trial comparing 3 versus 12 months antiprotozoal treatment for children with non-severe CT. The primary outcome is time to development of retinochoroiditis over two years. Secondary outcomes include impact of treatment on children with retinochoroiditis or intracranial calcifications at inclusion, neurodevelopmental outcome and quality of life indices (available at http://clinicaltrials.gov/ct2/show/NCT01202500).
Children diagnosed with CT in the first three months of life are included whether or not *in-utero* treatment was given. Children with severe CT are excluded, defined by the presence at birth of at least one of the following signs: three or more cerebral calcifications, hydrocephaly, microcephaly, seizures and microphthalmia. Children with inflammatory retinal disease or hypersensitivity to the treatments are also excluded. The study began in July 2010 and recruitment is estimated to end in September 2016 (Wallon et al 2011).

**6.4 Outcome of infants treated postnatally for CT**

**6.4.1 Introduction**

Historic and recent studies which report on the benefits of postnatal treatment for CT are without comparative groups as clinicians are reluctant to assign infected infants to a non-treatment group (Koppe et al., 1986; Mombro et al., 1995; Gomez-Marin et al., 2007).

The only RCT ever performed was a prospective study from Brazil on 124 patients with a history of recurrent toxoplasmic retinochoroiditis, which demonstrated an effect of co-trimoxazole vs no treatment on the recurrence of retinochoroiditis. The study participants were not selected from a congenitally infected population, they were selected based on findings of recurrent ocular toxoplasmosis and it was not defined whether infection was congenital or acquired. Sixty-one patients received trimethoprim/sulfamethoxazole every three days and 63 patients received no treatment. Recurrences developed in four (6.6%) treated patients and in 15 (23.8%) controls (p = 0.01). Treatment was discontinued prematurely in four patients because of mild side effects. The conclusion was that long-term intermittent treatment with trimethoprim/sulfamethoxazole could reduce the rate of recurrent toxoplasmic retinochoroiditis (Silveira et al., 2002).

Studies of infants treated postnataley are generally from cohorts of infants born to mothers screened and treated antenatally. Few international studies exist which examine solely the benefits of postnatal screening, infant treatment with antiprotozoals and long term outcome in adulthood.
6.4.2 Historic reports of outcome of infants treated and untreated postnatally for CT

Historic studies demonstrated that patients with CT who did not receive treatment or received treatment limited to one month only experienced poor outcomes. Heinz Eichenwald and colleagues demonstrated the natural history of untreated symptomatic congenital toxoplasmosis in a case series of 156 patients from the 1940s, of whom 152 had overt neurological or generalised manifestations of disease. Mortality was 12%. Ninety-three percent of patients developed intellectual disability, 81% had seizures, 70% had other neurological manifestations and 60% had severely impaired vision. Other sequelae included hydrocephalus or microcephaly in 33% and 15% had deafness. In Eichenwald's series of children with generalised disease who received no treatment or treatment for one month, 81% had learning disability at four years and deterioration in cognitive function was also demonstrated (Eichenwald H F, 1959).

Other historic studies reported on the outcome of asymptomatic children with subclinical CT who were not recognised at birth (Alford, Jr. et al., 1974; Wilson et al., 1980). Wilson et al reported on 24 children with asymptomatic CT who were not diagnosed until clinical signs of CT appeared. At 8-year follow up, almost all had disease progression in the form of permanent ocular and or neurologic disability and in some children deterioration of intelligence quotient (IQ) was demonstrated.

A study by Patel and colleagues performed more than two decades ago reported on resolution of intracranial calcification in postnatally treated infants. Between January 1982 and March 1994, cranial computed tomography (CCT) was performed at birth and approximately one year later in 56 infants treated for CT. By one year of age, calcifications had diminished or resolved in 30 (75%) and remained stable in 10 (25%) infants who were treated for one year. In contrast, a small number of infants who were untreated or treated for one month or less had intracranial calcifications that increased or remained stable during their first year of life. Resolution of intracranial calcification was associated with improved neurological outcome (Patel et al., 1996).
Historic studies may not be comparable to current or recent studies as improved outcomes over time compared with older studies may be also due to advances in diagnostics, drug treatment regimens, medical care and technologies for children with neurological involvement.

**6.4.3 Outcome of infants treated in France**

Long-term follow up studies of congenitally infected infants in France are of infants treated pre- and postnatally with favorable visual outcomes. Due to the existence of routine systematic antenatal screening in France, only a minority of congenitally infected infants are undetected antenatally or not treated before birth. Hence studies in French cohorts, which demonstrate benefits and favorable outcomes in treated infants encompass outcome with both antenatal and postnatal treatment. In a French study of 327 infected infants followed for 14 years, conclusions were that retinochoroiditis may occur years after birth in treated CT but overall prognosis was satisfactory when infection was diagnosed and treated early (Wallon et al., 2004).

A prospective cohort study performed over 16 years in Marseilles France aimed to determine the prognosis of treated CT in Europe (Faucher et al., 2012). Treatment was administered antenatally with rovamycine as soon as maternal infection was detected and with pyrimethamine and sulfadoxine if toxoplasma PCR was positive in amniotic fluid. Postnatal treatment with pyrimethamine and sulfadoxine was prescribed for one year. Of 127 children with confirmed CT, 24 (18.9%) presented with ocular lesions overall, despite pre- and postnatal treatment, eight of whom had visual impairment. Eleven children (8.7%) presented with ocular lesions at birth, mostly macular. Sixteen children (12.6%) developed ocular lesions during follow-up, mostly peripheral. In some cases the first ocular lesion occurred as late as 12 years after birth. Conclusions were that the overall prognosis of CT in Europe was good but there was a low risk of late ocular manifestation. Late lesions were less often macular but nevertheless sometimes caused visual impairment.

A study in France reported on quality of life and long term visual function for 126 adults treated for CT *in-utero* and postnatally. Individuals were monitored regularly up to a mean age of 22 years. Overall, 58.8% manifested ocular lesions and
12.7% had reduced visual function (Peyron et al., 2011). Conclusions were encouraging as the data revealed that treated CT had little effect on quality of life, and visual function was only slightly impaired in a minority of individuals.

A similar study is currently in progress in Lyon in a larger cohort to determine long-term ocular outcome in CT infants treated pre- and postnatally. Preliminary results for 22-year follow up of a cohort of CT infants demonstrate that 65% of infants never developed retinal lesions, 30% developed retinal lesions at some stage in the first 20 years of life, and in many cases the first retinal lesion occurred beyond infancy and during the first decade of life (Wallon M – personal communication).

### 6.4.4 EMSCOT studies on postnatal treatment for CT and outcome

The EMSCOT multicentre study recruited and followed up 293 children with CT for four years. However most of the publications that arose from the study focused primarily on the effect of prenatal screening and treatment on infant sequelae rather than the effect of postnatal screening and infant treatment.

The EMSCOT multicentre study demonstrated that postnatal treatment did not prevent ocular lesions: 5% of treated children had retinochoroidal lesions at birth, 20% at five years, and 30% at eight years of age (Garcia-Meric et al., 2010). However, without comparative groups the researchers refrained from concluding whether there would have been similar outcomes with no treatment.

The EMSCOT study group questioned the effect of postnatal treatment and regular scheduled ophthalmic examinations for infants with asymptomatic CT. One of the studies concluded that in Europe, if children with CT had no retinochoroiditis in early infancy, the risk of subsequent ocular disease was low and postnatal treatment and repeated ocular assessments were unnecessary during childhood (Freeman et al., 2008).

In another EMSCOT related study, infected and uninfected infants born to infected mothers had the same developmental outcomes but parents of infected infants were significantly more anxious about the child’s vision which was reinforced by repeat funduscopcy. Freeman et al from the EMSCOT study group demonstrated that children at highest risk of retinochoroiditis were those with fetal ultrasound
abnormalities, intracranial abnormalities and those with non-ocular manifestations of CT by four months age. By contrast, children with no retinochoroiditis by four months of age had a low risk of developing retinal lesions by the age of four years, regardless of other clinical manifestations.

Freeman et al proposed an alternative strategy in which postnatal treatment and follow-up are tailored to the child’s prognosis. It was suggested that children with no clinical manifestations should be offered a short 3-month course of antiprotoszoal treatment or no treatment; instead of regular funduscopy throughout childhood parents should be advised to consult an ophthalmologist if the child developed eye problems and visual acuity should be tested during routine school based screening at three to four years. Prior to this age ophthalmic evaluation should be performed at birth and at yearly intervals if the fundus was normal at birth (Freeman et al., 2008).

As demonstrated earlier by Thulliez et al, conclusions based on analysis of large grouped multi-centre cohorts may obscure significant findings from rigorous separate studies that were part of these larger combined analyses.

In France, where rigorous follow-up of congenitally infected infants takes place, regular scheduled ocular examinations are welcomed and praised by parents of infected infants and patients followed into the second decade of life. In a French study of 102 adult patients surveyed with CT, 98% stated that follow up was useful and expressed wish to continue with follow up. Patients perceived long term monitoring of their condition to be positive and reassuring. The conclusion was that without general agreement or guidelines on how patients with congenital toxoplasmosis should be monitored, the patient's wishes are important in making a decision (Beraud et al., 2013).

The final conclusions of the EMSCOT working group on postnatal treatment for CT in their cohort of children, most of who also received antenatal treatment, were that:

1) The diagnosis of CT needed to be confirmed;
2) In Europe the risk of severe CT and recurrent chorioretinitis was low, and treatment and regular ophthalmoscopy was likely to be beneficial only to
children with early clinical manifestations and or retinochoroiditis who were at high risk of recurrences;

3) The effectiveness of postnatal treatment should be evaluated systematically, ideally in a randomised controlled clinical trial.

**6.4.5 Outcome of infants screened postnatally and treated in Denmark**

Denmark was the only country in Europe that had a stand-alone national newborn screening programme for CT unlinked to antenatal screening. Screening utilised neonatal DBS to determine the incidence of CT and the programme operated from 1999 to 2007. The results and conclusions from this study are discussed in subsequent chapters in parallel with the national newborn screening programme for CT in Ireland.

**6.4.6 Outcome of infants treated postnatally for CT in South America**

Routine screening of women or infants for CT does not take place in South American countries primarily due to lack of funds and resources, hence there is paucity of data on toxoplasma seroprevalence, incidence of CT and outcome. Regional and provincial studies of limited duration have been performed in Columbia and Brazil to estimate the seroprevalence of toxoplasma antibody, disease burden of CT and infant prognosis. High maternal seropositivity has been reported in certain regions, up to 70% in some areas of Brazil (Vaz, 2011). The incidence of CT has been found to vary significantly between cities in Columbia, possibly reflecting disparity between public health policies and resources applied in different regions. A multicentre newborn screening programme for congenital toxoplasmosis in Colombia estimated that in some provinces the incidence was as high as 1 in 1,000. Cord blood screening was analysed from newborns in 19 maternity services from seven different cities in five geographic regions of Columbia between March 2009 to May 2010 during which time 15,333 cord blood samples were collected. Congenital infection was confirmed in 15 infants; 7 were symptomatic, and 3 died within the first month of life. A significant correlation was found between a high incidence of congenital toxoplasmosis and cities with higher mean annual rainfall (Gomez-Marin et al., 2011)
A 3-year pilot newborn screening programme in the Brazilian state of Rio Grande do Sul estimated that one per 4,800 live births were congenitally infected. In a larger prospective study the prevalence of CT in Brazil was estimated at one per 3,000 live births (Neto et al., 2004).

By contrast with Europe, studies from South America have demonstrated that *T. gondii* causes more severe ocular disease in congenitally infected children. In Brazil, infants with CT usually manifest active ocular lesions in the first three months of life and compared with Europe the risk of recurrence at four years was higher with mostly macular lesions and visual impairment (Sauer et al., 2011).

Gilbert et al examined ocular sequelae of CT in Brazil compared with Europe. Prospective cohorts of children with CT identified by neonatal screening in Brazil and neonatal or prenatal screening in Europe between 1992 and 2003 were compared using the same protocol in both continents (Gilbert et al., 2008). Three hundred and eleven children with CT were included in the study; 30 from Brazil and 281 from Europe. Median follow up was 4.1 years in Europe and 3.7 years in Brazil.

Fifteen of 30 (50%) children in Brazil had retinochoroiditis during the first year versus 29 of 281 (10%) in Europe. Children in Brazil had larger lesions, which were more likely to be multiple and to affect the posterior pole. In Brazil, visual impairment was predicted for most affected eyes but not in Europe.

The differences in infant outcome between the two continents has been associated with geographic variations in parasite strain, genotype and virulence (Khan et al., 2006; Vaudaux et al., 2010). Virulent parasite strains found in Brazil are not common in Europe (Pernas et al., 2014). In addition, resistant *T. gondii* mutant and atypical strains have been reported which are refractory to conventional treatments and this may explain disease severity and poorer outcomes in congenitally infected infants from South America (Aspinall et al., 2002; Meneceur et al., 2008).
In general, more aggressive clinical disease has been reported in North and South America compared with Europe. In Brazil, toxoplasmic retinochoroiditis has been reported as a leading cause of blindness.

6.4.7 Outcome of children screened and or treated postnatally for CT in North America

6.4.7.1 Newborn screening for CT in the states of Massachusetts and New Hampshire

In the states of Massachusetts and New Hampshire a newborn screening programme was implemented in 1986 and 1988 respectively. Newborn IgM screening in Massachusetts revealed an incidence of CT of approximately 1 case per 10,000 live births, which was consistent with a transmission rate of 10%. Factors that strongly predicted congenital infection were mother's country of birth outside the U.S.A., mother's educational level and higher parity (Jara et al., 2001).

Forty percent of congenitally infected infants identified by screening in the two states have either ocular or intracranial signs on evaluation at birth. In a follow up of a sub-group of 46 treated infants, only one child had long-term neurological sequelae secondary to a cerebral lesion present at birth, four (10%) had eye lesions that may have developed postnatally (a macular lesion in one child and minor retinal scars in three). Conclusions were that routine neonatal screening for toxoplasmosis identifies congenital infections that are subclinical, and early treatment may reduce the severity of long-term sequelae (Guerina et al., 1994).

6.4.7.2 The National Collaborative Chicago-based Congenital Toxoplasmosis Study (NCCCTS): outcome of postnatal treatment for CT

The NCCCTS is an ongoing study in the U.S.A with continued enrollment of infants with confirmed or probable CT. Routine antenatal screening for CT in the U.S.A does not take place and hence infants referred to the trial are not treated in-utero unless toxoplasmosis was suspected or sought for specific reasons antenatally.

Participants with confirmed CT are fully evaluated, assigned to 12 months antiprotozoal treatment (24 months if severe disease) and followed up
prospectively. The follow up period is currently up to mid-childhood years; the earlier recruits from 1981 to 1994 were followed up to mid teenage years. The patient population is mainly symptomatic.

The trial reported outcome of referred cases of infants treated for the first year of life with pyrimethamine and sulfadiazine. The trial also had non-treated groups as it included children with CT referred to the study after the age of one year. In the group of early recruits from 1982-1992, 24% had cognitive impairments overall. Seventy-six percent of treated children who had ocular, systemic or neurological manifestations of infection at referral had normal development at follow-up (McAuley et al., 1994).

In a subsequent NCCCTS analysis of a cohort recruited from 1981 up to 2004, McLeod et al demonstrated the impact of one year of treatment on 120 infected infants who received antiprotozoal treatment from shortly after birth for 12 months (McLeod et al., 2006). The 120 infants were randomly assigned to one of two 1-year regimens of combination therapy. One group received daily sulfadiazine, thrice weekly folinic acid, plus two months of daily pyrimethamine followed by thrice-weekly pyrimethamine for the remainder of the year. The second group received daily sulfadiazine, thrice weekly folinic acid, plus six months of daily pyrimethamine followed by thrice-weekly pyrimethamine for the remainder of the year. Children were followed up at predetermined intervals for neurodevelopmental progress, ocular and audiology examinations for 10 to 15 years.

The major findings of the cohort of 120 in the NCCCTS are summarised in figure 6.1. McLeod et al compared the results from the NCCCTS to historical controls untreated or treated for one month from the studies by Eichenwald et al. The trial results concluded that treatment with antiprotozoals did not prevent chorioretinitis, but almost all outcomes were better than children untreated or treated for one month in earlier decades (p < 0.01 to p < 0.001).

Sub groups from the NCCCTS have been prospectively studied to provide results and conclusions on treated vs untreated CT. Phan et al reported on a prospective longitudinal study in the U.S. to determine the incidence of new chorioretinal
lesions in 132 children with CT treated for one year (Phan et al., 2008a). The results are summarised in figure 6.2a.

Phan et al also reported on a second prospective longitudinal study with a different cohort of children from the NCCCTS diagnosed with CT beyond the first year of life and hence did not receive treatment. The sub-group consisted of 25 children who were evaluated and followed up in Chicago between 1981 and 2005 for new chorioretinal lesions (Phan et al., 2008b). The results are summarised in figure 6.2b.

By comparison of treated and untreated groups, conclusions were that antiprotozoal therapy contributed to a more favourable outcome and new central chorioretinal lesions were uncommon in children who were treated with antiprotozoals during the first year of life compared with those who were untreated. Recurrences occurred after 10 years in both treated and untreated groups indicating that long-term follow-up into the second decade of life was important in assessing the efficacy of treating toxoplasmosis during infancy.

6.5 Summary of issues and controversies associated with postnatal screening and treatment for CT

In summary, debate remains regarding the benefits of newborn screening and treatment for CT, as some areas require further elucidation. Further study is required, as of now:

1) The benefits of early newborn treatment with currently available regimens have not been evaluated in a large RCT and concerns remain regarding the adverse effects of treatment;
2) There is a paucity of data on the effect of asymptomatic CT, treated or untreated on developmental and visual outcome long term;
3) The optimum duration of infant treatment is not known.

The experts who campaigned for screening and its associated benefits, e.g., screening increases awareness of CT among healthcare providers and pregnant women, have raised questions about contemporary screening recommendations. Studies from Europe, the NCCCTS and The New England newborn screening programme in Massachusetts U.S.A have identified epidemiological factors
associated with increased risk of CT such as maternal ethnicity and dietary habits. Hence data from newborn screening programmes could be used to identify and target the at-risk population with education programmes to increase awareness (Cook et al., 2000; McLeod et al., 2006).

Degrees of intellectual impairment may be present in asymptomatic infants, which may not manifest for many years. Such children need to be offered interventions in a timely manner to maximise their outcome. The longitudinal NCCCTS demonstrates that the benefits of treatment of CT are significant and long lasting even for children with milder disease. More studies on long-term prospective follow up of infants with asymptomatic CT are necessary to determine how the disease affects outcome.

Postnatal screening for CT and cost-benefits remain controversial in low endemic areas such as Western Europe. However, the majority of studies that report on screened and or treated infant populations in Europe demonstrate good outcomes overall. Treated CT in Europe is associated with a low risk of severe neurological sequelae, intraocular reactivation and visual dysfunction. Experts who campaign for newborn screening argue that future mothers and their offspring cannot afford to wait years for additional data to be accumulated from RCTs to evaluate the effectiveness and safety of screening and treatment, but rather more effort should be made to evaluate the cost effectiveness of identification and treatment of all cases of CT (Kim, 2006).
120 Infants with Congenital Toxoplasmosis

- Mild or No Disease (n=12)
- Moderate (n=12)
- Severe (n=96)

**After 1 yr Tx antiprotozoals**
- 100% had normal cognitive, neurologic and auditory outcomes
- Only 9% developed new eye lesions

**After 1 yr Tx antiprotozoals**
- 100% had normal auditory outcomes
- >72% had normal cognitive and neurologic outcomes
- Only 36% developed new eye lesions
- 85% had impaired vision (most had retinal disease at birth)

- 11 deaths (9 pneumonia in children with severe CNS disabilities, 2 accidents)

---

Figure 6.1: McLeod 2006; Outcome of Treatment for Congenital Toxoplasmosis, 1981-2004: NCCCTS
Prospective Longitudinal Study of 108* Children Evaluated for New Chorioretinal Lesions (Phan et al, 2008)

<table>
<thead>
<tr>
<th>34 (31%)</th>
<th>had at least one new lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 15 children (14%) developed new central lesions</td>
<td></td>
</tr>
<tr>
<td>• 27 (25%) developed new peripheral lesions</td>
<td></td>
</tr>
<tr>
<td>• 10 (9%) had more than one occurrence of new lesions</td>
<td></td>
</tr>
<tr>
<td>• 13 (12%) had new lesions in both eyes</td>
<td></td>
</tr>
<tr>
<td>• 14 (41%) had lesions diagnosed at age 10 or later</td>
<td></td>
</tr>
</tbody>
</table>

* All received antiprotozoals from birth to 1 year

Prospective Longitudinal Study of 25* Untreated Children Evaluated for New Chorioretinal Lesions (Phan et al, 2008)

<table>
<thead>
<tr>
<th>18 (72%)</th>
<th>had at least one new lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 13 children (52%) developed new central lesions</td>
<td></td>
</tr>
<tr>
<td>• 11 (44%) developed new peripheral lesions</td>
<td></td>
</tr>
<tr>
<td>• 6 (24%) had both central and peripheral lesions</td>
<td></td>
</tr>
<tr>
<td>• 7 (39%) had new lesions in both eyes</td>
<td></td>
</tr>
<tr>
<td>• 13 (52%) had lesions diagnosed at age 10 or later</td>
<td></td>
</tr>
<tr>
<td>• 4 (22%) had new lesions detected at more than one visit</td>
<td></td>
</tr>
</tbody>
</table>

* All diagnosed with CT at >1 year

Figure 6.2: comparison of subgroups from the NCCCTS treated (blue box) and untreated (purple box) for CT
CHAPTER 7 - Literature review: surveillance and prevention programmes in Europe for congenital toxoplasmosis

7.1 Introduction
Epidemiological surveillance of CT is defined as ongoing systematic collection, analysis and interpretation of data for the purpose of describing and monitoring CT. Prevention of CT refers to primary prevention with educational strategies, or screening programmes to identify CT antenatally or postnatally and facilitate interventions aimed at maximising infant outcome. In Europe, systems for surveillance and or prevention of CT are variable and depend on seroprevalence in the population, incidence of CT and cost effectiveness of programmes. Some countries in Europe have defined CT as a public health issue and implemented both national surveillance and prevention programmes (Binquet et al., 2004).

Public health policies vary markedly across Europe regarding the prevention of CT. In addition, there can be discrepancies between national recommendations and routine regional practices of prenatal or neonatal screening (Stray-Pedersen, 2003; Signorell et al., 2006). Many countries in Europe have implemented prenatal screening programmes and other countries and regions have performed newborn screening for limited periods. In general, practices and policies vary due to lack of consensus on interpretation of pregnancy seroconversion and treatment efficacy pre- and postnatally (Ricci et al., 2003; Thiebaut et al., 2007). This is largely due to the lack of RCTs to compare outcome of screened versus non-screened groups to provide the level of evidence on which firm conclusions can be drawn to guide clinical practice. Debates are ongoing as to which are the best methods for controlling CT, however the debate has not always been based on accurate epidemiological data (Petersen, 2007).

7.2 The EUROpean TOXOplasmosis prevention project (EUROTOXO)
A joint European initiative, The EUROpean TOXOplasmosis prevention project (EUROTOXO), was a ‘consensus initiative’ working group which reviewed several thousand published papers on the subject of toxoplasmosis during pregnancy and childhood (Thiebaut et al., 2006; Benard et al., 2008). This was set up in 2004 by three groups; the Institute of Child Health London, United Kingdom (UK), the Staten Serum Institute Copenhagen, Denmark, and the Institute of Public Health Epidemiology and Development, Bordeaux, France. The EUROTOXO project
supported the aforementioned SYROCOT project and both groups had common investigators and participating institutions.

The purpose of EUROTOXO was:
1) To determine national public health policies, recommendations and systems in Europe for epidemiological surveillance and prevention of CT;
2) To review the burden of toxoplasma infection in pregnant women, fetuses, newborns and children in order to evaluate the need for CT prevention programmes;
3) To determine the frequency of the prevented disease and hence evaluate the rationale and efficacy of the different screening programs performed in Europe;
4) To define the implications of current scientific knowledge for future research agenda and policy decisions on how best to prevent CT and its consequences;

7.3 Congenital toxoplasmosis surveillance in Europe
In November 2004 the EUROTOXO group distributed a questionnaire adapted from the U.S. Centers for Disease Control and Prevention (CDC) to a panel of surveillance gatekeepers from the 35 European WHO members regarding surveillance systems for toxoplasmosis implemented in their country (table 7.1). Twenty-eight of 35 countries responded to the survey and seven countries gave no response The information from Denmark and France was updated in July 2007 (Benard et al., 2008). Of the 28 countries that responded, 12 did not have a surveillance system for toxoplasmosis, congenital or acquired.

Sixteen countries, mostly situated in central or Eastern Europe, reported surveillance of toxoplasmosis. In 12 of 16 countries the event under surveillance was symptomatic toxoplasmosis, congenital or not (figure 7.1 and table 7.2). However surveillance of symptomatic toxoplasmosis in the general population does not distinguish between congenital and acquired toxoplasmosis. In 2007, only four countries reported specific surveillance for CT to detect symptomatic as well as asymptomatic cases; these included at a national level France, Germany and Denmark and at a regional level, Campania in Italy.

In France specific surveillance for CT began in May 2007, 29 years after implementation of antenatal screening (Villena et al., 2010). CT cases are notifiable for fetuses, newborns and children up to age one year (Garcia-Meric et
In Germany CT cases have been notifiable since 2001. In Italy CT surveillance confined to the Campania region has been in effect since 1997 (Stagni et al., 2009). In Denmark nationwide CT surveillance was linked to a systematic neonatal screening programme implemented in 1999 and discontinued in July 2007 (Roser et al., 2010).

In Ireland, toxoplasmosis, congenital or acquired, asymptomatic and symptomatic has been a notifiable disease since 2004. Approximately 35 to 50 cases of toxoplasmosis are reported in Ireland each year, which represent both congenital and acquired infection (available at http://www.hpsc.ie/AZ/Zoonotic/Toxoplasmosis/EpidemiologicalData/ accessed November 2015).

In the UK, since 1975, the health event under surveillance was symptomatic toxoplasmosis, congenital or acquired, and since August 2002 children presenting with CT are reported. Approximately 400 cases of toxoplasmosis, congenital and acquired, are diagnosed in the UK per year (Gilbert et al., 2006). There have been no recent changes in the number of countries in Europe that employ CT surveillance.

7.4 Congenital toxoplasmosis prevention policies in Europe
Congenital toxoplasmosis prevention programmes in Europe are influenced by the rate of maternal seroprevalence, the incidence of CT, the disease burden of CT and not least funding and cost-effectiveness. In Europe, the overall toxoplasma seroprevalence in pregnant women is approximately 30% to 50%. Some countries in Western Europe and northern countries (Scandinavia and the UK) quote seroprevalence rates of less than 30% (Petersson et al., 2000; Lappalainen, 2003).

One study of women attending antenatal clinics in an ethnically diverse population in central London found seroprevalence for T. gondii was 17.32% in 2,610 samples tested (Flatt et al., 2013). The overall incidence of CT in Europe is estimated at 1 to 5 cases per 10,000 live births (Benard et al., 2008).

In 2005, the EUROTOXO group dispatched surveys to the 36 WHO European members. In addition a literature review of Medline was performed to document national policies in existence for primary prevention of CT, screening programmes and the associated cost. Twenty-seven countries (75%) responded to the survey.
In 2005, five European countries officially had a mandatory prenatal screening programme in effect: France, Italy, Austria, Slovenia and Lithuania. Among the other countries, 18 had no specific national pre- or postnatal screening policy, 10 of which employed primary prevention with maternal education on risk avoidance during pregnancy. Overall primary prevention was recommended on a nationwide basis in 18 of 27 European countries that responded. In addition, prenatal screening was routinely practiced in several countries at regional levels in the absence of national guidelines, and some countries also employed newborn screening at a regional level in the absence of a national policy to determine the incidence of CT (figure 7.2 and table 7.3).

Monthly testing of pregnant women has been employed in Italy since 1998 where the seroprevalence rate is 40% (Meroni et al., 2009; Pinto et al., 2012). In the Campania region of Italy the prevalence of CT was estimated at 1.38 per 10,000 live newborns (Stagni et al., 2009). This was based on the follow up of infants born to mothers with proven gestational toxoplasmosis and on referred cases of CT up to age one year. Three-monthly testing of pregnant women was recommended in Austria since 1975 however compliance with the antenatal screening was poor with subsequent under-reporting of seroconversion in pregnancy and missed opportunities to diagnose congenital infections (Knerer et al., 1995; Prusa et al., 2015). The seroprevalence in pregnant women varied according to region and was quoted as 33-40% (Sagel et al., 2011; Aspock, 2012). Slovenia employed three monthly prenatal screening since 1995 (Logar et al., 1995). In Lithuania women were tested twice during pregnancy and at delivery but the implementation date of the programme was unknown.

Since the EUROTOXO survey in 2005, the only country that changed its CT prevention policy was Switzerland. In the Basel region of Switzerland cord blood screening was performed from 1982 – 1999; 64,622 cord blood samples were collected representing more than 90% of neonates from the region, with confirmatory paired mother and infant serology where necessary. In the Lausanne region a retrospective newborn study was conducted from 1995 – 2006. Maternal seroprevalence and the incidence of CT in Switzerland was estimated from the data collected from both studies. In 1996, the seroprevalence in pregnancy was 46%. The incidence of CT was 4.3 per 10,000 live births and clinical disease was observed in one of 16,250 neonates. This study concluded that the prevalence of
maternal toxoplasma antibody had declined over the 17 year period under study (Berger et al., 1992) and the prevalence of CT also markedly declined during the study period from 0.08% to 0.012% (Signorell et al., 2006).

In 2009 the Swiss Working Group on Toxoplasmosis (SWGT) discontinued random screening in pregnancy. The SWGT claimed that the prevalence of toxoplasma infections had decreased, the incidence of CT was much rarer than initially expected (35 cases in four years), screening and treatment during pregnancy was considered not to be beneficial and it was recommended that primary preventive strategies should be strengthened to avoid infection in pregnancy (Stricker et al., 2009).

Newborn screening was reported in the Poznan region of Poland from 1996 to 1998. Newborn bloodspot cards were screened for toxoplasma IgM from 1996 to 1998. The seroprevalence of toxoplasma antibody was 43.7%, the incidence of CT was 1 in 2,000 and the neonatal test sensitivity was 86.7% (Paul et al., 2000;Switaj et al., 2006).

The EUROTOXO survey identified two countries, the Netherlands and the UK, that had national recommendations which specifically stated that there should be no screening, either pre- or postnatally. In the Netherlands, the antenatal seroprevalence was 40% and screening was not recommended because of unfavourable cost effectiveness and the lack of data demonstrating a significant treatment impact on long-term ocular outcome (Havelaar et al., 2007). In the UK the seroprevalence in pregnant women was 8% to 10%. The existing policy was that screening for toxoplasmosis should not be offered during the antenatal or newborn periods due to the lack of evidence on disease frequency, severity and treatment efficacy (Joynson et al., 1988;Gilbert et al., 2002). The policy was based on a review for the UK National Screening Committee (NSC) on antenatal and newborn screening for toxoplasmosis which was carried out in 2001 (NSC website www.screening.nhs.uk/toxoplasmosis).

The UK NSC review estimated that CT affected 1 in 10,000 live births, of which less than 5% had severe neurologic impairment and 20% to 30% would have intracranial or ocular lesions by three years of age. The policy was reviewed in July 2006 based on a knowledge update and no changes were made. It was
agreed that the policy should be revised again if significant new evidence emerged.

In 2010 The National Institute for Health and Clinical Excellence (NICE) reviewed the evidence relating to screening for CT as part of the revision of the 2003 and 2008 Antenatal Care Guideline and recommended that:

1) Routine antenatal serologic screening for toxoplasmosis should not be offered in the UK as the risks of repeated tests, unnecessary treatment, amniocentesis associated risks and termination of pregnancies with unaffected fetuses may outweigh the benefits of potential reduction in the number of cases with CT;

2) Pregnant women should be informed of primary prevention to avoid exposure to infection (available at: http://www.nice.org.uk/guidance/cg62/chapter/1-Guidance).

In 2005 at the time of the EUROTOXO survey there were no existing policies for prevention of CT in Ireland and that has remained unchanged. Currently, women are tested antenatally if there is a suspicion of maternal infection or a congenitally infected fetus. Obstetric units provide free literature on risk factors for seroconversion. However there are no national recommendations for primary prevention by education of pregnant women or reinforcement of avoidance of risk factors during pregnancy. Toxoplasma antibody prevalence in women of childbearing age in Ireland was determined by a study which employed anonymous testing of newborn DBS cards and this study is detailed in the subsequent chapter.

7.5 EUROTOXO conclusions

The conclusions drawn from the EUROTOXO review of toxoplasmosis surveillance and prevention programmes were that there was an overall lack of CT surveillance in Europe to determine the true disease burden and hence to assess the effectiveness of and the need for existing prevention programmes. In several European Union (EU) and European Economic Area (EEA) countries, national surveillance systems did not collect key data items needed to identify CT cases.

In addition, a high degree of heterogeneity was identified amongst European countries and a broad lack of evidence with regard to many aspects of CT was
recognised. Due to limited data, epidemiological parameters such as incidence of seroconversion in susceptible pregnant women or incidence of sequelae in congenitally infected infants could not be analysed.

A definite conclusion was that the prevalence of toxoplasmosis among pregnant women in Europe had decreased over the years. The decline did not necessarily reflect a decrease in the incidence of toxoplasmosis acquired during pregnancy but probably reflected a decline in incidence during childhood that rendered more pregnant women susceptible to infection. EUROTOXO also concluded that published data on the burden of CT in Europe was limited and available information was not sufficient to introduce or adjust national policies.
Figure 7.1: Map of Surveillance systems for toxoplasmosis in Europe

(Source: Benard et al, Eurosurrveillance 2008; 13(4-6): p3)
Figure 7.2: Geographical representation of nationwide and regional policies for prevention of CT in Europe

(Source: EUROTOXO-panel 3-Version 12/12/2005)
Table 7.1: European correspondents contacted to participate in the survey on epidemiological surveillance of toxoplasmosis

### Countries that participated in the survey

<table>
<thead>
<tr>
<th>Country</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>Renéfild Strauss, Federal Ministry for Health, Family and Youth, General Directorate of Public Health, Vienna</td>
</tr>
<tr>
<td>Belgium</td>
<td>Germaine Hanquet, Scientific Institute for Public Health, Unit of Epidemiology, Ministry of Social Affairs, Public Health and Environment, Brussels</td>
</tr>
<tr>
<td>Benin and Nigeria</td>
<td>Semra Cavajuga, Institute for Epidemiology and Biostatistics, School of Medicine, University of Sarajevo, Sarajevo</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>Mira Kogunaruova, National Centre of Infectious and Parasitic Diseases, Department of Epidemiology, Sofia</td>
</tr>
<tr>
<td>Cyprus</td>
<td>Olga Kalkoua, on behalf of Dr. Chrysanta Hadjiantasaistou, Chief Medical Officer, Medical and Public Health Services, Ministry of Health, Nicosia</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Petr Kodym, National Reference Laboratory for Toxoplasmosis, National Institute of Public Health, Prague</td>
</tr>
<tr>
<td>Denmark</td>
<td>Henrik Vedel Nielsen, Unit for Mycology and Parasitology, Statens Serum Institut, Copenhagen</td>
</tr>
<tr>
<td>England and Wales</td>
<td>Robert Smith, Public Health Laboratory, Service of Communicable Disease Surveillance Centre Wales, Cardiff</td>
</tr>
<tr>
<td>Estonia</td>
<td>Kusla Kutsar, Department of Communicable Diseases, Health Protection Inspectorate, Tallinn</td>
</tr>
<tr>
<td>Finland</td>
<td>Marja Lappalainen, Department of Virology, Hospital District of Helsinki and Uusimaa, Helsinki</td>
</tr>
<tr>
<td>France</td>
<td>Isabelle Villena, National Reference centre for toxoplasmosis, Rennes; Véronique Goulet, Department of Infectious Diseases, Institut de Veille Sanitaire, Saint-Maurice</td>
</tr>
<tr>
<td>Germany</td>
<td>Katharina Alpers, Department for Infectious Disease Epidemiology, Gastrointestinal Infections, Zoonoses and Tropical Infections, Robert Koch Institute, Berlin</td>
</tr>
<tr>
<td>Greece</td>
<td>Yantis Tsilentis, Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, University of Crete, Faculty of Medicine, Heraklion</td>
</tr>
<tr>
<td>Ireland</td>
<td>Gartina D’Flanagan, HSE-Health Protection Surveillance Centre, Dublin</td>
</tr>
<tr>
<td>Italy</td>
<td>Silvina Buffalano, Perinatal Infection Unit, Department of Paediatrics, Federico II University of Naples; Marta Grazia Pompa, Communicable Disease Unit, DG Health Prevention, Ministry of Health, Rome</td>
</tr>
<tr>
<td>Latvia</td>
<td>Irena Lucenko, Division of Epidemiology of Infectious Diseases, State Public Health Agency, Riga</td>
</tr>
<tr>
<td>Lithuania</td>
<td>Bronius Morkūnas, Centre for Communicable Disease Prevention and Control, Vilnius</td>
</tr>
<tr>
<td>Malta</td>
<td>Tanya Mielilo Fenech, Disease Surveillance Unit, Department of Public Health, Ministry of Health, Malta</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Laetitia M. Kortbierv, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening (LLS), National Institute of Public Health and the Environment, Bilthoven</td>
</tr>
<tr>
<td>Norway</td>
<td>Hans Blystad, Department of Infectious Disease Epidemiology, Norwegian Institute of Public Health, Oslo</td>
</tr>
<tr>
<td>Poland</td>
<td>Malgorzata Sadkowska-Todys, Laboratory of Zoonoses, Department of Epidemiology, National Institute of Hygiene, Warsaw</td>
</tr>
<tr>
<td>Portugal</td>
<td>Juditha Calatino, General Health Directorate, Lisbon</td>
</tr>
<tr>
<td>Romania</td>
<td>Adriana Pistoll, General Department of Public Health, Service of Prevention and Control of Communicable Diseases, Ministry of Health and Family, Bucharest</td>
</tr>
<tr>
<td>Scotland</td>
<td>Lynn Brown, Zoonoses Section, Health Protection Scotland, Glasgow</td>
</tr>
<tr>
<td>Slovakia</td>
<td>Maria Avdichtova, Department of Epidemiology, Regional Institute of Public Health, Baska Bystrica</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Jurek Logar, Institute of Microbiology, Medical Faculty, Ljubljana</td>
</tr>
<tr>
<td>Sweden</td>
<td>Johan Lindh, Department of Parasitology, Mycology and Water, Swedish Institute for Infectious Disease Control, Solna</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Karin Boubaker, Infectious Diseases Section, Division of Communicable Diseases, Swiss Federal Office of Public Health, Public Health Direction, Bern</td>
</tr>
</tbody>
</table>

### Countries that were asked but did not participate in the survey

<table>
<thead>
<tr>
<th>Country</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albania</td>
<td>Eduard Kakarirti, Department of Epidemiology, Institute of Public Health, Praga</td>
</tr>
<tr>
<td>Croatia</td>
<td>Ira Gjenero-Margin, Croatian Public Health Institute, Department of Epidemiology of Infectious Diseases, Zagreb</td>
</tr>
<tr>
<td>Hungary</td>
<td>Márta Mihály, ‘Johan Béla’ National Centre for Epidemiology, Budapest</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>Robert Hamer, National Service of Infectious Diseases, Centre Hospitalier de Luxembourg, Luxembourg</td>
</tr>
<tr>
<td>Macedonia</td>
<td>Kristina Vlaskovska, Medical Faculty Skopje, University &quot;Ss. Kiril i Metodij&quot; Institute of Epidemiology, Biostatistics and Medical Informatics, Skopje</td>
</tr>
<tr>
<td>Spain</td>
<td>Luisa Sanchez Servano, Sección de Sistema de Información Microbiológica, Vigilancia de Salud Pública, National Centre of Epidemiology, Hospital Carlos III, Madrid</td>
</tr>
<tr>
<td>Serbia-Montenegro</td>
<td>Danica Masanovic, Sanitary Inspection of the Ministry of Health of Montenegro, Podgorica</td>
</tr>
</tbody>
</table>

(Source: Benard et al, Eurosurveillance 2008; 13(4-6): p2)
### Table 7.2: Characteristics of European epidemiological surveillance programmes for toxoplasmosis (Eurotoxo 2007)

<table>
<thead>
<tr>
<th>Country</th>
<th>Year started</th>
<th>Population under surveillance</th>
<th>Case definition</th>
<th>Reporting sources</th>
<th>Frequency of analysis</th>
<th>Frequency of surveillance reports</th>
<th>Surveillance report distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>1999</td>
<td>Live newborns</td>
<td>Detection of IgM on blood filter sample confirmed by IgA, IgM and IgG profile in newborn and mother</td>
<td>Statens Serum Institut</td>
<td>NA</td>
<td>Annually</td>
<td>Healthcare authorities</td>
</tr>
<tr>
<td>France</td>
<td>2007</td>
<td>Fetuses, newborns and infants</td>
<td>PCR, mouse inoculation, cell culture or immunocytochemistry on body tissues or fluids; detection of specific IgM or IgA antibodies; neosynthesis of specific IgG, IgM or IgA antibodies; persistence of IgG until one year of age</td>
<td>Laboratories qualified for antenatal or postnatal diagnosis</td>
<td>NA</td>
<td>NA</td>
<td>Healthcare authorities</td>
</tr>
<tr>
<td>Germany</td>
<td>NA</td>
<td>Live newborns and infants</td>
<td>Detection of Toxoplasma gondii in body tissues or fluids; detection of specific IgM or IgA antibodies; persistently stable specific IgG titre or a single elevated specific IgG titre</td>
<td>Laboratories</td>
<td>Continuous</td>
<td>Quarterly and annually</td>
<td>Free access on the Website of the Robert Koch Institute</td>
</tr>
<tr>
<td>Italy</td>
<td>1997</td>
<td>Live newborns</td>
<td>Persistence of IgG until one year of age</td>
<td>Social workers, Paediatricians, Neonatologists</td>
<td>Annually</td>
<td>Annually</td>
<td>National Health Institute, physicians, Regional Public Health Surveillance on Infectious Diseases</td>
</tr>
</tbody>
</table>

**Surveillance systems specifically dedicated to congenital toxoplasmosis**

- **Bulgaria**: NA All EU (notifiable disease) Physicians, Laboratories, Epidemiologists Annually Monthly and annually Ministry of Health, National Centre of Health Information, National Centre of Infectious and Parasitic Diseases
- **Cyprus**: 2004 All EU (notifiable disease) All registered medical practitioners Weekly Twice a year Physicians
- **Czech Republic**: 1970 All EU (notifiable disease) Epidemiologists Monthly Monthly and annually Epidemiologists, Physicians Laboratories
- **England and Wales**: 1975 All EU (notifiable disease) Toxoplasma Reference Unit Swansea Monthly Quarterly and annually Electronic distribution (http://www.hpa.org.uk/hpconf)
- **Estonia**: 1997 All EU (notifiable disease) General practitioners Laboratories Monthly Monthly and annually Health protection Inspectorate and Ministry of Social Affairs
- **Ireland**: 2004 All EU (notifiable disease) All registered medical practitioners laboratories Weekly and annually Weekly and annually Physicians, Public health departments and population (Website)
- **Latvia**: 1995 All EU (notifiable disease) Physicians, Epidemiologists Annually Monthly and annually Ministry of Health, Physicians
- **Lithuania**: 1992 All EU (notifiable disease) All registered medical practitioners Monthly and annually Monthly and annually Territorial healthcare institutions, Ministry of Health, European surveillance networks, WHO
- **Malta**: 2004 All EU (notifiable disease) Physicians Laboratories Continuous Weekly, monthly and annually Physicians, Ministry of Health, WHO
- **Poland**: 1962 All EU (notifiable disease) Physicians Occasionally Quarterly and annually Public administrations, research institutions sanitary stations
- **Scotland**: 1988 All EU (notifiable disease) Laboratories Continuous Available on Website Free access for all (on demand)
- **Slovakia**: 1975 All EU (notifiable disease) Physicians Monthly and annually Monthly and annually Physicians, Ministry of Health

**Surveillance systems dedicated to toxoplasmosis (congenital or not)**

- **Bulgaria**: NA All EU (notifiable disease) Physicians, Laboratories, Epidemiologists Annually Monthly and annually Ministry of Health, National Centre of Health Information, National Centre of Infectious and Parasitic Diseases
- **Cyprus**: 2004 All EU (notifiable disease) All registered medical practitioners Weekly Twice a year Physicians
- **Czech Republic**: 1970 All EU (notifiable disease) Epidemiologists Monthly Monthly and annually Epidemiologists, Physicians Laboratories
- **England and Wales**: 1975 All EU (notifiable disease) Toxoplasma Reference Unit Swansea Monthly Quarterly and annually Electronic distribution (http://www.hpa.org.uk/hpconf)
- **Estonia**: 1997 All EU (notifiable disease) General practitioners Laboratories Monthly Monthly and annually Health protection Inspectorate and Ministry of Social Affairs
- **Ireland**: 2004 All EU (notifiable disease) All registered medical practitioners laboratories Weekly and annually Weekly and annually Physicians, Public health departments and population (Website)
- **Latvia**: 1995 All EU (notifiable disease) Physicians, Epidemiologists Annually Monthly and annually Ministry of Health, Physicians
- **Lithuania**: 1992 All EU (notifiable disease) All registered medical practitioners Monthly and annually Monthly and annually Territorial healthcare institutions, Ministry of Health, European surveillance networks, WHO
- **Malta**: 2004 All EU (notifiable disease) Physicians Laboratories Continuous Weekly, monthly and annually Physicians, Ministry of Health, WHO
- **Poland**: 1962 All EU (notifiable disease) Physicians Occasionally Quarterly and annually Public administrations, research institutions sanitary stations
- **Scotland**: 1988 All EU (notifiable disease) Laboratories Continuous Available on Website Free access for all (on demand)
- **Slovakia**: 1975 All EU (notifiable disease) Physicians Monthly and annually Monthly and annually Physicians, Ministry of Health

**Notes:**
- NA: Not available; WHO: World Health Organization;
- Regional surveillance system (Campania country)
- Distinction between acquired and congenital toxoplasmosis since 1999
- Distinction between acquired and congenital toxoplasmosis since 1997

(Source: Benard et al, Eurosurveillance 2008; 13(4-6): p4)
Table 7.3: National public health policies and local routine practices to prevent CT in Europe 2005

<table>
<thead>
<tr>
<th>Country</th>
<th>Nationwide policies recommended</th>
<th>Public health insurance coverage</th>
<th>Local routine practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary prevention to pregnant women #</td>
<td>Screening policy</td>
<td>Rhythm</td>
</tr>
<tr>
<td>Albania</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>Information</td>
<td>Prenatal</td>
<td>Three monthly</td>
</tr>
<tr>
<td>Belgium</td>
<td>Leaflets</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Bosnia</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Bulgaria</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>No</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Croatia</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Cyprus</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>Information</td>
<td>Neonatal</td>
<td></td>
</tr>
<tr>
<td>England &amp; Wales</td>
<td>Information</td>
<td>No screening</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>No</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Information</td>
<td>Prenatal</td>
<td>Monthly</td>
</tr>
<tr>
<td>Germany</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>No</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>NA</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>No</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Information</td>
<td>Prenatal</td>
<td>Monthly</td>
</tr>
<tr>
<td>Latvia</td>
<td>No</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Lithuania</td>
<td>Leaflets</td>
<td>Prenatal</td>
<td>Twice birth</td>
</tr>
</tbody>
</table>
Table 7.3: National public health policies and local routine practices to prevent CT in Europe 2005

<table>
<thead>
<tr>
<th>Country</th>
<th>Nationwide policies recommended</th>
<th>Public health insurance coverage</th>
<th>Local routine practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary prevention to pregnant women #</td>
<td>Screening policy</td>
<td>Rhythm</td>
</tr>
<tr>
<td>Luxemburg</td>
<td>NA*</td>
<td>NA*</td>
<td></td>
</tr>
<tr>
<td>Macedonia</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Malta</td>
<td>Leaflets</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>Leaflets</td>
<td>No screening</td>
<td>1990</td>
</tr>
<tr>
<td>Norway</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>Leaflets</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>Information</td>
<td>No policy</td>
<td>2000</td>
</tr>
<tr>
<td>Romania</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Scotland</td>
<td>No</td>
<td>No screening</td>
<td></td>
</tr>
<tr>
<td>Serbia &amp; Montenegro</td>
<td>NA</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Slovakia</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Slovenia</td>
<td>Information</td>
<td>Prenatal</td>
<td>Three monthly</td>
</tr>
<tr>
<td>Spain</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>NA</td>
<td>No policy</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>Information</td>
<td>No policy</td>
<td></td>
</tr>
</tbody>
</table>

* Refused to participate in study; NA, No answer
# Leaflets, written information on congenital toxoplasmosis given during the obstetric visit; Information referred to oral information only

(Source: EUROTOXO-panel 3-Version 12/12/2005)
CHAPTER 8 - Susceptibility of pregnant women in Ireland to toxoplasma infection and proposal for national newborn screening for congenital toxoplasmosis

8.1 Introduction
The subject of this chapter is a report on a previous study that was performed to determine toxoplasma seroprevalence in women of child bearing age in order to support a proposal for newborn screening for CT in Ireland (Ferguson et al., 2008).

The incidence of CT in Ireland was previously not known. The overall incidence of CT in Western Europe is low, with 1 to 5 cases per 10,000 live births reported (Benard et al., 2008). Due to the fact that the avirulent type II strain of *T. gondii* predominates in Europe, CT in European countries is associated with a low rate of severe disease and a good outcome in cases detected and treated early (Peyron et al., 2006;Gilbert et al., 2008). However, most countries in Europe that have reported on CT employ antenatal screening and treatment, which also influences incidence and outcome.

In the absence of antenatal screening, the incidence of CT in Ireland could only be determined by newborn screening which most importantly detects the asymptomatic cases that comprise the majority of infants with CT in Europe. The incidence of CT will be influenced by various factors including the prevalence of immunity among women of childbearing age and the rate of acquisition of primary infection in pregnancy (Kravetz et al., 2005;Levine, 2006).

Prior to an application for national newborn screening for CT, an estimate of the seroprevalence of toxoplasma antibody in pregnant women in Ireland was first required in order to determine the potential risk for pregnancy seroconversion. Hence in 1996, a study was undertaken in Ireland to determine the prevalence of toxoplasma antibody in women of childbearing age and hence estimate the risk for primary toxoplasmosis in pregnancy.

As detection of toxoplasma antibodies in neonatal blood reflects maternal exposure, maternal antibody seroprevalence was determined by anonymised testing of residual blood from newborn metabolic screening cards.
8.2 Objectives
This background study prior to newborn screening for CT aimed to a) determine the seroprevalence of toxoplasma antibody in women of child-bearing age and hence b) estimate the susceptibility of pregnant women to toxoplasma infection in order to c) support an application to fund a pilot newborn screening programme for CT in Ireland.

8.3 Methods

8.3.1 The seroprevalence study
The prevalence of toxoplasma antibody in the pregnant population would be determined by testing of newborn heel blood routinely collected for metabolic screening on filter paper cards. This testing would be performed at the Microbiology Laboratory at the Rotunda Hospital Dublin.

8.3.2 Estimation of seroprevalence in pregnant women
A random sample of DBS was selected from newborn screening cards referred to NNBSL throughout 1996. The sampling method was anonymous and unlinked. To estimate geographical variation in seroprevalence throughout Ireland, when the screening cards were sampled, the DBS were placed into Petri dishes labelled with the names of the individual counties as recorded on the screening card. Blood was eluted from each spot and a modified latex agglutination test (Eiken) was used to detect all classes of toxoplasma antibody (Parker et al., 1992). Simulated positive and negative DBS were included as controls.

8.3.3 Geographical analysis of seroprevalence
The seroprevalence of toxoplasma antibody was estimated for the whole country and for each county. The data was analysed for county of maternal residence by 95% confidence intervals and into tertiles of lowest, medium and highest seroprevalence.

8.3.4 Analysis of maternal age and seroprevalence by county
Data on maternal age for each county was compiled by the Central Statistics Office (CSO) of Ireland for 1996, and the influence of average maternal age in each county in relation to seroprevalence was assessed.
8.3.5 Sensitivity and specificity of the modified Eiken latex agglutination test
Sensitivity and specificity of the modified Eiken latex agglutination test were calculated. Following completion of the seroprevalence study, a selection of both positive and negative DBS were submitted to the Toxoplasma Reference Laboratory at St. George's Hospital, Tooting, London for testing. The method used was a one well Sabin Feldman dye test (SFDT) with a level of sensitivity of approximately 31 international units per millilitre (IU/mL). The DBS used for the DT were selected from 10,000 DBS spots stored from the second half of the seroprevalence study as follows:

1) Positive group: with a positive rate of 24.64%, 963 specimens will give 95% confidence +/- 2%. Every third specimen was selected, followed by every 10th until 1,000 spots were selected.
2) Negative Group: with a negative rate of 75.36%, 1,112 DBS will give 95% confidence +/- 1%. Every seventh negative was selected initially then every 20th until 1,112 spots had been selected.

8.3.6 Proposal for pilot newborn screening for CT and application for funding
A full report of the results from the seroprevalence study was compiled for a proposal for a pilot period of national newborn screening for CT and an application was made to the Department of Health and Children (DoHC) for funding.

8.4 Results
8.4.1 Seroprevalence data
A total of 20,252 newborn bloodspot cards were tested which represented 40.2% of the 50,390 registered live births in 1996. A total of 4,991 DBS were positive for toxoplasma antibody, representing 24.64% of the screened population (95% confidence interval: 24.1 - 25.2). The antibody seroprevalence of approximately 25% indicated that 75% remained susceptible to toxoplasma infection in pregnancy.

8.4.2 Geographical variation in seroprevalence
Table 8.1 details the total number of births registered in each county, the number screened, the seroprevalence by county and the 95% confidence intervals. The sample ranged from 29.98% to 53.36% of births registered in each county. The
highest number of births registered was in county Dublin (15,215) from which 42.35% were sampled. The lowest birth rate was in county Leitrim (280 births) from which 32.64% of newborn cards were sampled.

Analysis of the data by confidence intervals demonstrated that the seroprevalence in one county, Dublin, was below the mean and six counties had rates above the mean. The geographical distribution of counties with rates below or above the mean is shown in figure 8.1. Analysis of the results by tertiles of lowest, medium and highest seroprevalence demonstrated that lower seroprevalence tended to be in counties in the southern and eastern parts of the country and higher seroprevalence rates were in the northerly half of the country. One county, Longford, had a rate of 41.29% which was higher than the rate in the rest of the country and was double the rate observed in county Dublin, for which the rate was 19.92%.

8.4.3 Maternal age and seropositivity
The average maternal age did not differ significantly between counties. The average maternal age in relation to descending seropositivity is shown in table 8.2. Excluding Dublin, the lowest seroprevalence was in counties Waterford and Louth with seropositivity of 21.13% and 22.30% respectively. Data for Waterford and Louth had tight confidence intervals reflecting the percentage of registered births which were sampled. The average maternal age was not significantly different between Waterford and Louth (average maternal age 29.03 years and 28.68 years) when compared with Longford (average maternal age 29.50 years).

8.4.4 Sensitivity and specificity of the modified Eiken test
The sensitivity of the modified Eiken test employed for the seroprevalence study was 98.39% and the specificity was 84.42%. With the Eiken test, every 202 per 1,000 DBS that screened positive were false positives by the DT, and 11 of 1,123 negative screens were false negatives by the DT. The false positive rate of the Eiken test was calculated at 15.37% and the false negative rate was 1.36%. The likelihood ratio for a positive test was 6.42 and the likelihood ratio for a negative test was 0.02.
8.5 Discussion

8.5.1 Seroprevalence data and implications
This study demonstrated that in Ireland, approximately 75% of pregnant women were toxoplasma non-immune and potentially at risk for seroconversion in pregnancy. This study to date has been the only large report and analysis of toxoplasma seroprevalence in women of childbearing age in Ireland (Ferguson et al., 2008).

8.5.2 Geographical variation in seroprevalence
The geographical variation in seroprevalence suggested that the risk of exposure to toxoplasma varied in different parts of Ireland (figure 8.1 and table 8.1). Seropositivity rate in Dublin was below the mean and the rates in six counties, Longford, Westmeath, Cavan, Donegal, Meath and Mayo were above the national mean. The low rate in Dublin was expected and consistent with urban versus rural differences in seroprevalence (Carellos et al., 2014). Areas of highest seroprevalence were mainly contiguous and concentrated in the northern half of the country.

8.5.3 Maternal age and seropositivity rates
Toxoplasma seropositivity has been shown to increase with age (Remington, 2011). In this study, as the data was county agglomerated averages on age and serostatus, conclusions about the possible association between maternal age and variation in seroprevalence by county could not be made. Such conclusions can only be made based on individual results of age and seropositivity.

8.5.4 Sensitivity and specificity of the modified Eiken test
The modified Eiken latex agglutination method was chosen for DBS screening in this study because it was able to detect total toxoplasma antibody, was simple to perform and the procedures could readily be semi-automated making it ideal for testing large numbers of DBS samples. The sensitivity of 98.39% and the specificity of 84.42% were similar to those in previous reports (Patel et al., 1994). A false positive rate of 15.37% was acceptable for a screening test whilst a false negative by this method was uncommon with a likelihood ratio for a false negative test of 0.02.
8.5.5 Anticipated rate of CT in Ireland
Previous studies have estimated the incidence of CT in Ireland. In 1987, Coffey et al estimated that the rate of all congenital infections in a cohort of pregnant women attending a Dublin maternity hospital was 2 per 1,000 live births (Coffey et al., 1987).

A study that included Wales and Scotland, both with a similar population to Ireland, yielded antenatal seropositivity rates comparable to Ireland, with anticipated rates of congenital infection of 2 per 1,000 (Joynson, 2003).

A study by Gilbert et al performed between 2002 and 2004 reported that the incidence of symptomatic CT due to congenital and postnatally acquired infection in Ireland and the UK was 0.16 per 10,000 (Gilbert et al., 2006). However, less than 10% of congenitally infected infants are symptomatic.

8.5.6 Congenital toxoplasmosis in Ireland
The incidence of CT, and the severity and prognosis in Ireland was unknown. There was no information on the incidence of asymptomatic CT and outcome. Cases of CT reported to the Health Protection Surveillance Centre (HPSC) in Ireland for the purpose of surveillance are primarily infants who are symptomatic at birth or children incidentally identified with ocular toxoplasmosis or other signs (available at [http://www.hpsc.ie/AZ/Zoonotic/Toxoplasmosis/EpidemiologicalData/AnnualReports](http://www.hpsc.ie/AZ/Zoonotic/Toxoplasmosis/EpidemiologicalData/AnnualReports)).

8.5.7 Proposal for national newborn screening for CT in Ireland
The low seroprevalence of toxoplasma antibody amongst women of childbearing age in Ireland suggested that the majority remained susceptible to seroconversion in pregnancy with the possibility of infant congenital infection.

The true rate of CT in a population can only be determined by antenatal or postnatal screening. Newborn screening in most other European countries and regions are linked to antenatal screening programmes. In Ireland, isolated screening of the newborn population was more feasible than antenatal screening as constraints in health care resources and funding could not support development of antenatal screening even for a limited time period. It was less costly to initiate newborn screening for CT using DBS routinely collected for a pre-existing newborn metabolic screening programme. To assess the possibility of mass
screening of newborn filter paper cards for CT using toxoplasma IgM, the Irish toxoplasma working group reviewed data from other European countries that had performed newborn screening using this method.

8.5.7.1 Newborn screening for CT in Poland
In Poland, newborn screening was performed at a regional level in Poznan for two years and four months. Postnatal screening of DBS for toxoplasma-specific IgM using an ELISA assay, and if positive further analysis of DBS for IgG and IgA antibodies was performed. An incidence of one per 2,000 live births was reported. The sensitivity of the IgM assay was 86% (Paul et al., 2000).

Screening in Ireland could not be based on the Polish programme as the population characteristics in Poznan were dissimilar to that of Ireland. In addition the newborn screening tests used in Poland were not available or feasible for mass screening in Ireland.

8.5.7.2 National newborn screening for CT in Denmark
Denmark was the only country in Europe that embarked on a stand-alone nationwide screening programme for CT unlinked to antenatal screening. Subsequent to the Irish seroprevalence study in 1996, studies performed in Denmark to determine toxoplasma seroprevalence and the feasibility of newborn screening for CT using filter paper cards were published.

The first study, published in 1999, was a pre-screening study in Denmark of a similar purpose to the Irish study in 1996 to determine seroprevalence in pregnant women aimed at supporting newborn screening for CT. The prevalence of toxoplasma antibody in women of childbearing age in Denmark was 28% (Lebech et al., 1999).

In a second study, retrospective analysis of newborn filter paper cards from children with known CT was performed to determine the rate of recovery of toxoplasma IgM in a population without antenatal screening and treatment. The study published in 2002 demonstrated that in newborns with CT, testing of DBS for IgM within 10 days of birth would yield a false positive rate of 0.2 per 1,000 and no false negatives. Screening would identify approximately 75% of congenital infections in untreated mothers (Sorensen et al., 2002). The study concluded that
with low rates of false positivity and false negativity, newborn screening was feasible in areas with low seroprevalence, particularly with large populations.

Nationwide newborn screening for CT in Denmark was initiated in 1999 and continued for nine years until 2007. Screening utilised DBS card eluates obtained from newborns and samples were analysed for toxoplasma-specific IgM antibodies. Confirmatory serology was performed on infants and their mothers where infection was suspected.

The newborn screening programme in Denmark was ongoing in 2003 when the proposal for screening in Ireland was put forward to the DoHC. Population demographics for Denmark and Ireland were similar. Denmark was a setting with a low toxoplasma seroprevalence of 28% amongst women of childbearing age, comparable with the seroprevalence of 25% in Ireland. It was clear that newborn screening using routinely collected infant heel blood for detection of IgM was simple and feasible in Denmark. Thus, the newborn screening programme for CT in Ireland would be based on the Danish screening protocol.

8.5.8 Application to the DoHC to fund newborn screening for CT

8.5.8.1 Research proposal, ethical approval and funding
With approximately 75% of women of childbearing age found to be *T. gondii* non-immune, it was desirable to determine the true incidence and outcome of CT in Ireland. As demonstrated by newborn screening programmes elsewhere, the detection of specific IgM on neonatal bloodspots was feasible, moderately sensitive, highly specific and low cost (Guerina et al., 1994; Lebech et al., 1995). The findings in the seroprevalence study performed in Ireland in 1996 were included in a proposal to the DoHC in 2003 to provide funding for a pilot study of newborn screening for CT. The objectives of the pilot study were to determine:

1) The feasibility of newborn screening for toxoplasma IgM using DBS;
2) The incidence of congenital toxoplasmosis;
3) The outcome of infected infants to early treatment and intervention, determined by follow up during the first decade of life.

Ethical approval for linked newborn screening for CT was obtained from the ethics committee at The Children’s University Hospital (TCUH) Dublin. The proposal was
then submitted by the Consultant Microbiologist and the Director of the National Newborn Bloodspot Screening Laboratory (NNBSL) at TCUH to the Secretary Manager of the hospital and thereafter to the DoHC. Funding was subsequently provided by the DoHC under the area of “Prevention of Handicap”, for a 2-year screening programme. Some additional funding for the study was provided by the Child Health Foundation at TCUH Dublin. Following granting of funding, the toxoplasma working group for project implementation comprised: a consultant chemical pathologist (director of the NNBSL), a consultant microbiologist and a paediatric infectious diseases (ID) consultant. Testing of DBS would be performed at the NNBSL at TCUH, Dublin. The programme was initiated on July 1st 2005 following two years of necessary preparatory ground-work.

8.6 Conclusions

The seroprevalence of toxoplasma antibody amongst women of childbearing age in Ireland was 25%, which implied that 75% of pregnant women remained susceptible to primary infection in pregnancy. The results of this study supported a proposal to the DoHC for newborn screening for CT and approval and funding were granted.
Figure 8.1: Counties in Ireland with toxoplasma seroprevalence above or below national average

<table>
<thead>
<tr>
<th>County</th>
<th>Screen Positive Births</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longford</td>
<td>41.3%</td>
<td>[33.5 – 49.5]</td>
</tr>
<tr>
<td>Westmeath</td>
<td>32.8%</td>
<td>[27.4 – 35.7]</td>
</tr>
<tr>
<td>Cavan</td>
<td>30.9%</td>
<td>[25.8 – 38.4]</td>
</tr>
<tr>
<td>Meath</td>
<td>30.3%</td>
<td>[25.8 – 33.2]</td>
</tr>
<tr>
<td>Donegal</td>
<td>30.0%</td>
<td>[26.3 – 33.8]</td>
</tr>
<tr>
<td>Mayo</td>
<td>29.1%</td>
<td>[25.4 – 33.4]</td>
</tr>
<tr>
<td>Dublin</td>
<td>19.9%</td>
<td>[19.0 – 20.9]</td>
</tr>
</tbody>
</table>
**Table 8.1:** Number of births registered per county, number screened and percentage positive, with 95% confidence intervals in each county

<table>
<thead>
<tr>
<th>County</th>
<th>No. of Births</th>
<th>No. Screened</th>
<th>% of Births Screened</th>
<th>% Positive</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork</td>
<td>5849</td>
<td>2313</td>
<td>39.54</td>
<td>25.46</td>
<td>23.7 - 27.2</td>
</tr>
<tr>
<td>Carlow</td>
<td>605</td>
<td>219</td>
<td>36.19</td>
<td>30.13</td>
<td>24.1 - 36.2</td>
</tr>
<tr>
<td>Clare</td>
<td>1215</td>
<td>430</td>
<td>35.39</td>
<td>25.81</td>
<td>21.7 - 30.0</td>
</tr>
<tr>
<td>Cavan</td>
<td>708</td>
<td>212</td>
<td>29.94</td>
<td>32.07</td>
<td>25.8 - 38.4</td>
</tr>
<tr>
<td>Dublin</td>
<td>15215</td>
<td>6444</td>
<td>42.35</td>
<td>19.92</td>
<td>19.0 - 20.9</td>
</tr>
<tr>
<td>Donegal</td>
<td>1746</td>
<td>582</td>
<td>33.30</td>
<td>30.06</td>
<td>26.3 - 33.8</td>
</tr>
<tr>
<td>Galway</td>
<td>2534</td>
<td>993</td>
<td>39.18</td>
<td>26.18</td>
<td>23.4 - 28.9</td>
</tr>
<tr>
<td>Kildare</td>
<td>2189</td>
<td>926</td>
<td>42.30</td>
<td>25.48</td>
<td>22.7 - 28.3</td>
</tr>
<tr>
<td>Kilkenny</td>
<td>956</td>
<td>362</td>
<td>37.86</td>
<td>28.17</td>
<td>23.5 - 32.8</td>
</tr>
<tr>
<td>Kerry</td>
<td>1547</td>
<td>616</td>
<td>39.87</td>
<td>24.51</td>
<td>21.1 - 27.9</td>
</tr>
<tr>
<td>Longford</td>
<td>411</td>
<td>155</td>
<td>37.71</td>
<td>41.29</td>
<td>33.5 - 49.5</td>
</tr>
<tr>
<td>Louth</td>
<td>1224</td>
<td>529</td>
<td>43.21</td>
<td>22.30</td>
<td>18.8 - 25.9</td>
</tr>
<tr>
<td>Limerick</td>
<td>2463</td>
<td>1055</td>
<td>42.83</td>
<td>27.77</td>
<td>25.1 - 30.5</td>
</tr>
<tr>
<td>Laois</td>
<td>712</td>
<td>281</td>
<td>38.97</td>
<td>27.04</td>
<td>21.9 - 32.2</td>
</tr>
<tr>
<td>Leitrim</td>
<td>280</td>
<td>97</td>
<td>34.64</td>
<td>30.92</td>
<td>21.9 - 41.1</td>
</tr>
<tr>
<td>Meath</td>
<td>1470</td>
<td>580</td>
<td>39.45</td>
<td>29.48</td>
<td>25.8 - 33.2</td>
</tr>
<tr>
<td>Mayo</td>
<td>1377</td>
<td>503</td>
<td>36.52</td>
<td>29.42</td>
<td>25.4 - 33.4</td>
</tr>
<tr>
<td>Monaghan</td>
<td>634</td>
<td>240</td>
<td>37.85</td>
<td>23.75</td>
<td>18.4 - 29.1</td>
</tr>
<tr>
<td>Offaly</td>
<td>829</td>
<td>296</td>
<td>37.70</td>
<td>29.39</td>
<td>24.2 - 34.6</td>
</tr>
<tr>
<td>Roscommon</td>
<td>517</td>
<td>166</td>
<td>32.10</td>
<td>30.72</td>
<td>23.8 - 38.3</td>
</tr>
<tr>
<td>Sligo</td>
<td>730</td>
<td>295</td>
<td>40.41</td>
<td>27.21</td>
<td>22.0 - 32.2</td>
</tr>
<tr>
<td>Tipperary</td>
<td>1822</td>
<td>699</td>
<td>38.86</td>
<td>27.18</td>
<td>23.9 - 30.5</td>
</tr>
<tr>
<td>Waterford</td>
<td>1433</td>
<td>511</td>
<td>35.65</td>
<td>21.13</td>
<td>17.7 - 24.7</td>
</tr>
<tr>
<td>Westmeath</td>
<td>959</td>
<td>449</td>
<td>46.80</td>
<td>31.40</td>
<td>27.4 - 35.7</td>
</tr>
<tr>
<td>Wicklow</td>
<td>1475</td>
<td>686</td>
<td>46.50</td>
<td>24.34</td>
<td>21.1 - 27.4</td>
</tr>
<tr>
<td>Wexford</td>
<td>1490</td>
<td>613</td>
<td>41.14</td>
<td>27.40</td>
<td>23.9 - 30.9</td>
</tr>
</tbody>
</table>
Table 8.2. Percentage positive in each county & mean maternal age

<table>
<thead>
<tr>
<th>County</th>
<th>% Positive (descending)</th>
<th>Mean maternal age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longford</td>
<td>41.29</td>
<td>29.50</td>
</tr>
<tr>
<td>Westmeath</td>
<td>31.40</td>
<td>28.92</td>
</tr>
<tr>
<td>Leitrim</td>
<td>30.92</td>
<td>30.37</td>
</tr>
<tr>
<td>Roscommon</td>
<td>30.72</td>
<td>30.74</td>
</tr>
<tr>
<td>Carlow</td>
<td>30.13</td>
<td>28.71</td>
</tr>
<tr>
<td>Cavan</td>
<td>30.07</td>
<td>30.14</td>
</tr>
<tr>
<td>Donegal</td>
<td>30.06</td>
<td>29.11</td>
</tr>
<tr>
<td>Meath</td>
<td>29.48</td>
<td>30.31</td>
</tr>
<tr>
<td>Mayo</td>
<td>29.42</td>
<td>30.33</td>
</tr>
<tr>
<td>Offaly</td>
<td>29.39</td>
<td>29.16</td>
</tr>
<tr>
<td>Kilkenny</td>
<td>28.17</td>
<td>30.17</td>
</tr>
<tr>
<td>Limerick</td>
<td>27.77</td>
<td>29.30</td>
</tr>
<tr>
<td>Wexford</td>
<td>27.40</td>
<td>29.00</td>
</tr>
<tr>
<td>Sligo</td>
<td>27.21</td>
<td>30.17</td>
</tr>
<tr>
<td>Tipperary</td>
<td>27.18</td>
<td>29.19</td>
</tr>
<tr>
<td>Laois</td>
<td>27.04</td>
<td>29.52</td>
</tr>
<tr>
<td>Galway</td>
<td>26.18</td>
<td>30.56</td>
</tr>
<tr>
<td>Clare</td>
<td>25.81</td>
<td>29.90</td>
</tr>
<tr>
<td>Kildare</td>
<td>25.48</td>
<td>29.46</td>
</tr>
<tr>
<td>Cork</td>
<td>25.46</td>
<td>29.83</td>
</tr>
<tr>
<td>Kerry</td>
<td>24.51</td>
<td>29.89</td>
</tr>
<tr>
<td>Wicklow</td>
<td>24.34</td>
<td>29.40</td>
</tr>
<tr>
<td>Monaghan</td>
<td>23.75</td>
<td>29.11</td>
</tr>
<tr>
<td>Louth</td>
<td>22.30</td>
<td>28.68</td>
</tr>
<tr>
<td>Waterford</td>
<td>21.13</td>
<td>29.03</td>
</tr>
<tr>
<td>Dublin</td>
<td>19.92</td>
<td>29.24</td>
</tr>
</tbody>
</table>
CHAPTER 9 - Methods employed for implementation of a newborn screening programme for congenital toxoplasmosis in Ireland

9.1 Introduction
A preliminary toxoplasma seroprevalence study was undertaken in Ireland in 1996 and described previously (chapter 8) to support an application to fund a 2-year pilot study of newborn screening for CT (Ferguson et al., 2008).

Prior to implementation of the programme, approximately two years of preparatory ground work was required to research and test methods in order to decide upon the most feasible and practical approach for mass screening of the newborn population. Arrangements with a toxoplasma reference laboratory were necessary for serological confirmation of screen positive cases. A management protocol for infants with confirmed congenital infection was devised based on existing best practice recommendations at the time. Initiation of the programme required education and training of relevant personnel plus nationwide dispatch of information on the programme and follow-up protocol for screen positive infants and those with confirmed CT.

9.2 Objectives
To describe 1) methods and protocols employed for implementation and execution of a national newborn screening programme for CT and 2) challenges encountered.

9.3 Methods

9.3.1 The steering committee for implementation of the CT screening programme
The national pilot study of newborn screening for CT (referred to hereafter as the CT screening programme) was implemented in conjunction with the NNBSL located at TCUH Dublin and was funded by the DoHC.

The CT screening programme was implemented, directed, supervised and coordinated by a steering committee comprising five members as follows: the director of the NNBSL, (a consultant chemical pathologist); two programme supervisors, (a consultant microbiologist and a consultant ID paediatrician); the CT
screening programme co-ordinator, (then a paediatric ID non-consultant hospital doctor); the programme scientist (a medical scientist at the NNBSL who performed the screening tests and quality controls).

Prior to initiation of the CT programme, the steering committee agreed the following: 1) the definition of CT, 2) study methodology and inclusion and exclusion criteria, 3) methods for community and hospital medical staff education and programme implementation, 4) laboratory screening methodology and arrangements for infant and mother recall, 5) arrangements with the Toxoplasma Reference Laboratory (TRL) at Singleton Hospital Swansea UK for confirmatory and additional toxoplasma specific laboratory investigations if necessary, and 6) arrangements for evaluation, treatment and follow-up of infants with positive serology.

9.3.1.1 The role of the CT screening programme co-ordinator
The CT programme co-ordinator had both clinical and non-clinical roles prior to initiation of the screening programme and during the recruitment, treatment and follow-up of infants detected.

Prior to programme initiation, the co-ordinator sent a standard letter to all consultant paediatricians, directors of midwifery and public health nationwide to outline the screening project. The co-ordinator designed the leaflets and consent forms necessary for the study, ensured all units were supplied with same and accepted queries from clinicians and public health staff in relation to programme initiation.

The overall role of the programme co-ordinator following programme initiation was to:

1) Accept positive screening results, co-ordinate and facilitate confirmatory mother/infant serology;
2) Accept and assist with interpretation of results from the TRL, communicate confirmatory results with parents and clinicians;
3) Co-ordinate serial confirmatory testing and clinical evaluation with a local paediatrician if necessary to out rule CT in false positive cases;
4) Manage confirmed positive cases;
5) Facilitate elective admission for evaluation and treatment initiation, meet with parents during elective admission;
6) Facilitate infant monitoring for toxicity whilst on treatment, acquire blood results, liaise with parents and service providers for treatment adjustments;
7) Provide follow-up clinical and developmental assessments with scheduled reviews at an ID clinic;
8) Co-ordinate the various aspects of clinical follow-up (ophthalmological, audiological, radiological, interventional, psychosocial);
9) Provide support and encouragement to parents to maximise compliance during treatment and follow-up.

**9.3.2 Definition of congenital toxoplasmosis**
According to international criteria, a case of CT is a fetus, newborn or infant aged less than one year with at least one of the following (Lebech et al., 1996; Stronati et al., 2003)

1) *T. gondii* in body tissues or fluids by PCR, inoculation of mice, cell culture or immunocytochemistry
2) Specific IgM or IgA antibodies demonstrable and persistent in infancy
3) Specific IgG antibodies within the first 12 months of life plus other serological or clinical features that define CT
4) Persistent IgG positivity up to and beyond one year of age

Severe CT was defined by the presence at birth of at least one of the following signs: microphthalmia, hydrocephaly, microcephaly, seizures, ≥ 3 cerebral calcifications, extensive visual impairment (Olariu et al., 2011; Remington, 2011).

**9.3.3 Study methodology and inclusion and exclusion criteria**
All newborn infants were eligible for inclusion. Testing was linked to the existing National Newborn Screening Programme. A specific parental consent form with “opt-out” was developed by the programme co-ordinator and provided to all centres at which newborn screening took place. In addition, a concise information leaflet was devised specifically for provision to parents/guardians at the time of heel sample collection; this was attached to the consent form (Appendix 1). DBS on a filter paper card (previously referred to as the Guthrie card) were collected from the newborn infants’ heel at 72-120 hours of age as routine procedure, but in addition were tested for toxoplasma IgM antibody. DBS sampling
was deferred in infants who had blood transfusion prior to 72 hours of age; these infants were tested 72 hours post transfusion.

Premature infants who routinely require serial filter paper cards tested for metabolic screening until establishment of full feeds were only screened once for CT on the first card and not on each repeat card, as detection of toxoplasmosis was not dependant on feeding status. For CT screening, collection of additional heel blood from infants was not required; residual blood from the filter paper card was used so that testing of the five other conditions routinely screened for was not compromised.

9.3.4 Methods for community and hospital medical staff education and programme implementation

Initiation of the programme was preceded by eight weeks of education and training of staff responsible for collecting heel blood samples. Workshops were held and lectures were delivered nationwide by the director of the NNBSL to public health staff and newborn screening liaison midwives.

In addition, the programme co-ordinator developed an information leaflet for health care professionals (Appendix 2) and distributed these nationwide to staff involved in the collection of the heel samples and in the follow-up of those infants with an initial positive screening test. Targeted staff included: directors of midwifery, screening liaison midwives at maternity units, directors of public health, all consultant paediatricians and consultant neonatologists, all consultant obstetricians, and directors of neonatal units. The information leaflet included the contact details for the CT screening programme co-ordinator, who dealt with all queries prior to programme initiation.

9.3.5 Laboratory methodology: screening of dried blood spots for toxoplasma IgM

9.3.5.1 Calibration of assays

From 2003 to 2004 prior to initiation of the programme, the laboratory methodology was set up and validated at the NNBSL Dublin. Calibration of assays and comparison with external quality controls were undertaken by the designated medical scientist at the NNBSL supervised by the director of the NNBSL. The
programme scientist was responsible for performing the screening assays on DBS during the two years of newborn screening and two other scientists were also trained to assist with screening tests. Two IgM screening tests were performed on DBS at the newborn screening laboratory, the AutoDELFIA IgM and ISAGA IgM (Appendices 3 and 4).

9.3.5.2 The AutoDELFIA IgM assay
The first screening test performed on DBS was the quantitative AutoDELFIA® IgM assay using a commercial kit (PerkinElmer Life and Analytical Sciences) reported to have a sensitivity of 100% and a specificity of 96.5% (Appendix 3). This fluoroimmunometric assay is specifically designed to detect toxoplasma IgM antibodies in DBS on filter paper and it’s use is well documented (Lebech et al., 1995; Sorensen et al., 2002). Factors that may affect the AutoDELFIA assay include improperly collected DBS and exposure to heat and humidity. The test may not detect very early or late congenital infection (Tan et al., 2009).

The AutoDELFIA IgM assay is divided into two phases. The first phase detects all IgM antibodies. In the second phase, IgM antibodies are incubated and labelled with *T. gondii* antigen. Labelled antibody is then placed in an enhancement solution where fluorescent chelates are formed with *T. gondii* specific IgM. Fluorescence is proportional to the concentration of *T. gondii* IgM in the DBS sample. For the CT Screening Programme, the AutoDELFIA test was performed in triplicate, i.e., three wells were used for each DBS tested and the mean value calculated.

The AutoDELFIA kit manufacturer recommended interpretation of results set at a predetermined threshold based on a population of newborn infants screened in Brazil as follows: normal i.e. a negative test < 11.5 IU/mL; borderline 11.5-23.1 IU/mL; toxoplasma IgM positive >23.1IU/mL (Appendix 3). The manufacturer recommended that values be used as a guide only and laboratories should establish their own reference range based on indigenous population demographics, prevalence of toxoplasmosis, incidence of CT or risk estimates for CT.

The Brazilian population is demographically dissimilar to the Irish population. Newborn infants in Brazil belong to a population with a higher incidence of CT than
Europe and thus are at increased risk of congenital infection. Hence it was necessary for the NNBSL to calibrate and adapt the AutoDELFIA assay to suit the Irish population.

A positive result for newborn screening in the Irish population was set at a threshold of ≥ 4 IU/mL, based on contemporaneous guidelines for its use in Denmark, a country with similar population demographics to Ireland and where newborn screening for CT had been in effect since 1999. If the mean of the three AutoDELFIA values was ≥ 4 IU/mL, a qualitative test was then performed on DBS using a toxoplasma ISAGA IgM commercial assay (bioMérieux®), to detect toxoplasma IgM antibodies.

9.3.5.3 The ISAGA IgM assay
The ISAGA IgM assay is a qualitative assay based on a 2-step reaction (Appendix 4). Human IgM antibodies in the DBS sample bind with anti-human IgM monoclonal antibodies in the test wells. Addition of toxoplasma organisms yields agglutination in a positive reaction, and if negative, toxoplasma sedimentation occurs. The ISAGA IgM assay is reported by the manufacturer to have 96.9% specificity (95% confidence interval: 92.7% - 98.7%) and its use for screening and confirmation of CT has been documented in previous studies (Pinon et al., 2001).

Results were interpreted using the ISAGA index as per the manufacturer’s recommendations as follows: 0-5, negative; 6-8, borderline; 9-12, positive; >12 strongly positive. Infants with a positive ISAGA IgM ≥ 9 were categorised as a positive screening result. Confirmatory venous serology was taken from all infants with a positive ISAGA IgM and parallel testing of maternal serology was performed.

9.3.5.4 Second adaptation of the AutoDELFIA result interpretation
The CT screening protocol was modified to incorporate a second adaptation of the AutoDELFIA test in September 2005, two months after screening had commenced. The director of the screening programme and the supervising consultant microbiologist upon review of results obtained for the first two months of screening, decided a second adaptation of the AutoDELFIA test was necessary to suit the Irish population.
Infants who had a mean positive AutoDELFIA ≥ 50 IU/mL with a negative ISAGA IgM were categorised as a ‘borderline’ screening result and a repeat filter paper card was obtained for retesting of DBS. If the repeat card demonstrated a persistently high AutoDELFIA ≥ 50 IU/mL with a negative ISAGA IgM, the screening result was labelled borderline and mother and infant were recalled for confirmatory serology. This adaptation was necessary as a high AutoDELFIA result ≥ 50 IU/mL may have been indicative of early infant seroconversion following maternal infection late in trimester 3. This modification enhanced the sensitivity of the screening programme with retention of overall specificity as all infants thus detected underwent serological testing. If the mean AutoDELFIA value obtained from the repeat card was < 50 IU/mL with a negative ISAGA IgM, the screening result was negative and no further confirmation was necessary. The screening protocol is summarised in figures 9.1 and 9.2.

9.3.6 Arrangements for mother-infant recall for confirmatory testing

All screen positive results were communicated to the CT screening programme co-ordinator who then directly contacted the relevant screening liaison midwife with details of serology samples to be taken for dispatch by courier to TCUH to ensure a rapid result.

Mother and infant were recalled by the liaison midwife for venous sampling. Arrangements were made by the screening liaison midwife for the mother-infant pair to attend their local paediatric out-patient unit for venous sampling. Confirmatory samples from all units were sent to the dispatch laboratory at TCUH, where they were prepared for transport, sent by courier to the TRL in Swansea and the programme co-ordinator was informed. The programme co-ordinator informed the Reference Laboratory of all samples in transit and requested urgent processing.

9.3.7 Confirmatory serology

9.3.7.1 Tests performed and result interpretation

Confirmatory mother-infant venous samples were all analysed at the TRL Swansea. The Director of the TRL (Consultant Clinical Scientist) interpreted all confirmatory results for the CT screening programme. Confirmatory tests performed on infant serology included:

1) The DT;
2) An ELISA assay for the detection of toxoplasma specific IgM;
3) ISAGA IgM;
4) ISAGA IgA.

Blood and cerebrospinal fluid (CSF) were tested for toxoplasma-specific PCR in infants with confirmed congenital infection.

Tests performed on maternal postnatal serology were the DT, ELISA and ISAGA IgM. Avidity testing was also performed if applicable to determine timing of infection. Where indicated, maternal stored serology from the first antenatal visit was requested by the programme co-ordinator for dispatch to the reference laboratory for analysis to determine timing of pregnancy seroconversion.

9.3.7.2 The Sabin Feldman dye test
The DT is generally accepted as being the ‘gold standard’ reference test for the serological detection of *T. gondii*-specific immunoglobulin. This was an in-house test at the TRL, which utilised live toxoplasma organisms extracted from mice cells and was calibrated against an international control serum. Levels of *T. gondii* specific immunoglobulin are expressed in IU/mL. The test is not diagnostic of congenital infection as it detects all classes of toxoplasma immunoglobulin which may be maternal in origin (Reiter-Owona et al., 1999). Hence reference laboratories use the dye test in combination with IgM and IgA assays to diagnose CT. The DT usually remains positive for life following congenital or acquired toxoplasmosis.

9.3.7.3 The ELISA IgM
The ELISA test for toxoplasma specific IgM was also an in-house test at the TRL. Results were reported in semi-quantitative terms ranging from ‘negative’ to ‘strongly positive’. The ELISA test can detect IgM for six to nine months after primary infection and detection up to 12 months has been reported (Petersen et al., 2005).

9.3.7.4 The ISAGA IgM assay
The ISAGA IgM assay used by the reference laboratory was the commercial kit (bioMérieux®) that was also used for the ISAGA IgM screening test at the NNBSL and the method has been previously described. The ISAGA IgM assay can usually detect residual levels of IgM for approximately 12-18 months after primary
infection and in some cases up to two years (Gras et al., 2004). The ISAGA IgM indices used by the reference laboratory for result interpretation were as per the manufacturer's recommendations and previously described. The manufacturer's advise that in the newborn period, a borderline ISAGA IgM index and even a result in the negative category with an index of $\geq 3$ and $\leq 5$ may indicate CT, and in such cases repeat infant serology is recommended three weeks later for comparison of antibody levels.

9.3.7.5 The ISAGA IgA assay
The ISAGA IgA assay used by the TRL was also a commercial kit manufactured by bioMérieux® (Appendix 5). The principle and method was the same as for the ISAGA IgM test previously described. The ISAGA IgA index for CT was read as per the manufacturer's guide for serological interpretation of CT: $< 3$, negative reaction; $\geq 6$, positive reaction; $\geq 3$ and $< 6$, indeterminate. An indeterminate or borderline index required confirmation, using serum collected from the infant 10 to 14 days later. The kit manufacturers established IgA sensitivity as 86.84% (95% confidence interval 71.91 - 95.59%) and specificity as 89.24% (95% confidence interval 83.31 - 93.24%). The ISAGA test can detect residual IgA for 12 to 18 months following infection.

9.3.7.6 The IgG avidity test
IgG avidity measures the binding capacity of toxoplasma IgG molecules to toxoplasma antigen which provides an estimation of duration of infection and the age of the patient's immune response to the parasite (Holliman et al., 1994; Pietkiewicz et al., 2007).

The IgG avidity test used at the reference laboratory was an in-house enzyme immunoassay system with the method described as follows. Toxoplasma antigen is bound directly onto two wells of a microtitre plate. Maternal serum is incubated in the wells, and unbound immunoglobulin washed off. One well is incubated with urea to enable some immune complex dissociation and the other well is incubated with phosphate buffered saline. The urea containing well is further washed to remove any antibody dissociated from toxoplasma antigen. The amount of bound antibody in the well is determined colourimetrically with an enzyme labelled antibody specific for human IgG. The avidity index is calculated from the ratios of
antibody bound after urea-treated and untreated serum (incubated in phosphate buffered saline only).

As the immune response to a pathogen develops, an adaptive process occurs whereby successive generations of IgG molecules bind more tightly to the target antigen than earlier infection, i.e., avidity increases. A low avidity index indicates recently acquired infection; a high index is indicative of less recent infection.

Interpretation of avidity indices vary according to the laboratory assay used. Most avidity assays can differentiate between infection greater than three months old or less than three months old. The avidity method employed at the TRL was able to differentiate (to 95% probability) between sera collected less than three months (index < 30% or < 0.3) or greater than six months (index > 40% or > 0.4) after primary infection.

9.3.7.7 The toxoplasma-specific PCR test
The PCR test for toxoplasma detection is a highly sensitive, specific and rapid method by which as little as a single tachyzoite can be detected in clinical specimens (Chabbert et al., 2004; Abdul-Ghani et al., 2011). The PCR method is based on detection of specific nucleic acid sequences and does not necessarily confirm the presence of viable cells or active infection as the test is unable to discriminate between the tachyzoite and bradyzoite form of the parasite. Nevertheless, PCR is helpful in confirming active toxoplasmosis by detection in a range of specimens where only the active form of the parasite will typically be found in a congenitally infected infant, e.g., cerebrospinal fluid (CSF) and ocular fluids (Torres et al., 2013). In the CT screening programme, PCR testing by the reference laboratory was employed for the investigation of neonatal parasitaemia in the blood and CSF of infants with confirmed congenital infection.

9.3.7.8 Western Blot analysis
Western Blot was employed as a diagnostic aid in the CT programme for cases of equivocal or borderline infant confirmatory serology, when serial serologic tests could not confirm or out-rule the diagnosis of CT. The test is 75% to 99% sensitive and is dependent on the production of toxoplasma-specific antibody in adequate quantities by the infant to enable structural comparison with toxoplasma antibodies in maternal serum (Nielsen et al., 2005; Franck et al., 2008).
Testing was employed only in cases where maternal seroconversion was proven and the infant antibody profile was equivocal, i.e., suggestive of, but uncharacteristic of congenital infection. Additional venous sampling was not required from mother and infant for WB testing, analysis was performed on samples previously acquired for confirmatory serological testing. For WB analysis, confirmatory samples were dispatched from the TRL at Swansea to the Scottish TRL and National Lyme Borrelosis testing Laboratory at Raigmore Hospital, Inverness, Scotland.

**9.3.8 Follow-up of confirmatory serology results**
Confirmatory serology results were available approximately five to 15 days (median seven days) after samples were dispatched to the TRL at Swansea. A scientist from the TRL delivered results via telephone directly to the screening programme co-ordinator who then promptly contacted parents. If the first confirmatory serological tests were inconclusive, the programme co-ordinator requested infant recall for repeat venous sampling and if necessary serial venous sampling was performed during infancy to confirm infant status.

**9.3.8.1 Screen positive cases with serological exclusion of CT (false positive screen)**
Screen positive cases with serological exclusion of CT on the toxoplasma antibody profile analysed by the TRL (false positive screening results) were not further evaluated. Parents were offered a final confirmatory test for the infant at approximately 12 months of age to demonstrate loss of maternal antibody where applicable.

A screen positive case was labelled as a false positive screening result if any of the following was demonstrated in mother-infant confirmatory serology (Stronati et al., 2003):

1) No evidence of toxoplasma infection in mother and infant;
2) Infant serology results consistent with passive transfer of maternal antibody only;
3) Failure of the newborn to demonstrate specific IgM or IgA antibodies;
4) Infant IgM present in the newborn period was undetectable by three months of age;
5) Infant IgG present in the newborn period was undetectable by one year of age. This classification is detailed in Chapter 11.

9.3.9 Evaluation of infants with confirmed positive serology

Children with serologically confirmed CT were assigned to a standard of care management protocol which was divided into three major categories: 1) clinical evaluation 2) treatment with toxoplasma specific antiprotozoal medication for 12 months and 3) clinical monitoring following completion of treatment.

All confirmed cases of CT were referred by the programme co-ordinator to the paediatric ID service at a tertiary children’s hospital, primarily Our Lady’s Children’s Hospital (OLCH) Dublin or TCUH Dublin if more geographically convenient. Infants were admitted electively to hospital for one week for full clinical evaluation under the care of the Paediatric ID service. Blood was drawn for toxoplasma PCR, full blood count (FBC), liver and renal function. CSF was analysed for cell count, protein, glucose and toxoplasma PCR. Infants underwent physical and neurological examination, brain imaging, dilated funduscopy examination and audiology testing.

9.3.10 Infant treatment and monitoring protocol

9.3.10.1 Treatment regimen

All confirmed positive infants were assigned to treatment with toxoplasma specific antiprotozoals; pyrimethamine and sulfadiazine. Treatment duration for CT varies internationally (McLeod et al., 2006; Roser et al., 2010). For the Irish CT screening programme, a 12-month treatment regimen was employed.

Treatment was initiated with pyrimethamine, sulfadiazine and folinic acid (calcium leucovorin) whilst infants were in-patients. Pyrimethamine 2 mgs per kilogram (kg) once daily was first administered as a loading dose for two days, followed by one mg/kg once daily for the first six months then thrice weekly for the remaining six months of therapy. Sulfadiazine was administered at 100 mg/kg daily in two divided doses for one year. Leucovorin was administered at a standard dose of 15 mgs thrice weekly, usually Monday Wednesday and Friday for the duration of treatment and for one week following treatment discontinuation.
Prednisone (0.5 mg/kg twice per day) was added if there was evidence of active chorioretinitis or if CSF protein was greater than one gram per decilitre (g/dL) and was continued until resolution of active chorioretinitis and normalisation of CSF protein.

The screening programme co-ordinator met with parents of infected infants whilst they were in-patients to discuss clinical evaluation, the treatment protocol, potential medication side effects and follow-up management. Infants were discharged when evaluation was complete and treatment was established.

Monthly supplies of antiprotozoal treatment for infants were dispensed by local pharmacies for the 12-month treatment period. The screening programme co-ordinator regularly liaised with dispensing pharmacies to provide prescription updates.

9.3.10.2 Treatment monitoring for adverse effects
Whilst on treatment, infants were closely monitored by the programme co-ordinator for toxicity, mainly bone marrow suppression manifesting as neutropenia. Infants attended their local paediatric out-patient or day-ward service for blood monitoring during treatment. Adverse events were managed with treatment adjustments.

FBC results for all infants were sent by fax to the screening programme co-ordinator who made contact with parents via telephone to discuss any treatment adjustments.

Whilst receiving treatment, infants attended the paediatric ID clinic at OLCH or TCUH Dublin every three months for clinical and developmental review by the screening programme co-ordinator. At these visits toxoplasma antibody profile, FBC, liver and renal function and urinalysis were checked.

9.3.11 Clinical follow-up protocol following treatment completion
Ophthalmology examinations were performed 3 to 6 monthly for the first 18 months, then 6 monthly up to age 3 years, annually thereafter and more frequently if necessary. Audiology testing was performed annually for the first three years of life
or more frequently if an abnormality was recorded, then as necessary thereafter. Infants who had intracranial lesions at birth had repeat brain imaging performed following treatment completion or sooner if necessary.

Following treatment completion, infants were reviewed by the screening programme co-ordinator for developmental progress 6 monthly for 3 years. Annual review was then offered up to the age of 10 years.

The programme co-ordinator was the direct point of contact and the liaison clinician for all service providers during the programme execution and decade of follow-up of infected children. The programme co-ordinator collated all clinical data including progress reports from all interventional services where relevant.

9.4 Results
The pilot programme of newborn screening for CT in Ireland was successfully implemented and commenced on July 1st 2005 and continued for a 24-month period to June 30th 2007.

Congenitally infected infants recruited during this time were managed and followed up clinically thereafter during the first decade of life as per protocol. It was not necessary to alter laboratory methods or clinical management during the programme based on new or updated guidelines. The results obtained from each sub-division of the CT screening programme are detailed separately in subsequent chapters.

9.5 Discussion

9.5.1 Delay with initiation of screening
Successful execution of the CT screening programme required co-ordinated input nationwide from: the NNBSL, public health and hospital staff involved in collection of newborn bloodspots, newborn screening liaison midwives for each maternity and paediatric unit, and paediatric teams. In addition, liaison between microbiology laboratory services, the dispatch laboratory at TCUH Dublin and the TRL at Swansea was necessary for timely transport of samples from Ireland to the Reference Laboratory for confirmatory analysis.
The steering committee made every effort to ensure that all relevant clinical and laboratory services in Ireland were educated and provided with written information at least six weeks before the programme commenced.

Despite this, there was a delay in uptake of screening by one of the three Dublin stand-alone maternity hospitals, the Coombe Women and Infant University Hospital (CWIUH) and the neonatal unit at Tralee General Hospital, County Kerry. This delay occurred because relevant personnel were unaware of the programme details and the initiation date. Literature delivered to the department directors in May was either not distributed or viewed in a timely manner by key personnel at these two units.

Department directors at both units requested a delay beyond July 1st to acquire clarification of details of the screening programme and ensure all their staff were adequately informed. In addition a tertiary paediatric hospital, OLCH in Dublin, delayed the initiation of screening because hospital management requested that the consent form include their hospital logo. Subsequently a general consent form, devised specifically for the CT screening programme with the NNBSL logo, was employed nationwide (Appendix 1). All maternity and paediatric units in Ireland had commenced newborn screening for CT by the third week of July 2005.

9.5.2 Timing of informed consent

All newborn infants were offered CT screening. Informed consent was obtained from parents at the time of heel blood collection. Parents were provided with a brief information leaflet which outlined CT and the screening programme (Appendix 1). Parental consent to inclusion in the screening study automatically consented the mother and infant to serologic confirmation if necessary.

Prior to the initiation of screening, a minority of directors of public health expressed concern and dissatisfaction with this method as they were strongly opposed to mothers in the immediate post partum period being asked to provide consent to a study. Their argument was that as women were emotionally vulnerable on days three to five post partum they should not be asked to provide consent for their infants to enter a study and the CT screening information should be provided antenatally rather than at the time of heel blood collection. The steering committee considered this and decided against prenatal information as provision of printed
information to all antenatal units was not a feasible option both technically and practically. This method would have been reliant on midwives or obstetricians ensuring that the leaflets were distributed to all pregnant women before and during the 24-month period.

CT screening was a nationwide programme. The steering committee was not prepared to undertake the task of requesting all maternity units to accept the responsibility of providing pregnant women with the information leaflets for CT screening, nor would this have been approved of by the directors of maternity units. Literature provided to pregnant women may not be read prior to delivery or recalled postnatally. The pilot programme involved screening of infants, and the steering committee concluded that the best method for ensuring informed choice was to provide the information at the time of routine heel blood collection.

9.5.3 Two-step screening protocol for recovery of toxoplasma IgM

Mass screening of blood samples collected on filter paper cards for the presence of toxoplasma antibody has been documented both historically and recently (Parker et al., 1992; Sorensen et al., 2002). The laboratory assays and 2-step screening protocol employed by the NNBSL were based on the strategy in use at the time for screening of the Danish population. Step one of the screening protocol (AutoDELFIA PerkinElmer), recovered IgM from DBS, as did the second or confirmatory step of the protocol (ISAGA bioMérieux®).

Both assays relied on production of IgM in the newborn. Approximately 30% of congenitally infected newborns produce negligible quantities of IgM, some studies report that this may be as high as 50% (Gilbert et al., 2007). In addition, both screening tests could potentially not detect infants infected very early or very late in-utero when IgM concentrations in infant sera are low (Boyer et al., 2011). The steering committee discussed that ideally for a 2-step toxoplasma screening protocol, the tests should differ.

A preliminary study performed prior to establishment of the Danish newborn screening programme for CT demonstrated that testing of newborn filter paper cards for toxoplasma-specific IgM antibodies would identify 70% to 80% of infected cases (Lebech et al., 1995). Other options for newborn CT screening tests
were not available or feasible for screening in the Irish population and therefore two IgM assays were employed.

A report on the Danish screening programme was published in 2006 after the first four years of screening; 1999 to 2002, which demonstrated that toxoplasma IgM was easily recoverable from heel blood using the bioMérieux® ISAGA IgM assay (Schmidt et al., 2006a). This report was available during the second year of the CT screening programme in Ireland. Thus whilst it was not an ideal situation to use two screening tests for IgM which could have potentially missed some infected infants, it was reassuring to the steering committee that at least 70% of infants with CT would be easily detected by screening.

9.5.4 Recall for repeat filter paper cards where necessary
The second adaptation for AutoDELFIA result interpretation applied to any infant with a mean AutoDELFIA ≥ 50 and a negative ISAGA IgM. This was classified as a borderline or equivocal screening result that first required recall for a repeat filter paper card and if a similar result was acquired on the repeat card, mother and infant were recalled for confirmatory serology.

In the normal process of events for routine metabolic screening, if for any reason an infant requires a repeat filter paper card taken, the screening liaison midwife at the infant’s hospital of birth would contact the parents for a repeat card. This method was also continued for toxoplasma screening. Recall for repeat cards for toxoplasma screening did not generate excess parental anxiety as parents perceived the heel test to be routine postnatal infant care and were made aware from the outset that repeat cards may be necessary for re-testing of any of the conditions screened for on heel blood. Repeat cards were taken at a neonatal clinic. Parents were then advised that they would be further contacted only if the result was abnormal and confirmatory serology was necessary.

9.5.5 Mother and infant recall for confirmatory venous sampling
A screen positive result was released from the NNBSL to the programme co-ordinator who informed the relevant screening liaison midwife who then contacted the parent. Hence the parent’s first point of contact for a positive screen result was the screening liaison midwife.
Recall of infants by the screening liaison midwife for mother and infant venous sampling proved to be more complex than recall for a repeat filter paper card, as the liaison midwife automatically acquired the responsibility of explaining the screen positive result and the necessity for confirmatory testing.

Many parents requested more detailed explanation of CT and the implications of a confirmed positive result. In those cases the programme co-ordinator made telephone contact with parents to clarify that a screen positive result was not diagnostic and required confirmation, as it was not possible to predict the likelihood of CT based on the screening result. Parental discussion of the screening results with the programme co-ordinator was necessary in some cases to alleviate parental anxiety until confirmatory results were available.

**9.5.6 Dispatch of confirmatory samples to the Toxoplasma Reference Laboratory Swansea UK**

All confirmatory samples were to be sent on the same day of acquisition by courier from local microbiology laboratories to the dispatch laboratory at TCUH Dublin. From there, samples were prepared for transport and delivered within 24 hours by courier to the TRL in Swansea.

Minor obstacles were encountered with the dispatch protocol during the 24 months of screening. Laboratory directors from two peripherally located microbiology units questioned the need for dispatch of confirmatory samples to the TRL for analysis. Both directors requested that samples should instead be tested at the National Virus Reference Laboratory Dublin which provides specialist serology testing. Lack of awareness of the screening programme methods and cost issues were the reasons quoted in both instances. Prior to initiation of the CT screening programme, the director of the NNBSL had written to all directors of hospital laboratories nationwide which outlined the screening programme and the necessary co-operation from local laboratories to courier without delay confirmatory samples to TCUH for subsequent dispatch to the TRL.

Resistance to this step of the programme was a further example which highlighted the fact that important information regarding the screening programme was either not received or not read by relevant personnel, as both situations arose when the screening programme was well underway.
It was necessary for the programme co-ordinator to reinforce that it was essential to maintain consistency and have all confirmatory samples tested in the same Reference Laboratory using the same assays with results interpreted by an expert in the field. The costs incurred from sample dispatch to Swansea and confirmatory tests were funded by the DoHC and not by local microbiology units. Hence cost issues were not reasons to prevent sample dispatch to the TRL in Swansea. Both unit directors subsequently agreed to co-operate with the confirmatory aspect of the protocol.

9.6 Conclusions
A newborn screening programme for CT was successfully implemented in Ireland by a steering committee and piloted for 24 months. Minor obstacles were encountered with programme initiation. Practical problems arose during the course of programme execution. However prompt resolution of all issues was possible and there was no cause for programme interruption during the 24-month pilot screening period.
Figure 9.1: Summary of the CT screening protocol
Infant recall for repeat filter paper card

Repeat card mean autoDELFIA ≥ 50 with negative ISAGA IgM

Borderline screen result

Mother and infant serology dispatched to reference laboratory

Confirmatory **Dye test, ELISA IgM, ISAGA IgA/IgM**, maternal avidity

Congenital toxoplasmosis confirmed or out-ruled

**Figure 9.2: The second adaptation of the AutoDELFIA assay for the CT screening protocol**
CHAPTER 10 - Results from 24 months of national newborn screening for congenital toxoplasmosis and incidence derived from these results

10.1 Introduction
A pilot programme of national newborn screening for CT in Ireland took place from July 1st 2005 to June 30th 2007. The screening programme was phase two of a project to determine the incidence of CT. Phase one of the project was a seroprevalence study which demonstrated that 25% of women of child bearing age in Ireland were toxoplasma immune, hence 75% of pregnant women remained susceptible to primary toxoplasma infection in pregnancy (Ferguson et al., 2008).
A low incidence of CT has been reported in Europe with overall rates of one to five per 10,000 quoted (Benard et al., 2008). Mass screening of newborn filter paper cards for CT has been shown to be feasible in Europe (Lebech et al., 1995; Sorensen et al., 2002) and North America (Guerina et al., 1994).

This chapter primarily serves to provide details of screening results. A summary of confirmatory serology is described to demonstrate derivation of the incidence of CT. Detailed analysis of confirmatory serology is provided in subsequent chapters which describe false positive and confirmed positive infants.

10.2 Objectives
1) To determine feasibility of newborn screening for CT by recovery of IgM from DBS on filter paper cards.
2) To determine the incidence of CT in Ireland.

10.3 Methods

10.3.1 Population studied
All infants born within the 24-month period from July 1st 2005 to June 30th 2007 inclusive were offered screening for CT.

10.3.2 Newborn screening methods
Screening was 'opt-out' with informed parental consent obtained. Newborn screening methods were detailed in chapter 9.

A two-step protocol was used for toxoplasma screening of heel blood routinely
collected from all newborns between 72 and 120 hours after birth. The initial screening step utilised the quantitative AutoDELFIA assay for testing of DBS. All samples which had a detectable IgM ≥ 4 IU/mL were repeated in duplicate. If the mean AutoDELFIA was ≥ 4 IU/mL, the second or confirmatory screening step was performed on DBS with the ISAGA IgM assay. Positive results were released to the CT programme co-ordinator. A negative screening result was entered on the laboratory information system along with the results of the routine metabolic screen and released to relevant paediatric units. Screen positive infants with ISAGA IgM positive, and those with a borderline screen result (AutoDELFIA ≥ 50 IU/mL and negative ISAGA IgM), were recalled for serological confirmation.

10.3.3 Confirmatory protocol
Confirmatory serologic tests were described in chapter 9. Paired mother infant serology were analysed for toxoplasma antibody profile at the TRL Swansea. Where indicated, retrospective analysis of stored maternal blood from the first antenatal visit was analysed.

10.4 Results

10.4.1 Total number screened
During the 24-month recruitment period, parental consent was sought for screening of 144,927 infants. In total, samples were collected from 144,564 newborns during the 24-month recruitment period; 363 parents (0.25%) opted out of toxoplasma screening.

The time from heel blood sampling to acquisition of a screen positive result ranged from 3 to 23 days; median 6 days.

10.4.2 AutoDELFIA IgM screening results
During the 24-month period, 299 samples from the total infant population screened (0.2% or two per 1,000) had a positive result on step one of the screening protocol with a detectable blood AutoDELFIA IgM ≥ 4 IU/mL. Positive AutoDELFIA concentrations ranged from 4 to 1,300 IU/mL (figure 10.1).
10.4.3 ISAGA IgM screening results
Thirty of 299 infants (10%) with a positive AutoDELFIA ≥ 4 IU/mL (median 435 IU/mL) were positive on step two of the screening protocol with toxoplasma ISAGA IgM indices in the positive range of > 9.

Four of 299 infants (1.3%) had a positive AutoDELFIA ≥ 50 IU/mL (median 178 IU/mL) with a negative ISAGA IgM on initial newborn screening and repeat filter paper card and were categorised as a borderline positive screening result.

Thus during the 2-year CT screening programme, a total of 34 out of 144,564 infants (0.02% or two in 10,000) had a screening result that required serologic confirmation.

10.4.4 Confirmatory serology
Confirmatory serology results from the TRL were available between 5 and 15 days following dispatch from Ireland, median 7 days. Table 10.1 details infant screening results with corresponding confirmatory mother-infant serology for the 34 mother-infant pairs listed in their temporal order of occurrence by infant date of birth. Results of retrospectively tested antenatal booking blood samples are also displayed. Paired mother-infant venous sampling confirmed congenital infection in 15 of 34 infants and 19 false positive cases were identified.

Of 34 screen positive infants, 11 were born to women who were seropositive prior to pregnancy. Nineteen infants were born to women who seroconverted in pregnancy, 15 were congenitally infected and 4 were not infected (table 10.1 case numbers 15, 16, 28 and 29). Four infants were born to women with no demonstrable toxoplasma antibody (table 10.1 case numbers 6, 8, 9 and 20). Hence of 34 screen positive infants, 44% were true positive cases of CT and 56% were false positive.

10.4.5 Summary of screen positive cases and venous confirmatory serology
For the total population of 144,564 screened, serologic confirmation was required in 0.02%. The results obtained from screening of DBS and confirmatory serology are summarised in figure 10.2 and table 10.2.
10.4.6 Demographics of screen positive cases

The highest number of screen positive cases, 17 of 34 (50%), were detected in the third six-month period of screening, i.e., July to December 2006 (figure 10.3). Birth rate nationally was highest for the fourth six-month period of screening; January to June 2007 (data not shown).

Of the 34 screen positive infants, 10 (29%) were born in County Dublin and 24 (71%) in other counties. Figure 10.4 displays location of 34 screen positive infants compared with geographical representation of the maternal seroprevalence study.

Thirty of 34 screen positive infants were born to women with toxoplasma antibody demonstrable antenatally and or postnatally; 8 of 30 mothers (27%) with demonstrable antibody were resident in county Dublin and 22 (73%) in counties outside Dublin. Eleven of 34 screen positive infants were born to women who were seropositive prior to pregnancy, one was resident in county Dublin and the remainder outside Dublin. For 20 of 34 infants (59%), the mother was born in Ireland (figure 10.5).

10.4.7 Incidence of CT and false positive rate

The incidence of CT (true positive rate) based on the 24-month screening period was approximately 1 in 10,000 newborns screened. The false positive rate for the population screened was 1.3 per 10,000.

10.4.8 Analysis of screening tests

10.4.8.1 The AutoDELFIA IgM screen

Table 10.3 demonstrates statistical analysis of the AutoDELFIA screening test based on the results obtained from the total population screened. The positive predictive value of the AutoDELFIA assay for the population screened was calculated as 5.02% (95% CI: 2.83% - 8.14%). The negative predictive value of the AutoDELFIA was calculated as 100% (95% CI: 100% - 100%).

10.4.8.2 The ISAGA IgM screen

Table 10.4 demonstrates statistical analysis applied to the ISAGA IgM screening test used in step two or the confirmatory step of the screening protocol. The positive predictive value of the ISAGA IgM assay for the population screened was
calculated as 44.12\% (95\% CI: 27.19\% - 62.11\%). The negative predictive value of the ISAGA IgM was calculated as 100\% (95\% CI: 98.62\% - 100\%).

10.5 Discussion

10.5.1 Parental uptake of newborn screening for CT
During the 2-year period of screening for CT the opt-out rate was 0.25\%. Hence the uptake of newborn screening was greater than 99\% despite the fact that parents were informed of the test and CT study at the point of infant heel blood collection. Mothers who were aware of toxoplasmosis or screening programmes in other countries welcomed the study. Many other mothers expressed positive thoughts towards screening and diagnosis of a condition that may be unrecognised in the majority of infants and associated with sequelae in some. For those who opted out, the reason most stated was simply lack of adequate knowledge on the topic to allow inclusion of their infant in the study. A minority of parents who declined the test had no particular reason and simply did not wish for their infant to be included in a study.

10.5.2 Total number screened
The total number of 144,564 screened was based on 'in-laboratory' data for the 24 month period. The CSO quotes total number of births registered; the Health Service Executive (HSE) quotes the total number of births which includes still births and early neonatal deaths. Hence the CSO data and the HSE data differ from 'in-laboratory' numbers as not all live births may be registered and not all registered births may have DBS tested.

Therefore, for the CT screening programme, total results were based on the number of newborns who had filter paper cards tested. The total number was not affected by repeat cards necessary for infants with initial borderline screening results, or cards that needed to be repeated because of quality issues which may have rendered the sample insufficient or unsuitable for reporting. These were represented by a very small number in total and only the final card tested with the valid result was included in the total number.

For premature infants who routinely require many repeat filter paper cards tested for metabolic conditions until establishment of full feeding, one card was screened
for toxoplasma antibody in the newborn period and repeat cards for retesting of metabolic conditions did not include toxoplasma retesting. One could argue that screening for CT in premature infants could yield false negative results due to insignificant antibody production in this population. Premature infants all routinely undergo serial ophthalmology examinations for retinopathy of prematurity and cranial ultrasound imaging up to full term gestation. Hence subclinical disease if present should be detected (Freeman et al., 2005). In addition, it is likely that asymptomatic CT in a premature infant would at least have some effect on immature vulnerable organs and manifest as a systemic infectious process such as elevated transaminases or bone marrow dysfunction which are routinely monitored in a premature infant, and if persistent, congenital infection profile is usually sought.

10.5.3 Time taken for a positive screening result
The wide range of 9 to 23 days from heel blood collection to availability of a screening result was partially explained by delays in transport of samples from maternity and paediatric units nationwide to the NNBSL in Dublin. During traditional holiday periods and long weekends with national holidays, blood spot cards were not tested until the next routine working day. The longest delay of 23 days occurred on one occasion. Heel blood was collected on day five of life, results were available 23 days later when the infant was 28 days old. Delay in delivery of the filter paper card to the NNBSL was the reason provided in this case.

10.5.4 Recovery of toxoplasma IgM from newborn filter paper cards
Toxoplasma IgM was easily recovered from DBS on filter paper cards with a two step protocol that utilised AutoDELFIA and ISAGA assays. There were no major obstacles encountered with the AutoDELFIA or ISAGA IgM screening assays. The two-step screening process continued for the two years of newborn screening and at no point was it necessary to incorporate new or more sensitive and automated assays.

10.5.5 Timing of confirmatory results
The time taken for availability of confirmatory results ranged from 5 to 15 working days, median 7 days. During the 24 months, any delay beyond seven days coincided with the Christmas and new year holiday periods in 2005 and 2006. This was mainly due to a combination of busy courier services and staff operating on
an on-call basis only at the TRL during this time. Hence samples taken from the third week of December onwards were not processed until regular laboratory opening hours resumed in the first week of January.

A total of five infants were affected by delays encountered with confirmatory results over the 2005 and 2006 Christmas periods. Two were confirmed negative (table 10.1 case numbers 26 and 28) and three were confirmed positive (table 10.1 case numbers 4, 5 and 27). The delay in results generated excess parental anxiety, however the circumstances could not be altered for more timely results during the Christmas season.

**10.5.6 Spectrum of confirmatory results for screen positive cases**

A diversity of serology was encountered in the 34 mother-infant pairs that required confirmatory serology (table 10.1).

A full spectrum of serological results was demonstrated, from strongly positive IgM and IgA results in some confirmed cases, to borderline and equivocal serology in other confirmed positive and negative cases. The diagnosis of CT was difficult to confirm or exclude when equivocal results were encountered.

The diversity of serology displayed in our cohort highlighted the complexities of toxoplasma serology interpretation. In a small number of false positive cases, serologic exclusion of CT was not possible on the first confirmatory result and serial testing of the newborn was required to rule out CT (table 10.1 case numbers 14, 15, 16 and 28). There were two confirmed cases of CT with inconclusive serology (table 10.1 case numbers 4 and 27); one required input from the wider European expert community and even then, serologic confirmation was not possible in a timely manner (table 10.1 case number 4).

It would be more accurate to summarise that in the Irish study, whilst interpretation of infant serology was difficult in the newborn period in a minority of instances where the toxoplasma antibody profile was uncharacteristic of CT, it was eventually possible to confirm the correct diagnosis in all cases, albeit with repeat serial tests in infancy.
The overall experience in Ireland reinforced the recommendation that when screening populations for CT, it is important to have all confirmatory serology tested in a Reference Laboratory with interpretation led or assisted by an expert in the field.

Results of confirmatory serology are further detailed in the following two chapters.

**10.5.7 Demographics of screen positive cases**

Eleven infants screened positive in the first 12 months of the programme and 23 screened positive in the second 12 months. The finding that seventeen of 34 (50%) screen positive cases were obtained in the third six-month period of screening, i.e., July to December 2006 (figure 10.3) could not be explained by that being the period with the highest birth rate, which was found in the final six-month period (data not shown).

Approximately 40% of all infants born in Ireland are born in county Dublin. Of the 34 screen positive infants, 10 (29%) were born to women resident in county Dublin and the remainder in other counties.

Confirmatory serology demonstrated that in the cohort of 34 mothers, 30 had demonstrable antibody, seven of who (23%) were resident in County Dublin. In addition, of 11 women who were seropositive prior to pregnancy, only one was resident in county Dublin (table 10.1 case number 32). Despite the small number that screened positive, the geographical variation in seroprevalence demonstrated within the group of 34 mothers in this study was consistent with the preliminary seropositivity study performed in Ireland in 1996, which demonstrated that toxoplasma seroprevalence was below the mean in county Dublin (figure 10.4).

**10.5.8 Incidence of CT and false positive rate**

The incidence of CT in the Irish population was 1 in 10,000 which was similar to that calculated for Denmark where an incidence of 1.6 per 10,000 was derived from nine and a half years of newborn screening (Roser et al., 2010). The Irish incidence was also similar to the low overall incidence quoted for Europe; i.e., 1 to 5 per 10,000 (Benard et al., 2008).
In this study, 19 infants, representing more than half (56%) of the 34 screen positive cohort were subsequently confirmed negative on serological testing. The high yield of false positives in our study emphasised the need for timely follow-up with complete serological testing.

10.5.9 Positive predictive value of the screening assays

The two assays used for the screening protocol demonstrated that toxoplasma IgM was easily recoverable from filter paper cards. However, step 1 of the screening protocol had a low predictive value. An infant with a positive AutoDELFIA screen had a 1 in 20 chance of having CT confirmed. A negative AutoDELFIA screen excluded CT (table 10.3).

The median AutoDELFIA value was higher in confirmed positive infants compared with the median AutoDELFIA for screen positive infants who were subsequently confirmed negative (table 10.2). It can be questioned whether a higher threshold than ≥ 4 IU/mL set as the cut-off for a positive AutoDELFIA would have yielded less infants that required step two ISAGA IgM screening and hence less false positives overall. This question can only be answered by direct comparison of subgroups of the same population screened at a higher threshold for a positive AutoDELFIA.

The ISAGA IgM test was moderately predictive, an infant with a positive ISAGA IgM screen had less than a 50% chance of having CT confirmed, but a negative ISAGA IgM screen would reliably exclude CT. This was exemplified by the four cases that were ISAGA IgM negative on screening but released as borderline positive screening results due to high AutoDELFIA values > 50 IU/mL. In all four, mother-infant toxoplasma antibody profiles were negative (table 10.1 case numbers 6, 8, 9 and 20). This demonstrated that a negative ISAGA IgM screen was reliable and did not require serologic confirmation of negativity, regardless of the AutoDELFIA value.

No screen negative children born during the 24-month period were subsequently identified with CT, i.e., there were no false negative screening results.
10.5.10 Screening for CT compared with metabolic screening

Whilst the screening tests employed reliably excluded CT, ideally a higher positive predictive value for the second or confirmatory step (ISAGA IgM bioMérieux®) of the screening protocol would have been preferred as this would have reduced the number unnecessarily recalled for confirmatory venous sampling. This would be an important point for consideration if newborn screening for CT was to be considered for adoption into the routine metabolic screening programme.

Recall of 19 mother infant pairs represented only 0.01% of the total population screened. Whilst it could be argued that this still meant 19 mothers and infants were subject to unnecessary investigations with disruption of routine for repeat hospital visits with generation of parental anxiety, the necessary tests were blood tests which were not extensively invasive.

The incidence in Ireland of metabolic conditions routinely screened for on DBS is as follows:

1) Congenital hypothyroidism, one in 3,500;
2) Phenylketonuria, one in 4,500;
3) Galactosaemia, one in 19,000;
4) Homocysteinuria, one in 65,000;
5) Maple syrup urine disease, one in 125,000.

Whilst the purpose of this study was not to determine or justify whether newborn screening for CT should be considered for routine adoption into the newborn screening programme, the researchers attempted to address this issue based on the tests used for 24 months of newborn screening of CT and the results obtained. An argument could be made for incorporation of CT screening into the routine metabolic screening programme, as comparatively the incidence of CT is high enough to warrant screening. However when comparing screening of DBS for CT with screening of DBS for inherited metabolic disorders, factors other than the incidence in the population need to be considered, such as the positive predictive value of the screening test, the number that require confirmation, the number of true positives vs false positives, rate of prevented disease, data on long term outcome following interventions in infancy and benefits vs harms.
Data from the NNBSL in Dublin demonstrates that when infants screen positive for metabolic disorders, interventions are commenced by day 7 to 15 of life and in some instances prior to the screening result, if the infant is known to be in a high risk category for the disorder (available at http://www.hse.ie/eng/health/child/newbornscreening/newbornbloodspotscreening/bloodspotar2012.pdf accessed October 2015).

With CT, the diagnosis needs to be confirmed at an external Reference Laboratory prior to initiation of treatment, and in some cases, confirmation may take weeks. In addition it is preferable to perform full clinical evaluation for CT before initiation of treatment. In cohort studies of CT with good outcomes, infants commenced treatment between the first and third month of life (Phan et al., 2008a). Hence compared with inherited metabolic disorders, it is not critical to commence treatment for CT in the days immediately after birth, but rather, as soon as feasibly possible.

There is a paucity of data on long term outcome, visual function and quality of life in untreated asymptomatic CT compared with screened and treated asymptomatic groups. Perhaps answers to those questions and the availability of a toxoplasma screening test with a higher positive predictive value may justify or disprove the need for routine CT screening in our population in the future.

10.6 Conclusions
Newborn screening for CT was feasible. Toxoplasma IgM was easily recovered from DBS on newborn filter paper cards. Step one of the screening protocol had a low positive predictive value and the second or confirmatory step of the screening protocol was moderately predictive for CT. The screening tests reliably excluded CT.

The incidence of CT in Ireland was 1 in 10,000 which was similar to rates quoted for other European countries with similar populations.
Figure 10.1: Blood IgM concentration (AutoDELFIA) in 299 infants with a detectable initial screen
Figure 10.2: Outcome of the population screened for congenital toxoplasmosis

144,564 total newborn population screened on heel DBS

299 positive on DBS with first assay (AutoDELFIA IgM) of screening protocol

299 tested on DBS with second assay (ISAGA IgM) of screening protocol

30 positive on ISAGA IgM plus four borderline positive recalled for venous confirmatory sampling

19 confirmed negative

15 confirmed positive (congenital toxoplasmosis)
Figure 10.3: Infants screened per six month period over 24 months (n=34)
Figure 10.4: Geographical comparison of maternal seroprevalence of Toxoplasma antibody (map a) and number of screen positive cases by county (map b)
Figure 10.5: Maternal ethnicity for 34 screen positive infants
<table>
<thead>
<tr>
<th>Case No.</th>
<th>County of birth</th>
<th>Auto-DELFIA IgM IU/ml</th>
<th>ISAGA IgM Screen</th>
<th>Screen Result</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Kerry</td>
<td>206, 198, 218</td>
<td>Pos</td>
<td>Pos</td>
<td>4000 Neg Pos Neg</td>
<td>4000 Pos Pos Pos 0.07</td>
<td>125 Pos NT</td>
<td>Congenital infection. Maternal seroconversion trimester 2.</td>
</tr>
<tr>
<td>3</td>
<td>Waterford</td>
<td>166, 165, 179</td>
<td>Pos</td>
<td>Pos</td>
<td>4000 Neg Pos Pos</td>
<td>125 Pos NT NT NT</td>
<td>4000 Avidity 0.03 Pos NT</td>
<td>Congenital infection, symptomatic in-utero. Maternal seroconversion trimester 2. Antenatal diagnosis &amp; treatment from 28/40 gestation.</td>
</tr>
<tr>
<td>4</td>
<td>Dublin</td>
<td>210, 204, 218</td>
<td>Pos</td>
<td>Pos</td>
<td>125 250 500 500</td>
<td>500 Pos Pos Pos 0.17</td>
<td>Neg Neg NT</td>
<td>Congenital infection confirmed at 9 months. Equivocal infant serology. Maternal seroconversion trimester 3.</td>
</tr>
<tr>
<td>5</td>
<td>Tipperary</td>
<td>595, 558, 568</td>
<td>Pos</td>
<td>Pos</td>
<td>4000 SP SP SP</td>
<td>250 Pos SP SP 0.14</td>
<td>Neg Neg NT</td>
<td>Congenital infection. Maternal seroconversion trimester 3.</td>
</tr>
<tr>
<td>6</td>
<td>Dublin</td>
<td>187, 176, 174</td>
<td>Neg BD</td>
<td>Neg NT</td>
<td>Neg Neg NT NT</td>
<td>500 Neg Neg NT NT NT NT NT NT</td>
<td>Mother-infant serology negative. Borderline screen result confirmed negative.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Limerick</td>
<td>2, 7, 14</td>
<td>Pos</td>
<td>Pos</td>
<td>500 Neg Neg Neg</td>
<td>1000 Neg NT NT NT 500 Neg NT</td>
<td>Maternal infection prior to pregnancy. False positive screen.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Cork</td>
<td>77, 59, 65</td>
<td>Neg BD</td>
<td>Neg NT</td>
<td>Neg Neg NT NT</td>
<td>NT NT NT NT NT NT NT NT NT NT</td>
<td>Mother-infant serology negative. Borderline screen result confirmed negative.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Dublin</td>
<td>86, 75, 65</td>
<td>Neg BD</td>
<td>Neg NT</td>
<td>Neg Neg NT NT</td>
<td>NT NT NT NT NT NT NT NT NT NT</td>
<td>Mother-infant serology negative. Borderline screen result confirmed negative.</td>
<td></td>
</tr>
<tr>
<td>Case No.</td>
<td>County of birth</td>
<td>Auto-DELFIA IgM IU/ml</td>
<td>ISAGA IgM Screen</td>
<td>Screen Result</td>
<td>Infant Confirmatory Serology</td>
<td>Maternal Postnatal Serology</td>
<td>Maternal Antenatal Serology</td>
<td>Conclusion</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
<td>----------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dye test IU/ml</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
</tr>
<tr>
<td>10</td>
<td>Laoise</td>
<td>1108, 929, 953</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Galway</td>
<td>5, 3, 4</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Dublin</td>
<td>1070, 970, 948</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>SP</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Limerick</td>
<td>450, 432, 424</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Cork</td>
<td>9, 15, 15</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Dublin</td>
<td>12, 13, 9</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Galway</td>
<td>126, 120, 112</td>
<td>Pos</td>
<td>Pos</td>
<td>4000</td>
<td>4000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Galway</td>
<td>10, 7, 5</td>
<td>Pos</td>
<td>Pos</td>
<td>250</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Louth</td>
<td>10, 10, 11</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Louth</td>
<td>31, 31, 35</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Tipperary</td>
<td>89, 50, 72</td>
<td>Neg</td>
<td>BD</td>
<td>NT</td>
<td>NT</td>
<td>Neg</td>
<td>NT</td>
</tr>
</tbody>
</table>

Table 10.1: 34 screen positive results and confirmatory serology for 34 mother-infant pairs
<table>
<thead>
<tr>
<th>Case No.</th>
<th>County of birth</th>
<th>Auto-DELFIA IgM Screen</th>
<th>ISAGA IgM</th>
<th>ISAGA IgA</th>
<th>Auto-DELFIA IgM Screen</th>
<th>ISAGA IgM</th>
<th>ISAGA IgA</th>
<th>ISAGA IgA</th>
<th>ISAGA IgA</th>
<th>Avidity</th>
<th>ISAGA IgM</th>
<th>ISAGA IgA</th>
<th>ISAGA IgA</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Waterford</td>
<td>18, 22, 23</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>NT</td>
</tr>
<tr>
<td>22</td>
<td>Limerick</td>
<td>6, 2, 4</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>&gt; 0.40</td>
</tr>
<tr>
<td>23</td>
<td>Dublin</td>
<td>1289, 1276, 1299</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
<td>4000</td>
<td>SP</td>
<td>NT</td>
<td>Pos</td>
<td>VL</td>
<td>Neg</td>
</tr>
<tr>
<td>24</td>
<td>Cork</td>
<td>441, 282, 247</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>BD</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>0.20</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>25</td>
<td>Cork</td>
<td>894, 897, 908</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>SP</td>
<td>Pos</td>
<td>Pos</td>
<td>250</td>
<td>Pos</td>
<td>NT</td>
<td>0.20</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>26</td>
<td>Galway</td>
<td>9, 8, 10</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>28</td>
<td>Cork</td>
<td>18, 23, 23</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>250</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>2000</td>
<td>Pos</td>
<td>NT</td>
<td>Pos</td>
<td>0.20</td>
</tr>
<tr>
<td>29</td>
<td>Dublin</td>
<td>12, 16, 17</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>4000</td>
<td>BD</td>
<td>Pos</td>
<td>Pos</td>
<td>0.26</td>
</tr>
<tr>
<td>30</td>
<td>Galway</td>
<td>10, 11, 12</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>2000</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>0.76</td>
</tr>
</tbody>
</table>
Table 10.1: 34 screen positive results and confirmatory serology for 34 mother-infant pairs

<table>
<thead>
<tr>
<th>Case No.</th>
<th>County of birth</th>
<th>Auto-DELFIA IgM</th>
<th>ISAGA IgM Screen</th>
<th>Screen Result</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dye test IU/ml</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
<td>Dye test IU/ml</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
</tr>
<tr>
<td>31</td>
<td>Galway</td>
<td>737, 713, 674</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>32</td>
<td>Dublin</td>
<td>13, 19, 18</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>33</td>
<td>Dublin</td>
<td>161, 189, 171</td>
<td>Pos</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>34</td>
<td>West-meath</td>
<td>86, 91, 98</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td>Neg</td>
<td>BD</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Pos, positive; SP, strongly positive; VL, very low; Neg, negative; NT, not tested; BD, borderline; WB, Western Blot; IND, indeterminate; NA, not available.
### Table 10.2: Summary of infant and maternal results in those with a positive screen

<table>
<thead>
<tr>
<th></th>
<th>Newborn Screening tests</th>
<th>Infant’s Confirmatory test</th>
<th>Maternal Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Postnatal</td>
<td>Antenatal</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Auto-DELFIA IgM (IU/mL)</td>
<td>ISAGA IgM (IU/mL)</td>
</tr>
<tr>
<td>True Positive</td>
<td>15</td>
<td>435</td>
<td>Positive 15/15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92-1288</td>
<td>125-4000</td>
</tr>
<tr>
<td>Borderline</td>
<td>4</td>
<td>70</td>
<td>Negative 4/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67-179</td>
<td></td>
</tr>
<tr>
<td>False Positive</td>
<td>15</td>
<td>11</td>
<td>Positive 15/15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-119</td>
<td>250-4000</td>
</tr>
</tbody>
</table>

**N**, number
Table 10.3: Positive predictive value for the AutoDELFIA IgM screen

<table>
<thead>
<tr>
<th></th>
<th>Disease present</th>
<th>n</th>
<th>Disease absent</th>
<th>n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test positive</td>
<td>True positive (a)</td>
<td>15</td>
<td>False positive (c)</td>
<td>284</td>
<td>299</td>
</tr>
<tr>
<td>Test negative</td>
<td>False negative (b)</td>
<td>0</td>
<td>True negative (d)</td>
<td>144,265</td>
<td>144,265</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td>144,549</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Formula</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>a/(a+b)</td>
<td>100%</td>
<td>78.2% - 100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>d/(c+d)</td>
<td>99.8%</td>
<td>99.78% - 99.83%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>a/(a+c)</td>
<td>5.02%</td>
<td>2.83% - 8.14%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>d/(b+d)</td>
<td>100%</td>
<td>100% - 100%</td>
</tr>
</tbody>
</table>
### Table 10.4: Positive predictive value for the ISAGA IgM screen

<table>
<thead>
<tr>
<th></th>
<th>Disease present</th>
<th>n</th>
<th>Disease absent</th>
<th>n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test positive</strong></td>
<td>True positive (a)</td>
<td>15</td>
<td>False positive (c)</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td><strong>Test negative</strong></td>
<td>False negative (b)</td>
<td>0</td>
<td>True negative (d)</td>
<td>265</td>
<td>265</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>15</td>
<td></td>
<td>284</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Formula</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>( \frac{a}{a+b} )</td>
<td>100%</td>
<td>78.2% - 100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>( \frac{d}{c+d} )</td>
<td>93.31%</td>
<td>89.75% - 95.92%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>( \frac{a}{a+c} )</td>
<td>44.12%</td>
<td>27.19% - 62.11%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>( \frac{d}{b+d} )</td>
<td>100%</td>
<td>98.62% - 100%</td>
</tr>
</tbody>
</table>
CHAPTER 11 - Serological findings in the cohort of infants that were false positive on DBS screening for congenital toxoplasmosis

11.1 Introduction
Newborn screening for CT will detect true positive cases in addition to infants who screen positive but are subsequently confirmed negative, i.e., false positives. In countries with a low incidence of CT, screening may yield a high rate of false positivity (Lebech et al., 1999).

Newborn screening for CT in Ireland detected 34 screen positive cases in 24 months. For 19 infants who screened positive on DBS testing, subsequent serological testing confirmed that the DBS results were false positives. The incidence of CT was 1 per 10,000 and the false positive rate was 1.3 per 10,000. Serological exclusion of CT in infants may be complex, particularly if maternal seroconversion occurred in pregnancy. In such instances, serial testing of the toxoplasma antibody profile may be necessary, in addition to clinical evaluation to definitively rule out CT.

11.2 Objective
To demonstrate serological findings in screen positive infants confirmed negative by paired mother-infant toxoplasma antibody profiles.

11.3 Methods

11.3.1 Population studied
Nineteen infants with false positive toxoplasma screening results detected by 24 months of national newborn screening for CT.

11.3.2 Methods for confirmation of negative status
Infant status was confirmed by comparative analysis of paired mother-infant toxoplasma antibody profiles. Retrospective testing of stored maternal blood from the first antenatal visit was performed to estimate timing of maternal seroconversion. Where indicated, serial infant venous samples were tested for status confirmation. Clinical evaluation was performed if necessary to rule out CT whilst awaiting serological confirmation.
A final serology test was offered at age one year to all infants who were false positives on screening to demonstrate loss of maternal antibody.

11.4 Results

11.4.1 Summary of confirmation of infant status

Nineteen infants who screened positive on DBS were confirmed as false on follow-up testing in the immediate postnatal period, or in infancy by age 6 to 14 weeks.

11.4.2 Maternal demographics

Five of 19 (26%) infants were born in county Dublin and 14 (73%) were born to mothers resident in other counties. Figure 11.1 demonstrates maternal county of residence for 19 false positive cases, displayed against the geographical findings from the seroprevalence study. Ten of 19 infants (53%) were born to mothers of Irish ethnicity and 9 (47%) were born to migrant mothers. Maternal ethnicity is displayed in figure 11.2.

11.4.3 Summary of paired mother-infant confirmatory serology

Table 11.1 displays screening and confirmatory results for the 19 mother-infant pairs numerated in their temporal order of occurrence by infant date of birth. Serological confirmation was required in 4 of 19 (21%) infants with a borderline positive result on DBS screening, i.e. positive AutoDELFI A > 50 IU/mL and a negative ISAGA IgM. Paired serology revealed no evidence of toxoplasma antibody, maternal antenatal serology was not requested and infants were not further tested (table 11.1 case numbers 1, 3, 4 and 12).

Fifteen of 19 (79%) infants who screened positive on DBS had a detectable dye test on confirmatory serology, one also had transiently detectable IgM and IgA (table 11.1 case number 8). In all these 15 infants, passively transferred maternal antibody was demonstrated from maternal seroconversion prior to or during pregnancy. Eleven such infants were born to mothers with seroconversion prior to pregnancy.

Four infants were born to mothers with serological evidence of pregnancy seroconversion (table 11.1 case numbers 7, 8, 16 and 17). In three of these, ≥ 2 confirmatory tests were performed in early infancy to definitively exclude CT; one of these infants also underwent clinical evaluation whilst awaiting confirmation.
(table 11.1 case number 8). Thus in the total cohort of 19 infants; in order to exclude CT, repeat serological tests in early infancy were performed in 3 of 19 cases (16%) and limited clinical evaluation in 1 infant (5%). Eight of 15 infants with an initial positive DT had a final test at one year which was negative. Figure 11.3 summarises outcome for 19 screen positive cases confirmed negative.

11.4.4 Eleven screen positive infants confirmed negative: maternal seroconversion prior to pregnancy with passive transfer of antibody to infant

Comparative analysis of maternal postnatal and antenatal antibody profiles with avidity testing confirmed maternal seroconversion prior to pregnancy in 11 cases. Infant dye tests represented passively acquired maternal antibody, IgM and IgA were not detected in infant sera and CT was out ruled. Repeat confirmatory testing in early infancy was not indicated for any of the 11 infants. Confirmatory serology was repeated at six weeks in one case at maternal request to alleviate anxiety (table 11.1 case number 6).

Final serological testing was performed in 7 of 11 infants and negative dye tests were demonstrated at one year (table 11.1 case numbers 5, 6, 9, 10, 14, 15, and 18). The parents of 2 infants opted out of final serological testing at one year (table 11.1 case numbers 2 and 11). One family from the Irish travelling community could not be located after the newborn period (table 11.1 case number 13). Maternal antenatal serology was unavailable as the first presentation to antenatal services was at delivery. One Irish family emigrated to France prior to the infant's final test at one year (table 11.1 case number 19). No infant in this group underwent clinical evaluation in the newborn period.

11.4.5 Four screen positive infants likely uninfected: maternal seroconversion during pregnancy with passive transfer of antibody to infant

In four cases maternal seroconversion was estimated to have occurred during pregnancy: two in trimester 1, one in trimester 2, and one in trimester 3 (table 11.1 case numbers 7, 8, 16 and 17). In order to exclude CT, repeat serological tests were offered to all 4 in early infancy prior to the final test at one year. Repeat confirmatory tests were performed in 3 infants, one of whom additionally required limited clinical evaluation; 1 of the 4 infants had a final antibody test at 12 months.
1) In case number 7, low maternal avidity with a dye test of 4,000 IU/mL and a positive ELISA IgM were demonstrated antenatally and postnatally. The antenatal booking sample was from six months gestation. An avidity of 0.26 at six months gestation with a positive ELISA IgM suggested pregnancy seroconversion less than three months prior, likely in trimester 2 (table 11.1 case number 7). A small increment of the avidity to 0.37 in the postnatal period supported trimester 2 seroconversion. The infant was tested at birth and at six weeks. A positive DT was detected and infant IgM and IgA were not demonstrable. The infant's family were asylum seekers from Africa and they left the state before the infant was one year old.

2) Infant number 8 was born to a mother whose antenatal sample was from eight months gestation (table 11.1 case number 8). The avidity of 0.45 suggested maternal seroconversion more than three months prior; and combined with a high DT and a negative ELISA IgM, it was estimated that seroconversion occurred six to nine months prior to antenatal sampling, possibly in trimester 1 or peri-conceptual. Initial infant serology suggested CT. Two further confirmatory tests were performed, one at 6 weeks and one at 14 weeks of age at which point IgM and IgA were undetectable; dye test titres remained unchanged. Limited clinical evaluation was performed in this infant whilst undergoing confirmation. Cranial ultrasound scan and ophthalmology examination were normal at age three months. The infant's mother opted out of the final antibody test at one year.

3) Comparative maternal postnatal and antenatal serology for case number 16 confirmed pregnancy seroconversion, likely in trimester 3, given the avidity of 0.2. The infant was tested in the newborn period and at 12 weeks by which time the DT had decreased fourfold and IgM and IgA remained undetectable. Final testing at one year was not possible as the family relocated to their native country of Spain when the infant was age three months.

4) Infant number 17 was born to a mother with a low avidity of 0.26 and a borderline ELISA IgM postnatally. Antenatal serology from six months gestation demonstrated a positive ELISA IgM and a similar avidity of 0.24. A positive ELISA IgM at six months gestation with a borderline ELISA IgM postnatally suggested that at the time of postnatal sampling, the IgM response for detection
by ELISA was at an end and maternal seroconversion occurred six to nine months prior, possibly in trimester 1. Infant serology in the newborn period was not indicative of CT. The family were of the Roma community and could not be located for a repeat infant sample in the first six weeks of life. Infant DT at one year was negative.

11.5 Discussion

11.5.1 Demographics of women with false positive infants
The geographical distribution of the 19 false positive cases, and in particular the low occurrence of false positives in county Dublin, was consistent with the seroprevalence study performed in 1996 (figure 11.1). Fifteen of 19 women in the cohort had demonstrable antibody, only 3 of 15 women were residing in county Dublin.

Amongst the 19 false positive cases, almost half of the mothers were recent migrants. Toxoplasma antibody seroprevalence in a population varies with migration trends, particularly in regions where the incidence of disease in the indigenous population is low (Flatt et al., 2013), this was reflected in our study. Antibody seroprevalence in migrant women varies according to country of origin and dietary or lifestyle practices that may incur risk factors for infection. Whilst the numbers are too small to draw conclusions, one can assume that the false positive rate would be in lower in the Irish-born population.

11.5.2 Paired mother infant confirmatory serology

11.5.2.1 Summary of criteria for serological exclusion of CT in infancy
According to international criteria the diagnosis of CT is either unlikely or can be excluded in infancy if any one of the following is demonstrable in infant sera (Lebech et al., 1996; Remington, 2011):
1) No evidence of toxoplasma antibody in mother and infant;
2) Infant serology consistent with passively acquired maternal antibody;
3) Failure of the newborn to demonstrate specific IgM or IgA antibodies;
4) Infant IgM present in the newborn period was undetectable by three months of age;
5) Infant IgG present in the newborn period was undetectable by one year of age.

The classification system from Lebech et al separates the likelihood of *T. gondii* infection into five mutually exclusive categories: definite, probable, possible, unlikely, and not infected. Inclusion within a specific category is dependent upon the case definition, which is in turn derived from criteria based on serological, parasitological, and clinical information. Passively acquired maternal antibody in an uninfected infant can confound the interpretation of infant serology particularly when the infant profile is atypical or maternal antibody is slow to decline in infant blood.

Infants detected on screening due to passively acquired maternal antibody from seroconversion prior to pregnancy are not at risk of CT unless there is maternal immunocompromise or the primary infection was peri-conceptual. Therefore infants born to women with demonstrable seroconversion prior to pregnancy can generally have CT excluded in the newborn period based on the initial comparative mother-infant profile.

Uninfected infants born to women with pregnancy seroconversion may be contaminated with IgM during delivery and have an initially high titre DT. Thus serial testing may be necessary to exclude CT in addition to a final DT at one year of age to confirm negativity.

It is appreciated that pregnancy seroconversion may not result in an infected infant, and some of the uninfected cohort may not be captured for a final DT at one year. This has been demonstrated where prenatal screening programmes exist to detect maternal seroconversion. Remington et al described data from studies performed by Desmonts and Couvreur in historic cohorts of infected and uninfected children born to women with pregnancy seroconversion. Follow up serologic profiles of uninfected children did not demonstrate neo-synthesis of any class of toxoplasma specific antibody, whereas sera of infected children demonstrated synthesis of at least one class of toxoplasma specific antibody. Remington et al recommended that if newly synthesised specific antibody could not be demonstrated at the point of final testing albeit less than one year of age, the case could be classified as 'likely uninfected' as described by other researchers.
The principle of this classification system is applied in France where systematic prenatal screening will detect women who seroconvert in pregnancy without transmitting infection to the fetus. In such cases, the diagnosis of CT has to be serologically excluded in the newborn period, well before the age of one year. All infants born to women who demonstrate pregnancy seroconversion in France are tested following delivery and CT is excluded in the newborn period if any of the criteria 2) to 4) are met as above. Infants are monitored serologically for one year to demonstrate decline in antibody status until negativity is confirmed. Thus in cases where pregnancy seroconversion was demonstrated, the diagnosis of CT can be 'excluded' in early infancy and subsequently 'confirmed negative' with a DT obtained at one year. If a final test is not available to confirm negativity in the infant, the infant is classified as 'likely uninfected.'

Comparative analysis of paired mother-infant serology excluded CT in 19 infants that screened positive on DBS. Repeat serological tests in early infancy were recommended by the Director of the TRL for the 4 infants born to mothers with estimated pregnancy seroconversion (table 11.1 case numbers 7, 8, 16 and 17). The parents of 3 infants agreed to retesting after the first newborn sample; the parents of one other were uncontactable in early infancy, however this child had a negative DT at one year (table 11.1 case number 17).

Requests for repeat infant confirmatory samples were communicated by the CT programme co-ordinator to the infant's parents and local paediatrician. Whilst the need for repeat infant serology generated inconvenience and anxiety, parents were co-operative with recommendations and were subsequently reassured by negative confirmation.

**11.5.3 Serological exclusion of CT in 11 infants born to women with seroconversion prior to pregnancy: role of the avidity test in confirming timing of seroconversion**

In 8 of 11 mothers, dye test titres in maternal postnatal and antenatal sera were largely unchanged with absence of IgM and high avidity in both samples which was conclusive for seroconversion prior to pregnancy.
In 3 of 11 mothers, IgM or IgA was detected by ISAGA in maternal postnatal and or antenatal serology which made the timing of maternal seroconversion less conclusive (table 11.1 cases 6, 10 and 14). The avidity test in these cases was useful for its ability to reliably demonstrate that seroconversion occurred prior to pregnancy, and detectable IgM and IgA were attributed to the ability of the ISAGA assay to detect residual maternal immunoglobulin for 12 months or more. In one such case IgM detectable by ISAGA was borderline postnatally and positive on an antenatal sample from three months gestation (table 11.1 case number 6). The antenatal avidity test of 0.53 indicated infection prior to pregnancy or infection at least six months old. Infant serology on the first sample was clearly indicative of passive transfer of maternal antibody only and a repeat test was not indicated other than the final DT at one year. However maternal anxiety was generated from the positive antenatal IgM, which was most likely residual IgM from remote rather than recent maternal infection. The ISAGA assay can generally detect IgM for 12 months and up to two years in some cases which can confound interpretation of maternal serology (Gras et al., 2004). Due to the detection of antenatal IgM, the mother had concerns that her infant could be congenitally infected despite the reassuring infant confirmatory result. Attempts to alleviate anxiety were unsuccessful and a repeat infant test was performed at six weeks which had no demonstrable IgM or IgA. Infant DT was also negative at one year.

The serological profile in case number 10 was from a woman with known HIV positivity and primary toxoplasma infection documented three years previously (table 11.1 case 10). Antenatal and postnatal maternal serology were ISAGA IgM positive. Literature reports demonstrate that the toxoplasma antibody profile is generally unreliable in HIV positive individuals, who can have demonstrable IgM many years after initial toxoplasma infection (Patel et al., 1993; Fernandes et al., 2012) and who often require adjunctive methods to confirm primary infection or reactivation (Vidal et al., 2011; Sukthana et al., 2012). In this case, high avidity results indicated infection prior to pregnancy. The IgM was attributed to the seroconversion that had been documented three years previously. One could argue that with a background of HIV, detectable IgM could represent toxoplasma reactivation in pregnancy. There was no history of any symptoms of maternal reactivation at any time or any suspicion of reactivation during pregnancy, which would likely be symptomatic in an immunocompromised individual. The infant’s
serology in the newborn period and at one year proved that the maternal IgM was of no recent clinical significance.

In case number 14, maternal IgA was detectable postnatally and antenatally at five months gestation. The two possibilities were either maternal infection in pregnancy or maternal infection prior to pregnancy. The avidity result of 0.54 at five months gestation together with a negative ELISA IgM suggested that maternal seroconversion occurred at least six months prior, and detectable IgA was from infection prior to pregnancy. Infant serology was IgM and IgA negative in the newborn period and the dye test was negative at one year.

To summarise, for screen positive infants born to women who seroconverted prior to pregnancy, the diagnosis of CT was serologically out-ruled by the first confirmatory sample and further proven by the final test at one year where applicable. There was only 1 of 11 infants who required a repeat sample at six weeks to alleviate maternal anxiety rather than for serological confirmation.

11.5.4 Unusual maternal serological profile in one case of seroconversion prior to pregnancy

Comparison of postnatal and antenatal serology in one mother raised the question of possible maternal immunocompromise (table 11.1 case number 5). Maternal postnatal serology was consistent with infection prior to pregnancy with a dye test of 500 IU/mL and a borderline ISAGA IgM. Antenatal serology from five months gestation demonstrated a dye test of 32 IU/mL and a negative IgM. Based on the increase in the dye test from 32 IU/mL at five months gestation to 500 IU/mL four months later, the Director of the TRL queried whether the serological profile was from an HIV positive individual, given that in his experience, such an increase in the dye test over a short space of time with no corresponding evolution of IgM or IgA could represent either toxoplasma seroconversion or reactivation in an immunocompromised individual.

The mother was an African migrant. Maternal antenatal screen from five months gestation was HIV negative. Based on the explanation from the Director of the TRL that the serological profile was likely due to immunocompromise unless disproven, a repeat HIV test was requested which was taken by the mother’s general practitioner in the postnatal period; HIV status was confirmed negative.
Whilst there was no concern regarding the infant's toxoplasma status which was the purpose of recall for confirmatory testing, this case was unusual as the maternal toxoplasma serological profile necessitated exclusion of immunocompromise. This case highlighted that the toxoplasma antibody profile in a minority of pregnant women can be atypical, complex and varied as per literature reports (Fricker-Hidalgo et al., 2013).

11.5.5 Serological exclusion of CT in four screen positive infants born to women with estimated seroconversion in pregnancy

Serology interpretation for 3 of 4 women with estimated pregnancy seroconversion was confounded by late presentation for antenatal care (table 11.1 case numbers 7, 8 and 17). Thus timing of maternal seroconversion was not easily deduced and could only be estimated as best as possible as stored samples available for retrospective testing were from the sixth month of gestation in two cases (table 11.1 cases 7 and 17) and from the eighth month in one (table 11.1 case 8). All three women were recent migrants to Ireland with adverse social circumstances. Two were African asylum seekers (table 11.1 case numbers 7 and 8) and one was from the Roma gypsy community (table 11.1 case number 17).

One mother-infant pair posed a particular challenge for serological interpretation (table 11.1 case 8). Antenatal serology taken at eight months gestation suggested seroconversion in trimester 1 or in the preconceptional period. The possibility of seroconversion a year or more prior with persistently detectable IgM and IgA by ISAGA was also considered but thought to be less likely, given the equivocal avidity of 0.45 and the high dye test of 4,000 IU/mL.

Case reports of CT after preconceptional or periconceptional infection have been reported (Robert-Gangneux et al., 2009). For this infant it was necessary to perform a total of three serological tests up to 14 weeks; cranial ultrasound and retinal examination in the third month of life, to exclude the diagnosis of CT. Final testing at age one year was refused. The family were African asylum seekers with challenging social circumstances. The mother chose not to engage with services after the third confirmatory test. The mother communicated that infant evaluation and repeat testing generated additional stress and anxiety to a family with other existing challenges and issues.
Whilst a final antibody test at one year would have been preferable to definitively exclude CT, there was adequate serological proof that the infant was not congenitally infected. Infant IgM and IgA were undetectable at 14 weeks and serology at that point was consistent with passive transfer of maternal antibody only. Hence despite initial serology suggestive of CT, serological criteria were subsequently met for classification as a false positive case.

To summarise, in four infants who screened positive for CT, the diagnosis of CT was serologically out-ruled by repeat testing in early infancy for 3 of 4 infants (table 11.1 cases 7, 8, and 16) 1 of whom also required clinical evaluation which generated maternal anxiety. One infant had a final antibody test at one year (table 11.1 case number 17).

Retrospectively, for these four cases, repeat testing was absolutely necessary in one infant due to detectable IgM and IgA in the initial confirmatory sample and estimation of maternal seroconversion in trimester 1 (table 11.1 case number 8). For the remaining three with no demonstrable IgM or IgA on the first confirmatory sample, one could argue that it was not necessary to repeat serology for negative confirmation, as the first confirmatory result fulfilled criteria for exclusion of CT (Lebech et al., 1996; Stronati et al., 2003). However, with the knowledge that these three infants were born to mothers with estimated seroconversion in pregnancy and the fact that some congenitally infected infants may not synthesise IgM, serological exclusion of CT could only be proven with a repeat test in the newborn period to exclude appearance of IgA, or with a negative antibody test at age one year.

11.5.6 Summary of criteria met for serological exclusion of CT in 19 false positive cases
As previously stated, according to international criteria the diagnosis of CT can be excluded in infancy if any one of the following is demonstrable in infant sera (Lebech et al., 1996; Remington, 2011):

1) No evidence of toxoplasma antibody in mother and infant;
2) Infant serology consistent with passively acquired maternal antibody;
3) Failure of the newborn to demonstrate specific IgM or IgA antibodies;
4) Infant IgM present in the newborn period was undetectable by three months of age;
5) Infant IgG present in the newborn period was undetectable by one year of age.

The toxoplasma antibody profile of 19 mother/infant pairs excluded CT in the 19 screen positive infants as follows:

a) Four mothers and infants had no evidence of toxoplasma antibody;

b) Fourteen infants had no demonstrable IgA or IgM in the initial confirmatory sample, 8 of 14 also had a negative DT at one year

c) One infant with detectable IgM and IgA on the initial sample demonstrated loss of both immunoglobulins by week 14 of life (table 11.1 case number 8).

Figure 11.3 summarises outcome for the 19 screen positive cases with CT out-rulled serologically.

There are limitations to the currently available serologic assays used for diagnosing or excluding CT as they are reliant on infant antibody synthesis and may not detect very early or late congenital infection, or those infected infants who demonstrate a paucity of antibody production. However limited studies on postnatal screening and confirmation of CT in the absence of antenatal screening have shown that combination testing with ISAGA and ELISA for IgM and IgA obtained the most accurate results with sensitivity of 94% and specificity of 99.9%. Thus whilst it is possible that some congenitally infected infants can potentially be missed, the current best practice recommendation for serology testing with a combination of assays for IgM and IgA aims to maximise the diagnosis or exclusion of CT. In cases where maternal seroconversion was proven in pregnancy and the infant had no demonstrable IgM or IgA initially, repeat testing should be performed after the initial test in addition to a final antibody test at one year where feasible. However, if a serological test was not possible at one year, failure to demonstrate neo-synthesis of specific IgM or IgA after the initial test indicates that the infant is likely uninfected. Clinical evaluation should also be considered if indicated in such cases.

11.6 Conclusions
Newborn screening for CT produced false positive results in 19 infants born to mothers with serology ranging from no evidence of infection at any time (21%), to evidence of infection prior to (58%) and during pregnancy (21%). Paired mother-
infant toxoplasma antibody profiles out ruled CT in all 19 infants; 18 (95%) by the age of 6 weeks and one at age 14 weeks.

For the screen positive infants born to mothers who seroconverted prior to pregnancy, exclusion of CT was possible with the first confirmatory test. For mothers who seroconverted in pregnancy, detection of their infants by screening necessitated more extensive confirmation in the newborn period to exclude CT.
Figure 11.1: Geographical comparison of maternal seroprevalence (map a) and number of false positive cases by county (map b)
Figure 11.2: Maternal ethnicity for 19 screen positive infants confirmed serologically negative
Figure 11.3: Summary of 19 screen positive infants with serological exclusion of CT
### Table 11.1: Mother-infant toxoplasma antibody profiles for 19 screen positive infants with serological exclusion of CT

<table>
<thead>
<tr>
<th>Infant Case Number</th>
<th>County of Birth</th>
<th>Auto-DELFIA IgM Screen</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dye test IU/mL</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
</tr>
<tr>
<td>1</td>
<td>Dublin</td>
<td>187, 176, 174</td>
<td>Neg</td>
<td>BD</td>
<td>Neg</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>Limerick</td>
<td>2, 7, 14</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>Cork</td>
<td>77, 59, 65</td>
<td>Neg</td>
<td>BD</td>
<td>Neg</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>Dublin</td>
<td>86, 75, 65</td>
<td>Neg</td>
<td>BD</td>
<td>Neg</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>Galway</td>
<td>5, 3, 4</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>Cork</td>
<td>9, 15, 15</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>Neg</td>
</tr>
<tr>
<td>7</td>
<td>Dublin</td>
<td>12, 13, 9</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>Galway</td>
<td>126, 120, 112</td>
<td>Pos</td>
<td>4000, 4000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>9</td>
<td>Roscommon</td>
<td>10, 7, 5</td>
<td>Pos</td>
<td>250</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>Louth</td>
<td>10, 10, 11</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>11</td>
<td>Louth</td>
<td>31, 31, 35</td>
<td>Pos</td>
<td>1000</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>12</td>
<td>Tipperary</td>
<td>89, 50, 72</td>
<td>Neg</td>
<td>BD</td>
<td>Neg</td>
<td>NT</td>
</tr>
</tbody>
</table>
**Table 11.1:** Mother-infant toxoplasma antibody profiles for 19 screen positive infants with serological exclusion of CT

<table>
<thead>
<tr>
<th>Infant Case Number</th>
<th>County of Birth</th>
<th>Auto-DELFIA IgM Screen</th>
<th>Screen Result</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dye test IU/mL</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
</tr>
<tr>
<td>13</td>
<td>Waterford</td>
<td>18, 22, 23</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Limerick</td>
<td>6, 2, 4</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Galway</td>
<td>9, 8, 10</td>
<td>Pos</td>
<td>Pos</td>
<td>500</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Cork</td>
<td>18, 23, 23</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td>250</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Dublin</td>
<td>12, 16, 17</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td></td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Galway</td>
<td>10, 11, 12</td>
<td>Pos</td>
<td>Pos</td>
<td>1000</td>
<td></td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Dublin</td>
<td>13, 19, 18</td>
<td>Pos</td>
<td>Pos</td>
<td>2000</td>
<td></td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Neg, negative; BD, borderline; NT, not tested; Pos, positive; IND, indeterminate; NA, not applicable
CHAPTER 12 - Serological profiles and clinical findings in 15 infants with congenital toxoplasmosis

12.1 Introduction
Screening for CT facilitates early diagnosis of infected infants, particularly the asymptomatic group who would not be detected by routine paediatric review. Serologic confirmation of screen positive infants with suspected congenital infection is necessary prior to embarking on evaluation and a treatment regimen. Interpretation of infant serology can pose challenges in asymptomatic infants with inconclusive serology, in which case initiation of evaluation may assist diagnostic confirmation if ophthalmological, radiological or other signs are present in the newborn period. In Europe, approximately 75% to 85% of cases of CT are asymptomatic and hence have subclinical infection, some of who will have signs of CT when evaluated (Villena et al., 2010).

The clinical spectrum of manifestations of CT at birth ranges from absence of signs on evaluation to severe or obvious neurological symptoms, however the latter is rare in Europe (Peyron et al., 2006; Gilbert et al., 2008). Disease can manifest solely as a non-specific infectious systemic process e.g. abnormal CSF indices, liver or bone marrow dysfunction. Signs of mild dormant disease such as inactive chorioretinal scars or intracranial calcification without neurological dysfunction are the most common findings (Gras et al., 2005; Havelaar et al., 2007). Severe CT is defined as the presence at birth of at least one of the following signs: microphthalmia, hydrocephaly, microcephaly, seizures, ≥ three cerebral calcifications, extensive visual impairment (Olariu et al., 2011). The classic triad of CT; hydrocephalus, chorioretinitis and intracranial calcification is found in less than 2% of infected infants in Europe (Remington, 2011). Newborn screening for CT in Ireland detected 34 infants who screened positive on heel blood and 15 were confirmed with CT.

12.2 Objectives
To demonstrate 1) the toxoplasma antibody profiles of 15 infants with serologically confirmed CT; 2) estimated maternal trimester of seroconversion in pregnancy and 3) infant clinical findings on evaluation in the newborn period.
12.3 Methods

12.3.1 Population studied
Fifteen infants with confirmed CT.

12.3.2 Confirmatory methods
Infant toxoplasma antibody profiles were interpreted in conjunction with maternal results for confirmation of infant status and timing of maternal seroconversion. Where indicated, serial infant venous samples were tested.

12.3.3 Infant clinical evaluation
Following confirmation of CT, infants underwent full evaluation that included: physical and neurological exam, FBC, liver and renal function, blood and CSF analysis for toxoplasma PCR, intracranial imaging, ophthalmology and audiology assessments. For infants that required serial antibody testing, clinical evaluation was initiated prior to serological confirmation to aid diagnosis and consisted of dilated funduscopy and a cranial ultrasound scan.

12.4 Results

12.4.1 Summary of infant serology
Table 12.1 displays confirmatory serology for 15 mother-infant pairs numerated in their temporal order of occurrence according to infant date of birth. Thirteen infants were diagnosed with CT based on the initial toxoplasma antibody profile in the newborn period; one of whom also had the diagnosis confirmed in-utero (table 12.1 case number 3).

Two of 15 infants had inconclusive serology on the first confirmatory sample and required serial serology in addition to adjunctive testing with immunoblot methods (table 12.1 case numbers 4 and 12). Infant DT in the cohort of 15 ranged from 125 IU/mL to 4,000 IU/mL; 14 infants had a DT ≥ 1000 IU/mL and 1 had an initial DT of 125 IU/mL (table 12.1 case number 4).

In the confirmatory samples, IgM was detectable by ELISA in 8 of 15 (53%) infants and by ISAGA in 13 (87%). In 2 infants, IgM was detectable at borderline levels by ISAGA (table 12.1 case numbers 4 and 15). Fourteen infants (93%) had IgA detectable and 1 had no demonstrable IgA (table 12.1 case number 2).
Thirteen infants had serological confirmation of CT in the newborn period, based on analysis of one venous sample. Two infants with equivocal results in the newborn period required serial toxoplasma antibody profile testing and were confirmed positive later in infancy, one at nine months (table 12.1 case number 4) and one at three months of age (table 12.1 case number 12). Both infants had Western Blot analysis of serology taken at three months to aid confirmation; the result was negative in the former infant and positive in the latter. Hence for one infant, immunoblot did not assist with confirmation and further serial serological tests were subsequently necessary (table 12.1 case number 4).

12.4.1.1 Timing of maternal seroconversion for 15 congenitally infected infants

Maternal seroconversion in pregnancy was demonstrated for all cases of confirmed congenital infection (table 12.1). Timing of maternal seroconversion was estimated by comparison of maternal postnatal serology with retrospectively tested stored blood from the first antenatal visit, taking into account gestation at which the blood sample was taken. In addition, infant symptoms at birth, if present, were considered to determine timing of maternal seroconversion as accurately as possible.

Twelve of 15 women had negative dye tests on retrospectively tested stored samples from the first 15 weeks of pregnancy. One of 15 women had a positive dye test on a stored sample from trimester 2 (table 12.1 case number 2) and one other had trimester 2 seroconversion and infant infection confirmed antenatally (table 12.1 case number 3). Antenatal specimens were unavailable for testing in one case, where hospital facilities did not allow for storage of antenatal samples for more than four months (table 12.1 case number 12). All 15 women had avidity results less than 0.3 in the postnatal period suggestive of recent infection. Seroconversion was estimated to have occurred during the third trimester in 11 of 15 (73%) women.

Six of 11 women with trimester 3 seroconversion demonstrated postnatal serology with levels for IgM and/or IgA recorded as strongly positive. This together with low avidity indices supported the estimation of maternal infection and seroconversion in trimester 3 (table 12.1 case numbers 1, 5, 6, 7, 9 and 13). In the remaining 5 of 11 women with trimester 3 seroconversion (table 12.1 case numbers 4, 8, 10, 11 and 14), all 5 had IgM detected by ELISA and/or ISAGA with postnatal avidity.
indices of ≤ 0.2 and 4 were also IgA positive, one of whom had an infant with a strongly positive ELISA IgM (table 12.1 case number 11), all suggestive of trimester 3 seroconversion. Two of 15 women (13%) had serology consistent with trimester 2 seroconversion (table 12.1 case numbers 2 and 3).

Maternal postnatal serology in case number 2 demonstrated a DT of 4,000 IU/mL, positive ISAGA IgA and IgM and an avidity of 0.07. Retrospective testing of the first antenatal sample from 20 weeks gestation demonstrated a low dye test titre of 125 IU/mL, a positive ELISA IgM and a negative ISAGA IgA and IgM. The results suggested early seroconversion at the time of antenatal sampling or possibly within the previous four weeks, i.e., 16 to 20 weeks gestation. The postnatal increase in the DT, together with IgM and IgA detectable by ISAGA, previously negative at 20 weeks, supported trimester 2 seroconversion.

One woman in the cohort of 15 had trimester 2 seroconversion and infant congenital infection confirmed antenatally following investigations for an abnormal fetal ultrasound scan at 28 weeks gestation (table 12.1 case number 3). The first antenatal sample from 22 weeks gestation was toxoplasma antibody negative (data not displayed). Antenatal results displayed in the table were from serology taken at 28 weeks gestation following detection of fetal signs consistent with CT. Antiprotozoals were administered for the remainder of trimester 3. Maternal postnatal serology displayed in the table was taken three days postpartum.

Two of 15 women (13%) in the cohort had serology consistent with seroconversion likely in trimester 1 or trimester 2 (table 12.1 case numbers 12 and 15). Both cases demonstrated similar postnatal toxoplasma antibody profiles with a dye test of 2,000 IU/mL, borderline ELISA IgM, positive IgA and low avidity. The interpretation from the Director of the TRL was that the high dye test in conjunction with the borderline ELISA IgM suggested a serological profile towards the end of the IgM response detectable by ELISA, i.e., six to nine months old. The low avidity in both cases was attributed to delayed maturation associated with pregnancy. Stored antenatal serology was not available in one of these cases for comparative analysis (table 12.1 case number 12). In the other case, antenatal serology from the eighth week of gestation was negative (table 12.1 case number 15) and it was highly likely that maternal infection was at least six months old and occurred in
trimester 1 or early trimester 2 despite the postnatal avidity of 0.11, as the infant was symptomatic at birth.

12.4.2 Infant clinical evaluation

12.4.2.1 Summary of infant evaluation

The median age at infant evaluation was 4 weeks (range 3 days to 17 weeks). Infant evaluation in the newborn period is summarised in table 12.2. Two of 15 infants (13%) were symptomatic; one was diagnosed *in-utero* at 28 weeks gestation (table 12.2 case number 3) and the other had hydrocephalus at birth (table 12.2 case number 15).

Thirteen of 15 infants (87%) had a normal routine physical examination at birth with no symptoms of congenital infection. Four of 13 asymptomatic infants had signs of CT on further evaluation (table 12.2 case numbers 5, 7, 8 and 12). Thus in the cohort of 15 infants with CT, 6 (40%) had signs of CT on initial evaluation during the first weeks of life, only 2 of whom (13%) were symptomatic prior to detailed evaluation.

All infants were born at term i.e. at ≥ 37 weeks of gestation. Birth weights and head circumferences were within the normal percentile range for gestational age, with the exception of the head circumference of the infant with hydrocephalus.

12.4.2.2 Symptomatic infants

One infant was symptomatic *in-utero* with ascites and ventriculomegaly detected on a routine ultrasound scan at 28 weeks gestation (table 12.2 case number 3). Toxoplasma infection was confirmed by a positive amniotic fluid PCR at 28 weeks from which time maternal treatment was commenced. Evaluation of the infant at birth revealed ventricular dilatation, multiple areas of intracranial calcification and bilateral inactive chorioretinitis involving the right macula with absence of central visual potential in the right eye, i.e., unilateral central blindness.

One other symptomatic infant was born with congenital hydrocephalus (table 12.2 case number 15). The infant was born in a maternity unit located outside county Dublin. Birth head circumference was 40 centimetres (cm), which was above the 99th centile. The large head circumference was not evaluated further at birth. Hydrocephalus was first noted at age three weeks by a public health nurse (PHN)
who prompted evaluation; cranial ultrasound scan demonstrated significant ventriculomegaly. The infant was then referred by local paediatric services to TCUH Dublin for ventriculoperitoneal (VP) shunt insertion.

Cranial imaging performed at TCUH prior to shunt insertion on day 23 of life demonstrated aqueduct stenosis, extensive hydrocephalus and multiple foci of periventricular calcification. The positive newborn screening result was available on day 28 of life, five days post VP shunt insertion at which time the possibility of CT as a cause of the hydrocephalus was first considered. Serology confirmed CT. Further evaluation revealed a unilateral inactive chorioretinal scar. Both symptomatic infants in the cohort of 15 had severe CT by definition.

12.4.2.3 Asymptomatic infants
Thirteen of 15 infants (87%) were asymptomatic at birth. Four of 13 (31%) asymptomatic infants had signs of CT on further evaluation (table 12.2 case numbers 5, 7, 8 and 12). All 4 had inactive chorioretinal lesions; 2 of who also had intracranial calcification (Table 12.2 case numbers 5 and 8). Hence of the 13 asymptomatic infants, 31% had chorioretinal lesions and 15% had both intraocular and intracranial lesions. One asymptomatic infant (case number 5) with multiple intracranial calcifications had signs of severe CT by definition.

12.4.2.4 Infant blood and CSF analysis for toxoplasma PCR
Testing of whole blood for toxoplasma-specific PCR was negative in all 15 infants with CT. CSF-PCR was negative in 12 of 13 infants tested. One infant with equivocal confirmatory serology in the newborn period and in whom the diagnosis of CT was confirmed beyond six months of age, did not have CSF sampling performed (table 12.2 case number 4).

CSF sampling was unsuccessful in one infant (table 12.2 case number 12). One symptomatic infant with non-communicating congenital hydrocephalus had a negative PCR in CSF taken from spinal fluid and a positive PCR in a CSF sample taken directly from the ventricular area during VP shunt insertion (table 12.2 case number 15).
12.4.2.5 Ophthalmology assessment of 15 infants with CT
Active chorioretinitis was not detected in the cohort at initial evaluation. Six infants (40%) had inactive chorioretinal scars (table 12.2 case numbers 3, 5, 7, 8, 12 and 15). Three had a unilateral lesion (table 12.2 case numbers 7, 12 and 15) and three had bilateral lesions (table 12.2 case numbers 3, 5 and 8). Four of six infants with inactive chorioretinal lesions were asymptomatic at birth; two with bilateral and two with unilateral lesions.

In five of six infants, chorioretinal lesions were located peripherally with no visual impairment detected at birth. One infant with symptomatic CT in-utero had bilateral inactive chorioretinal scars and a large unilateral central macular scar with associated visual impairment (table 12.2 case number 3).

12.4.2.6 Neurology examination and intracranial imaging
Two infants with symptoms of CT had abnormal neurological signs detected by physical examination (table 12.2 case numbers 3 and 15). Both infants had central hypotonia with head-lag and one also had lower limb hyper-reflexia (table 12.2 case number 15). Thirteen asymptomatic infants, including four with subclinical signs, had a normal neurological examination.

Four of 15 infants (27%) had intracranial calcification detected on imaging in the newborn period, three had multiple foci and one had a single focus (table 12.2 case numbers 3, 5, 8 and 15). One infant with multiple punctate foci of calcification in both hemispheres and the right periventricular area was asymptomatic at birth but met criteria for severe CT (table 12.2 case number 5). One infant with multiple frontal lobe and periventricular calcifications had ventriculomegaly in-utero and at birth (table 12.2 case number 3). The infant with hydrocephalus had imaging that confirmed aqueduct stenosis with significant ventricular dilatation (table 12.2 case number 15). Intracranial imaging is demonstrated and discussed in further detail in chapter 15.

12.4.2.7 Intracranial and intraocular abnormalities
Four of 15 infants (27%) had both intracranial and intraocular lesions (table 12.2 case numbers 3, 5, 8 and 15), two were asymptomatic.
12.4.2.8 Audiology evaluation
All infants had normal audiology assessments.

12.5 Discussion

12.5.1 Summary of serologically confirmed infants with CT
For the confirmation of CT, best diagnostic performance is achieved by appropriate combinations of serological, PCR and or culture-based techniques where applicable. Combination testing is recommended to ensure that infected cases will not be missed (Pinon et al., 2001). In the cohort studied, serologic profile in the newborn period was consistent with congenital infection in 13 infants, for whom analysis of just one sample in the newborn period with a paired maternal sample was sufficient to serologically confirm the diagnosis of CT. Two of 15 infants required more extensive testing in order to confirm CT. The ISAGA IgA assay gave the highest yield in the newborn period with 93% of infants testing positive, followed by the ISAGA IgM test for which 87% of the cohort tested positive on the first confirmatory sample.

A French study (Wallon et al) to determine the diagnostic accuracy of IgM and IgA tests assessed these tests in the context of routine clinical practice on 233 newborns with CT and 661 healthy controls. Results demonstrated that the ISAGA IgA assay was 100% specific for detection of congenital toxoplasma infection in neonatal blood and the IgM assay was 98% specific. Sensitivities for IgA and IgM were 60% and 61% respectively. The authors recommended that these two assays should be used in combination, which increases the overall sensitivity to 73% and maintains specificity at 98% (Wallon et al., 1999a). The serological results from our cohort of 15 infected infants were consistent with the findings by Wallon et al.

Previous estimates that 30% of infants with CT and in some instances as many as 50% will not produce significant quantities of toxoplasma specific IgM (Remington, 2011) were not confirmed in our study, in which despite the small total number, only 2 of 15 (13%) infants had no significant IgM. Plausible explanations for lack of IgM in both infants were found. One asymptomatic infant was infected late in trimester 3 with equivocal serology throughout infancy (table 12.1 case number 4); the other was an infant with severe symptoms at birth consistent with early in-utero
infection, hence the IgM response had probably ended in the newborn period (table 12.1 case number 15).

12.5.2 Maternal seroconversion and the avidity test
Maternal serological profiles in all 15 cases were consistent with primary infection in pregnancy. Eleven women (73%) had seroconversion estimated in trimester 3, two in trimester 2 and two in trimester 1 or 2. In general the avidity index is initially low after primary host antigenic challenge and subsequently increases with time. The in-house IgG avidity test used by the TRL quantifies results as follows: sera taken early in infection (< 3 months) usually have avidity levels of < 0.3; most sera taken later in infection (> 6 months) demonstrate avidity levels > 0.40. Hence it is expected that women who seroconvert during trimester 3 will demonstrate avidity values of ≤ 0.3 in the postnatal period consistent with seroconversion less than three months prior. One mother in the cohort received antiprotozoal treatment following confirmation of an infected fetus. Hence in this case, maternal postnatal serology reflected a serological response to treatment (table 12.1 case number 3). Postnatal avidity results for the 15 mothers ranged from very low (VL) to 0.25 (table 12.1). Hence despite variation in trimester of seroconversion, the avidity was < 0.3 in all mothers with congenitally infected infants.

Persistently low postnatal avidity was seen in three mothers who most likely seroconverted prior to trimester 3 (table 12.1 case numbers 2, 12 and 15), which can be a confounding factor for interpretation of maternal serology in conjunction with infant serology for the diagnosis of CT. For these three, conclusions on timing of maternal seroconversion could not be drawn solely from the avidity value but from interpretation of the avidity result in conjunction with other serological indices and infant symptoms where applicable. This was exemplified by case number 15, where the maternal avidity of 0.11 suggested trimester 3 seroconversion, but infant hydrocephalus was consistent with infection no later than trimester 2.

Persistently low avidity in women who seroconvert during pregnancy at any gestation is well documented in the literature, and is thought to be a physiological process during pregnancy that delays the natural maturation of immunoglobulin (Buffolano et al., 2004).
12.5.3 Timing of maternal seroconversion correlated to infant symptoms and signs

Severity of infant disease is primarily influenced by the trimester in which maternal infection occurred, in addition to the virulence of the infecting parasite, and host genetic factors that control immunity (de Souza-e-Silva CH et al., 2013; Sloves et al., 2015). The majority of women in the cohort (73%) had seroconversion estimated in trimester 3, when the risk for transmission to the infant is highest but the risk for infant signs and associated sequelae is lowest.

Data analysed by Dunn et al for 554 French mothers with congenitally infected infants demonstrated that risks for infant transmission increased from 6% at 13 weeks to 72% at 36 weeks gestation, but fetuses infected in early pregnancy were more likely to be symptomatic. The authors also estimated from their study that women who seroconverted between 24 to 30 weeks carried the highest risk of having an infant with early clinical signs and long term sequelae (Dunn et al., 1999). However other studies conclude that the highest risk for infant signs in the brain and eyes occurs with transmission from the 10th week of gestation to the 24th week (Remington, 2011).

In our cohort of 15 congenitally infected infants, 11 infants were born to mothers with estimated trimester 3 seroconversion and all were asymptomatic at birth, 4 of whom had signs of CT on further evaluation consistent with inactive disease (table 12.2 case numbers 5, 7, 8 and 12).

Drastically different outcomes were observed for two infants born to mothers who seroconverted during the gestations associated with a high rate of severe infant signs (table 12.2 case numbers 2 and 3). In one case of maternal seroconversion in trimester 2 evident from retrospective testing of maternal serology at 20 weeks gestation with low level dye test and positive ELISA IgM demonstrated (table 12.2 case number 2), infant symptoms and signs were absent. In the second case of maternal seroconversion in trimester 2, fetal signs were evident in-utero from 28 weeks gestation with ascites and ventriculomegaly (table 12.2 case number 3). Evaluation at birth demonstrated that the infant had criteria for severe CT, with unilateral visual impairment and multiple calcific foci.
Different outcomes were also noted for two infants born to mothers with seroconversion in either late trimester 1 or early trimester 2 (table 12.2 case numbers 12 and 15). One infant was asymptomatic with a unilateral inactive chorioretinal scar detected on evaluation consistent with mild disease. The other infant had severe signs on evaluation with hydrocephalus and multiple intracranial calcifications.

These cases collectively highlight that infants born to mothers who seroconvert when the risk of damage to the developing fetus is generally high, can have entirely different outcomes as demonstrated by comparison of two infants born to mothers infected in trimester 2 and two born to mothers infected either late in trimester 1 or at some point during trimester 2.

In both sets of comparative cases maternal seroconversion likely occurred at the same time. There are two possible explanations for the dramatically different infant outcomes. One is that in the cases with mild or no infant disease manifested, whilst maternal seroconversion occurred in the earlier half of pregnancy, maternal transmission to the infant may have been delayed to later in pregnancy. Studies from large French cohorts that employ antenatal screening have demonstrated that transmission of T. gondii infection to the infant may be delayed in some instances following maternal seroconversion in pregnancy, and there may be a time lapse between maternal infection and transmission to the infant during which maternal antiprotozoal therapy may be beneficial. Whilst this theory has not been proven in RCTs, it is the premise under which the French antenatal screening programme operates.

French studies have shown that in some instances, women with proven seroconversion in pregnancy and negative amniotic fluid PCR, give birth to infants with confirmed CT (Hohlfeld et al., 1994). As the false negative rate associated with toxoplasma PCR is very low (Bhalla et al., 1999;Romand et al., 2004), it is likely that in some instances women who acquire infection in pregnancy do not transmit to the fetus until many weeks later. Hence if extrapolated to seroconversion in pregnancy during a high risk period for fetal signs, such as trimester 1 or 2, delayed transmission to trimester 3 when the risk for fetal organ damage is low will result in an asymptomatic infant or an infant with mild signs.
This delay is sometimes referred to as the prenatal incubation period, and the delay between maternal and fetal infection can vary. Transmission can occur during initial maternal parasitaemia, or be delayed for 16 weeks or longer. The parasite may be present in the placenta but infect the fetus after a delay, even long after maternal parasitaemia ceases (Thulliez, 2001). This in part explains why infants born to mothers with similar timing of seroconversion can have contrasting manifestations.

Variation in disease manifestation amongst congenitally infected children within a given population has also been explained by variation in parasite and host genetics. On large continents such as South America, parasite strain can vary amongst regions in a particular country. Differences in parasite genetics are not likely to be applicable to our pregnant population as in Europe the type II strain of *T. gondii* predominates and is associated with low disease burden and benign disease (Peyron et al., 2006). All women in the studied cohort were residing in Ireland for the duration of pregnancy.

However, host genetic factors that influence immunity offers an explanation as to why amongst women who seroconvert during the high risk period, some will have uninfected infants, some will have infants with severe signs and some will have infants with mild or no signs (Liu et al., 2014). Jamieson and colleagues studied mother-infant pairs from the European EMSCOT trial and the North American NCCCTS group and demonstrated that host genetic and epigenetic factors play a role in the pathogenesis of brain and eye disease in CT. The effect of helper T-cells on protective cytokines at the maternal-fetal interface can influence infant outcome. The authors concluded that maternal cell mediated immunity against *T. gondii* infection during pregnancy will either contribute to maternal-fetal transmission or protect against it to varying degrees (Jamieson et al., 2008).

This explanation has been supported by a recent study from Poland on the placentas of congenitally infected infants. Wujcicka and colleagues demonstrated that expression of Toll-like receptors (TLRs) on placental cells were involved in immunity against *T. gondii* and protection against dissemination from the placenta to the fetus. The expression of TLRs varied with placental cell type, gestational age and parasite strain. The study suggested that a high level of induction of TLRs occurs against type II strains of *T. gondii* compared with the type I strain. The
authors suggested that genetic alterations in TLRs alters placental immunity against *T. gondii* and hence protection against transmission to the fetus. Further research has been proposed in this particular area (Wujcicka et al., 2014).

12.5.4 Infant toxoplasma PCR

Analysis of CSF for *T. gondii* PCR has been shown to aid the diagnostic confirmation of CT in infants born to mothers who were not treated antenatally (Olariu et al., 2014). A positive PCR in infant blood or CSF signifies parasitaemia or active disease, and is usually demonstrable in infants with symptoms evident at birth or infants with severe disease (Hohlfeld et al., 1994; Wallon et al., 2010). However the yield from PCR analysis in our cohort was low, likely due to the high percentage of asymptomatic infants.

One infant diagnosed *in-utero* by positive toxoplasma PCR in amniotic fluid had a negative blood and CSF-PCR in the newborn period. This may be due to treatment *in-utero* with antiprotozoals for several weeks prior to delivery. Positive CSF-PCR was documented in only one infant (7%), who had such severity of symptoms in the newborn period that a positive CSF-PCR was expected (table 12.2 case number 15). The positive PCR from the periventricular area in this infant with congenital hydrocephalus indicated parasitaemia within the brain during the newborn period.

12.5.5 Congenitally infected infants with inconclusive serology

12.5.5.1 Summary

Two asymptomatic infants with CT had equivocal serology in the newborn period that could not confirm or out-rule CT for a number of months (table 12.1 case numbers 4 and 12). Repeat samples were serially tested for antibody profile at the TRL in Swansea.

Following three sets of equivocal results, both infants required additional testing with WB technique. The director of the TRL dispatched paired mother-infant samples to the Scottish Toxoplasma Reference Laboratory and National Lyme Borrellosis testing laboratory at Raigmore hospital, Inverness, Scotland UK for WB testing. WB analysis was negative in one case (table 12.1 case number 4) and positive in the other (table 12.1 case number 12).
In the interim, whilst awaiting confirmation, infant evaluation was performed. One infant had no signs on evaluation performed at three months (table 12.2 case number 4) whilst the other with a positive WB had an inactive chorioretinal scar detected which also enabled confirmation (table 12.2 case number 12). Hence one infant was confirmed positive at age 13 weeks by a positive WB and demonstration of a clinical sign consistent with CT, whilst the other with no signs of CT and negative WB required serial toxoplasma antibody profile tests during the first year of life to confirm CT.

12.5.5.2 Serological profile of congenitally infected infant case number 4

Table 12.3 summarises the serological profile for this infant during the first year of life. The infant's antibody profile supported the finding that some infants with CT will not produce any IgM or IgM in significant quantities.

Another confounding element of the serologic profile in this case was the low level DT quantified in the first three months. Western Blot and other immunoblot assays have been reported as useful adjunctive tools for serological diagnosis of CT in newborns with low level toxoplasma antibody and absence of specific IgA and IgM. For analysis by WB technique, *T. gondii* antigen is added to maternal and infant sera and antibody bands are compared following electrophoresis. Bands of infant antibody in a congenitally infected case will contain proteins unique to infant antibody, which enables differentiation between passively transferred maternal IgG antibodies and antibodies synthesised by the infant (Ho-Yen et al., 2000; Nielsen et al., 2005; Franck et al., 2008).

WB technique failed to confirm CT in this infant at 12 weeks. A negative result was reported based on the fact that there were no unique infant antibody bands or unique infant proteins bound to antibody in infant serum compared with maternal serum. In addition the total quantity of antibody in infant serum was sparser than that in maternal serum and hence it was concluded that at three months of age, the infant profile was consistent with passively transferred maternal antibody.

WB and other immunoblot techniques rely on production of adequate antibody by the infant, and in this case it is likely that the quantity of infant antibody was not suffice for the test to yield a positive result at the time of analysis. This infant was
likely infected late in trimester 3. The only serologic evidence of possible CT in the newborn period was the positive IgA in the first confirmatory test. For serology interpretation and recommendations in this case, the Director of the TRL liaised with European toxoplasma experts. Recommendations were to withhold treatment based on the first three sets of equivocal results obtained up to the age of six weeks (table 12.3). All experts were in unanimous agreement that the first three serial tests did not confirm or rule out CT. The advice was to await results of repeat testing at 12 weeks. This decision was supported by the supervisor of the Danish newborn toxoplasma screening programme which was ongoing at that time. The overall agreement was that it was more important to confirm the diagnosis rather than initiate anti-toxoplasma treatment based on inconclusive serology.

The negative WB result obtained from testing of the 12-week serology sample led some experts to conclude that the infant was not infected. However there was lack of agreement among the expert community regarding the conclusion drawn from the negative WB result and serology thus far. Some voices insisted that the negative WB result did not rule out congenital infection, and more importantly, persistent IgA at the age of 12 weeks confirmed CT and the infant should be treated.

At this point, due to the lack of agreement amongst the expert community, the advice from the director of the TRL was that the diagnosis of CT in this case could only be ascertained by demonstration of persistently positive antibody at or beyond one year of age. Subsequently, the diagnosis of CT was strongly suspected from results of the serologic profile at age six months, despite the previously negative WB. At six months, apart from a persistent IgA, there had been an overall increasing trend in the DT from birth, and whilst this was explained to be insignificant as there had not been a four-fold rise between two consecutive tests, the DT was not decreasing, which would be expected with passively transferred maternal antibody.

It became clear by the age of nine months that it was not necessary to await serological testing at one year to confirm that this was in fact a case of CT. Hence CT was confirmed at nine months of age based on persistent IgA thus far and this was supported by the results obtained at age 12 months (table 12.3).
Following confirmation in late infancy, advice was sought from European experts regarding initiation of infant treatment. Arguments were put forward for and against treatment initiation at nine months. The group for treatment argued that in theory the organism may remain active without treatment in infancy and treatment should commence albeit at nine months. The group against treatment argued that there was no evidence to support initiation of treatment at almost one year of age in an asymptomatic immunocompetent child, at which point potential toxicity from treatment outweighed benefits. The advice from the latter group was that it was more beneficial to adopt a conservative approach with regular monitoring and early intervention if ocular disease occurred in the future. The infant’s parents opted for the latter approach. The infant had been evaluated at age six weeks and continued with regular clinical evaluation as per the CT management protocol. The inability to confirm the diagnosis in this case was associated with much parental anxiety. The parents stated that they had experienced emotional stress for almost a year. Not only was the diagnosis inconclusive for many months, but at three months the parents were told that it was possible the infant was not congenitally infected based on the negative WB. At that point the parents were hopeful that was the end of the diagnostic dilemma, only to be told that further testing was necessary for a definitive conclusion, which led to a positive diagnosis at nine months. Following the diagnosis the parents then faced the further dilemma of a decision regarding treatment initiation for one year.

This case demonstrated the difficulty associated with diagnostic confirmation in an asymptomatic infant with an equivocal serological profile. Whilst the tests used for confirmation of CT in Europe are generally reliable with high specificity and sensitivity when used in combination, challenges remain for infants who produce low-level antibody, as demonstrated by failure of WB to aid with diagnostic confirmation. The falsely negative WB was attributed to the fact that at the point when the analysis was performed, the infant was not producing antibody in significant quantities to be detected or distinguished from maternal antibody by WB methods.

The most important conclusion drawn from this case was that in infancy, the sole presence of IgA, even with a very low DT, can indicate CT and is diagnostic if persistent at 12 weeks. The experience encountered with this case supports the
previously discussed finding by Wallon et al that the ISAGA IgA assay was 100% specific for detection of congenital toxoplasma infection in neonatal blood.

12.5.5.3 Serologic profile of congenitally infected infant case number 12
The first confirmatory sample taken at age three weeks from case number 12 appeared to be consistent with congenital infection with a high DT, positive IgM and IgA detected by ISAGA. However the advice from the director of the TRL was that the sample should be repeated in three weeks time for further confirmation, as although the ISAGA IgM result was released as a positive result, the actual index was between borderline and positive, i.e., an agglutination index of 8 to 9 was obtained. A repeat sample was recommended to determine whether IgM detectable by ISAGA was infant or maternal in origin (table 12.1 case number 12). It could be questioned why the first serological profile obtained at age three weeks was not taken as confirmatory, as ISAGA IgA and IgM were both positive and the IgM result was not released as borderline. It was thought best to accept the advice from the Director of the TRL, as quality and technical issues with result interpretation are frequently encountered by Reference Laboratory experts.

Subsequent confirmatory tests at 6 and 13 weeks demonstrated a serological profile similar to that obtained from case number 4. Positive IgA with disappearance of IgM was demonstrated and hence the diagnosis could not be confirmed or excluded on three serology tests up to the age of 13 weeks. However WB analysis was positive on the sample taken from the infant at age 13 weeks (table 12.1 case number 12). Infant clinical evaluation was initiated whilst awaiting results of WB analysis and an inactive chorioretinal scar was detected in the right eye.

Compared with case number 4, the difference with this case was that a positive WB at three months confirmed the diagnosis. In addition, the presence of an intraocular lesion consistent with CT enabled confirmation of the diagnosis beyond doubt even if the WB was negative. The inactive chorioretinal scar, indicated that the disease was active in fetal life. The infant had a high DT and it can be assumed that the infant was actively producing antibody, which enabled WB confirmation despite the fact that confirmatory serology was equivocal. In the former infant (case number 4) with equivocal serology there were no signs of CT on evaluation. A significant immune response had not yet been mounted by the infant and this was reflected by the negative WB result.
Case number 12 further highlighted that some infants with CT do not produce significant quantities of IgM in the newborn period, and for those infants who have persistence of IgA beyond six weeks of life, the diagnosis of CT must be strongly suspected. Had case number 12 a negative WB result at 13 weeks and no signs of CT on evaluation, the diagnosis would have been confirmed all the same and the infant assigned to treatment, based on the lesson learned from case number 4.

Other methods for early postnatal diagnosis of CT have been examined. Researchers have reported on the use of recombinant antigens and interferon assays for the diagnosis of CT. Conclusions are that these methods are effective, particularly when the serological diagnosis is not conclusive. However in the populations studied some cases also required two serial samples between months one to three of life in order to confirm CT. Expense and availability of the tests limit their use (Buffolano et al., 2005; Chapey et al., 2015). Studies in the area of improving early diagnosis of CT are welcome, particularly for those infants with equivocal serology.

**12.5.6 Infant clinical evaluation**

In countries with a low incidence and disease burden of CT, newborn screening primarily identifies asymptomatic infants who would have been otherwise unrecognised. This study demonstrated that whilst a small number of infants were identified by newborn screening, the majority would not have been detected by routine newborn paediatric examination or developmental surveillance in infancy and childhood.

The findings on initial evaluation in our study were compared with the findings from newborn screening in Denmark (1999-2007), where the screening methods, incidence of CT (1.6 per 10,000) and population demographics were similar to that of Ireland. Interim results from the first four years of screening in Denmark were published during the CT screening programme in Ireland (Schmidt et al., 2006a). Compared with the findings from newborn screening in Denmark where 27% of the cohort with CT had clinical signs at initial evaluation, a higher rate was demonstrated in the Irish cohort where 40% of infants had clinical signs at first assessment (table 12.4). Severe and symptomatic disease was uncommon in the
both the Irish and Danish cohorts. The symptomatic disease rate for the population screened in Ireland was 0.13 per 10,000.

Apart from Denmark, it may not be altogether accurate to directly compare infant presentation at birth in the Irish cohort with that of other European countries where infants are identified through antenatal screening. However the results from the Irish CT screening programme were similar to that for other European countries that have demonstrated that overall, severe symptomatic disease is uncommon in Europe (Gilbert et al., 2008; Cortina-Borja et al., 2010).

The absence of severe congenital disease in the Irish study is in part explained by the finding that in Europe, the predominant strain of *T. gondii* is the avirulent type II strain (Peyron et al., 2006). The EMSCOT study reported an association between congenital infection and preterm delivery, particularly in infants born to mothers who seroconverted before 20 weeks gestation (Freeman et al., 2005). Shorter gestation was not identified in our cohort of 15 infants, even in the 2 infants with severe signs (table 12.2 case numbers 3 and 15).

12.5.6.1 Symptomatic infants

Both symptomatic infants (table 12.2 case numbers 3 and 15) by definition met the criteria for severe CT, defined as the presence of at least one of the following signs on evaluation at birth: microphthalmia, hydrocephaly, microcephaly, seizures, ≥ three cerebral calcifications, extensive visual impairment (Olariu et al., 2011).

One symptomatic infant was diagnosed *in-utero* (Table 12.2 case number 3) and the other in the newborn period with hydrocephalus (Table 12.2 case number 15). The latter infant’s signs in the newborn period comprised the classic triad found in CT, defined as hydrocephalus, intracranial calcification and chorioretinitis. Even though the infant’s chorioretinal lesion was inactive at diagnosis, all the manifestations of the triad would have been active in fetal life. It was unusual that such gross hydrocephalus was not detected at any point during routine antenatal scans. According to the antenatal history, a booking visit was attended at eight weeks gestation. Routine ultrasound scans were performed throughout pregnancy. It is assumed that, given the degree of hydrocephalus present at birth at 37 weeks,
ventriculomegaly would have been evident at some point in the later months of gestation.

It is also perplexing that the infant's large head circumference was not noted as abnormal in appearance and hypotonia was not detected at birth. It is standard policy that all infants receive a thorough newborn examination prior to discharge from the maternity unit, which includes a newborn neurological exam with fundus visualisation to elicit newborn red reflexes. Hence routine newborn clinical examination should have revealed major abnormalities, which were evident when the infant was evaluated at age three weeks following referral for further management of hydrocephalus and prior to VP shunt insertion. Apart from the head circumference above the highest percentile, the infant had head lag, dilated forehead and scalp veins, eyes in the 'sun-setting' position, central hypotonia and exaggerated lower limb reflexes. It is likely such signs were present from birth even if to a lesser degree than at three weeks. The clinical records of the mother and infant could not be accessed from the birth hospital to provide answers to these questions.

It is also remarkable that with such gross hydrocephalus, feeding continued without issues in the interim and signs of raised intracranial pressure such as vomiting or seizures did not develop. According to the infant's mother, she noted nothing of concern during the three weeks. The infant's head size, facial appearance and poor tone did not cause concern as the infant fed well and was not excessively irritable.

This infant's scenario was a case of symptomatic CT with neurological signs that were undetected until age three weeks. This contrasts with the first symptomatic infant who was promptly diagnosed and treated when in-utero signs of fetal infection were first detected (table 12.2 case number 3). For this infant, it seems reasonable to speculate that the 28 week gestation signs of early ventricular dilatation and ascites would have progressed without intervention, and presentation at birth would have been gross hydrocephalus as in the aforementioned infant. Symptomatic CT most often presents neurologically, either at birth or during infancy. A wide spectrum of neurological symptoms has been demonstrated in large cohort studies and includes hydrocephaly, microcephaly, seizures, encephalopathy, or a subtle neurologic syndrome; however the most
The common neurological manifestation is obstructive hydrocephalus (McLeod et al., 2006; Hutson et al., 2015). Clinically apparent severe disease can also present with multisystem disease in the absence of neurological symptoms, but this is less common (Armstrong et al., 2004).

Neither of the symptomatic infants in the cohort of 15 had evidence of systemic involvement other than the CNS. There was no evidence of hepatosplenic, pulmonary or bone marrow involvement in the newborn period.

12.5.6.2 Asymptomatic infants
Asymptomatic infants are also referred to as infants with subclinical infection, i.e., serologically confirmed infection but with no apparent symptoms at birth to suggest CT. In some instances, infants initially deemed to be asymptomatic can have evidence of a systemic infectious process, such as depression of bone marrow cell lines, elevated transaminases or abnormal CSF biochemical indices. The asymptomatic infants in this study all had normal blood counts, CSF biochemical indices and liver and renal function on evaluation in the newborn period.

Asymptomatic infants can also develop general signs of systemic infection that may have delayed onset later in the newborn period or infancy such as fever, rash, prolonged jaundice, seizures and pneumonitis, none of which occurred in the 13 asymptomatic infants, 4 of who had intraocular signs of CT on evaluation including 2 with intracranial signs. Thus in the cohort of 13 asymptomatic infants, 4 had evidence of subclinical disease, one of whom had signs of severe CT (table 12.2 case number 5).

12.6 Conclusions
In the cohort of 15 infants, 13 (87%) had serological profiles typical of CT and were confirmed in early infancy. Two infants (13%) had inconclusive serology and required more extensive serological testing in addition to clinical evaluation to assist diagnostic confirmation which was made later in infancy. Eleven of 15 mothers (73%) were estimated to have seroconverted in trimester 3.

Six of 15 (40%) infants had signs of CT on initial evaluation, only two of whom (13%) were symptomatic; one had been diagnosed *in-utero*, the other in the newborn period. The majority of infected infants (87%) were asymptomatic.
However one third of the asymptomatic infants had lesions of CT detected only because of screening. Clinical findings in our cohort of infants with CT and the low rate of disease severity were consistent with European data.
Table 12.1: 15 infants with confirmed congenital toxoplasmosis; screening results and confirmatory serology (15 mother-infant pairs)

<table>
<thead>
<tr>
<th>Infant Case Number</th>
<th>AutoDELFIA IgM IU/mL</th>
<th>ISAGA IgM Screen</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Trimester of Maternal Seroconversion (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dye test IU/mL</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
</tr>
<tr>
<td>1</td>
<td>188, 182, 191</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>206, 198, 218</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>166, 165, 179</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>210, 204, 218</td>
<td>Pos</td>
<td>125</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>595, 558, 568</td>
<td>Pos</td>
<td>4000</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
</tr>
<tr>
<td>6</td>
<td>1106, 926, 953</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>1070, 970, 948</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>8</td>
<td>450, 432, 424</td>
<td>Pos</td>
<td>1000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>1289, 1276, 1298</td>
<td>Pos</td>
<td>2000</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
</tr>
<tr>
<td>10</td>
<td>441, 282, 247</td>
<td>Pos</td>
<td>1000</td>
<td>BD</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>11</td>
<td>894, 897, 908</td>
<td>Pos</td>
<td>1000</td>
<td>SP</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>12</td>
<td>265, 285, 277</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>13</td>
<td>737, 713, 674</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>14</td>
<td>161, 189, 171</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>15</td>
<td>86, 91, 98</td>
<td>Pos</td>
<td>2000</td>
<td>Neg</td>
<td>BD</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Pos, positive; SP, strongly positive; VL, very low; Neg, negative; NT, not tested; BD, borderline; WB, Western Blot; NA, not available
Table 12.2: Initial evaluation summary for 15 congenitally infected infants

<table>
<thead>
<tr>
<th>Infant Case number</th>
<th>Blood PCR</th>
<th>CSF PCR</th>
<th>Ophthalmology</th>
<th>Brain imaging</th>
<th>Audiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
<td>B/L inactive chorioretinal scars RT central blindness</td>
<td>Multiple foci calcification. Mild ventricular dilatation</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>Neg</td>
<td>N/A</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
<td>B/L inactive chorioretinal scars</td>
<td>Multiple foci calcification</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>Neg</td>
<td>Neg</td>
<td>Unilateral inactive chorioretinal scar</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
<td>B/L inactive chorioretinal scars</td>
<td>Single focus of calcification</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>Neg</td>
<td>N/A</td>
<td>Unilateral inactive chorioretinal scar</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>Neg</td>
<td>Neg</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>Neg</td>
<td>Ventricular sample positive</td>
<td>Unilateral inactive chorioretinal scar</td>
<td>Congenital hydrocephalus. Multiple foci calcification</td>
<td>N</td>
</tr>
</tbody>
</table>

Neg, negative; N, normal; B/L, bilateral; RT, right; N/A, not available
Table 12.3: Serological profile for congenitally infected infant case number 4

<table>
<thead>
<tr>
<th>Age</th>
<th>Dye test IU/mL</th>
<th>ELISA IgM</th>
<th>ISAGA IgM</th>
<th>ISAGA IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>125</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>4 weeks</td>
<td>250</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>6 weeks‡</td>
<td>500</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>12 weeks† (WB)</td>
<td>1000</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>6 months</td>
<td>1000</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>9 months</td>
<td>500</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>12 months</td>
<td>500</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

‡Infant blood PCR negative at 6 weeks
†WB, Western Blot analysis demonstrated no unique infant antibody protein bands
Table 12.4: Signs on initial evaluation of cohorts screened for CT: Ireland compared with Denmark

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
</tr>
<tr>
<td>Total with clinical signs</td>
<td>6/15 (40%)</td>
<td>13/48 (27%)</td>
</tr>
<tr>
<td>Retinochoroiditis</td>
<td>6/15 (40%)</td>
<td>2/47 (4%)</td>
</tr>
<tr>
<td>Calcifications</td>
<td>4/15 (27%)</td>
<td>5/47 (11%)</td>
</tr>
<tr>
<td>Calcifications/ Retinochoroiditis</td>
<td>4/15 (27%)</td>
<td>4/47 (9%)</td>
</tr>
<tr>
<td>Calcifications/Retinochoroiditis/Hydrocephalus</td>
<td>1/15 (7%)</td>
<td>1/47 (2%)</td>
</tr>
</tbody>
</table>
CHAPTER 13 - Maternal demographics and risk factors for pregnancy seroconversion in 15 women with infected infants

13.1 Introduction

Primary toxoplasma infection in a seronegative individual can occur following ingestion of active toxoplasma oocysts (sporulated oocysts or sporozoites) or inactive latent cysts (bradyzoites). The rate of seroconversion within a population depends on antibody prevalence and exposure to risk factors.

Oocysts are shed in cat faeces and can survive in soil and water for approximately one year. Environmental sources of sporozoites include soil, a contaminated water supply and unwashed soil grown fruits and vegetables (Lass et al., 2012). Undercooked fresh meat and raw cured meat products are the main sources of bradyzoites that lie dormant in animal muscle tissue. Bradyzoites can be rendered nonviable by temperatures $> 67^\circ\text{C}$ or $< -12^\circ\text{C}$ (Bader et al., 1997). Freezing meat at $-20^\circ\text{C}$ for at least 18 to 24 hours followed by thawing can destroy tissue cysts (Dubey, 1974).

Of equal importance to reduce the risk of toxoplasma infection is the practice of sanitary kitchen techniques, such as thorough washing of utensils and cutting boards used for meat preparation, prior to re-use for other food products such as bread, fruit or vegetables.

European multicentre studies demonstrated that ingestion of rare meat most strongly predicted infection in pregnant women. Between 30% to 63% of infections in different centres were attributed to consumption of undercooked or raw cured meat products, whilst soil contact accounted for 6% to 17% of infection in pregnant women. Exposure to cats or kittens was not identified as a major risk factor for toxoplasma infection in Europe (Cook et al., 2000).

13.2 Objectives

1) To identify risk factors for toxoplasma infection in pregnancy in a cohort of women with infected infants.

2) To propose methods for targeting areas that could reduce exposure to risk factors for toxoplasma infection in pregnancy.
13.3 Methods

13.3.1 Population studied
Fifteen women with toxoplasma seroconversion in pregnancy that resulted in congenitally infected infants.

13.3.2 Methods
Maternal demographics were analysed.
Women were interviewed face to face by the CT screening programme co-ordinator. Specific questions were asked relating to lifestyle, dietary and culinary habits, potential exposure to contaminated food, soil or water.

13.4 Results

13.4.1 Summary of maternal demographics
Table 13.1 summarises details of 15 mothers with infected infants.

The median maternal age was 32 years. Five of the 15 women (33%) in the cohort were born outside Ireland and not of Irish ethnicity. Four of five were immigrants to Ireland within the previous 12 to 24 months and one had migrated to Ireland five years previously. No women reported travel outside Ireland during the pregnancy. Five of 15 (33%) women with congenitally infected infants were resident in Dublin city and the remaining 10 (67%) were resident in counties outside Dublin. Figure 13.1 displays the geographical distribution of CT compared with toxoplasma seroprevalence in women of childbearing age from a previous study in 1996.

13.4.2 Risk factors for toxoplasma infection during pregnancy
Table 13.2 displays individual maternal data for 15 women with congenitally infected infants.

Thirteen of 15 (87%) women reported lack of awareness of risk factors for acquisition of toxoplasma infection. Eleven of 15 (73%) women reported exposure to food risks, namely ingestion of undercooked meat or raw cured meat products during pregnancy; all 11 were unaware of the associated risks. The undercooked meats ingested were primarily beef and pork. None of the 11 women altered their habitual practices of cooking meat to rare or medium during pregnancy. In addition, women were unaware that air-dried, smoked and salt cured meats, collectively referred to as cured meat product e.g., salami, Parma and prosciutto
ham; are uncooked. Cured meat products were found to be a major dietary constituent of the four women from Eastern Europe (table 13.2 case numbers 5, 6, 9 and 15).

Ingestion of unwashed fruits and vegetables was not reported in this cohort. Two of 11 women reported other potential risk factors in addition to food risks. One had contact with cats. One reported a contaminated water supply from a well that served as the main source of water for drinking and domestic purposes; the well water frequently appeared discoloured (table 13.2 case numbers 5 and 13). Direct soil contact from regular gardening without gloves followed by inadequate hand hygiene was identified as a possible risk factor in one of 15 women.

No definite risks for toxoplasma infection were identified for three women (table 13.2 case numbers 2, 11 and 12) two of who had pre-conceptual knowledge of toxoplasmosis and the associated risks. Awareness of risk factors was not found to be increased in women who had previous pregnancies. One of 10 primiparous compared with one of five multiparous women had knowledge of associated risk factors (p=0.57, two-tail Fisher’s exact test).

13.5 Discussion

13.5.1 Maternal demographics
In the studied cohort, 67% of women with seroconversion in pregnancy and congenitally infected infants were of Irish ethnicity. Four of five migrant women were from Eastern Europe. For the purpose of this study the four countries; Lithuania, Poland, Russia and Estonia will be referred to as Eastern Europe. Poland is also classified as a central European nation and Estonia and Lithuania as Baltic nations. However, these countries are geographically represented within Eastern Europe on a world atlas and politically referred to as countries of the Eastern European region.

Overall toxoplasma seropositivity rates in Europe are quoted as 30% to 50%. Seropositivity in Mid and Western Europe is at the lower end of the range quoted, or less than 30% in some countries. Seropositivity in women of child bearing age in Eastern European countries is estimated at approximately 40% (Logar et al.,
1995) compared with the historical seroprevalence of 25% for Ireland (Ferguson et al., 2008).

Five women (33%) were resident in Dublin city and the remainder in counties outside Dublin. A previous study on toxoplasma seropositivity in women of childbearing age in Ireland demonstrated that the lowest antibody prevalence was in county Dublin (figure 13.1 map a). This can be partially explained by urban-rural differences and is consistent with reports from other countries that show a clear urban versus rural difference in toxoplasma seroprevalence (Carellos et al., 2014). The higher rate of toxoplasma seropositivity in rural areas is attributed to higher probability of risk exposure from: soil handling, consumption of fresh locally sourced meat, consumption of untreated water and the presence of cats in the neighbourhood.

Some 40% of infants born in Ireland are born in Dublin city. Given that maternal seropositivity of 19.9% in county Dublin was significantly below the mean compared with other counties (figure 13.1 map a), one would expect that up to 80% of pregnant women in Dublin could be at risk for seroconversion, with a higher rate of CT in Dublin as a result. This was not demonstrated. We proposed that the low antibody seroprevalence in Dublin reflects a low rate of exposure to risk factors for toxoplasma infection, with a corresponding low rate of toxoplasma seroconversion in the county's population. Hence the relatively low rate of CT in Dublin is simply explained by urban vs rural differences.

The median maternal age was 32 years. Three of 15 mothers were under age 30. Toxoplasma seroprevalence has been shown to increase with age (Nowakowska et al., 2014). A deduction cannot be drawn in that regard from the CT screening study as data from only 15 women with infected infants was analysed, rather than a large cohort of pregnant women. However, the aforementioned Irish seropositivity study (Ferguson et al., 2008) that utilised 20,252 DBS specimens to determine the seroprevalence of toxoplasma antibody in women of childbearing age demonstrated that the mean maternal age by county was similar throughout the country and the rate of seroprevalence did not increase with average maternal age (data presented in chapter 8, table 8.2).
Toxoplasma seroprevalence has also been shown to increase with parity or the number of pregnancies (Villena et al., 2010). Whilst conclusions cannot be drawn in this regard without a comparative group of seronegative women describing their parity, 10 of 15 women (67%) were of single parity and one woman (7%) was multiparous with more than three pregnancies (table 13.1). Hence in this study, despite the small cohort, primigravidas predominated. This would lead one to assume that in Ireland, multiparous women have previously seroconverted, are toxoplasma immune and are therefore under-represented in the cohort of women with infected infants. This supports the finding that toxoplasma seroprevalence increases with parity.

13.5.2 Lifestyle risk factors
The risk factors identified for pregnancy seroconversion were all related to lifestyle and environmental risks, which accounted for seroconversion in 12 of 15 (80%) women, all of whom were unaware of the associated risks (Ferguson et al., 2011).

Dietary habits and ingestion of food containing bradyzoite cysts was the possible mode of infection in 11 women (73%) who acquired infection in pregnancy. While women were aware of foods to avoid for salmonella or listeria infection, they were strikingly unaware of the hazards of eating undercooked meat and cured meat products during pregnancy. Uncooked air-dried and salt-cured meats were misperceived as being cooked due to the similar appearance and packaging of cooked and processed meats. Commercial techniques for processing ready-to-eat raw cured meats do not destroy T. gondii, and consumption of cured meat products has been long documented as a risk for infection in pregnancy (Warnekulasuriya et al., 1998).

Ingestion of undercooked meat and the lack of knowledge of the associated risks did not vary amongst socio-economic groups; however the small numbers prevent definite conclusions being drawn in this regard.

One woman, aware of risks associated with ingestion of undercooked meat in pregnancy had discontinued this practice during pregnancy. However exposure to sporozoites by soil contact from gardening followed by improper hand hygiene was a risk exposure as the mother was unaware of soil as a source for T. gondii infection (table 13.2 case number 3). Infant signs of CT were incidentally detected.
at 28 weeks gestation by a routine scan, which prompted maternal evaluation, confirmation of CT and maternal treatment with antiprotozoals from 30 weeks gestation. Following confirmation of CT the mother retrospectively reported symptoms of a mononucleosis-like illness at 24 weeks gestation that most likely represented acute toxoplasmosis, but medical attention was not sought. Symptoms of illness during pregnancy were also retrospectively reported in one other mother. Following postnatal confirmation of symptomatic infant infection, the mother described symptoms of swollen neck glands at approximately the 13th week of gestation, but medical attention was not sought (table 13.2 case number 15). It is possible that the reported maternal symptoms represented acute toxoplasma infection and seroconversion.

It has been suggested that severity of symptoms of toxoplasmosis in immunocompetent adults may depend upon the parasite stage ingested, with infections from sporozoite ingestion believed to elicit more clinically severe manifestations in the individual than ingestion of latent tissue cysts or bradyzoites (Krueger et al., 2014).

It was noted that of the 15 women in the cohort, the only two women who reported symptoms of illness during pregnancy were those whose infants developed severe signs. Whilst no conclusion can be drawn from this, one can speculate whether the presence of acute maternal symptoms due to parasite stage ingested correlated with disease severity in the infant. In addition, in both cases of symptomatic infant infection, one could question whether infant signs would have been less severe had prompt medical attention been sought antenatally to enable early detection, diagnosis and treatment of maternal *T. gondii* infection.

One woman reported an untreated contaminated domestic water supply sourced from a well as a possible environmental source of oocysts (table 13.2 case number 13). Local residents were not provided with at-home tap water treatment devices but were advised to boil all water prior to domestic use as the water appeared discoloured at all times. Whilst contaminated water was a possible source of infection, ingestion of undercooked meat was also a documented risk factor in this case. Modern water treatment systems will destroy oocysts in contaminated water, but in order to do so effectively, techniques employed must include filtering, coagulation, flocculation and settling (Krueger et al., 2014).
Chemical techniques such as chlorination will not destroy oocysts. Untreated or ineffectively treated water can contribute significantly to toxoplasma infection and has been associated with outbreaks in some regions of North and South America (Burnett et al., 1998).

Cats and kittens have long been associated with toxoplasma infection and in the past were considered to be the main source of toxoplasma infection in humans. Only one of the 15 women reported contact with cats, but in this case ingestion of cured meat was also documented (table 13.2 case number 5). Pregnant women should avoid contact with cat litter boxes which should be disposed of daily as the oocysts become infective 24 hours post excretion. Domestic cats can be helped to remain free of toxoplasma infection by being fed canned food and scavenging discouraged.

Thirteen women in the cohort with lack of awareness expressed concern about not receiving specific education related to toxoplasmosis at any stage during the pregnancy. Women who acquired infection from meat sources were angry and upset because simple educational methods were not employed during pregnancy. This was perceived by women to be the duty of attending obstetricians and midwives. However, women also admitted that they did not read the free literature available in antenatal clinics. Such literature usually provides general information on safe eating in pregnancy and highlights risks for acquiring toxoplasmosis. Language barriers were identified in the four women from Eastern Europe as an impediment to accessing and interpreting available information at antenatal clinics.

### 13.5.3 Areas identified for future targeting and intervention for CT prevention in Ireland

Congenital toxoplasmosis prevention programmes can be performed on three levels. The first level utilises primary prevention strategies to educate women on risk factors, increase maternal awareness and reinforce avoidance of risk exposure during pregnancy. The EUROTOXO project identified 18 of 36 countries surveyed in Europe that employed primary prevention strategies (introductory chapter 7, table 7.3).

Debate exists as to whether primary preventive strategies based on maternal pre- and post-conception education is an effective means of preventing CT.
Researchers who have extensively studied the effectiveness of this approach conclude that whilst primary prevention of toxoplasmosis is a desired, simple and inexpensive option, success relies on continuous reinforcement of education throughout pregnancy (Breugelmans et al., 2004).

The second level preventative strategy is routine antenatal screening for toxoplasma antibody, with seronegative women offered repeat screening at subsequent antenatal visits. The third level of CT prevention is routine screening of all newborn infants and treatment of those detected. In Ireland, women are not routinely screened for toxoplasmosis antenatally, nor is postnatal screening of infants routine practice. Hence maternal toxoplasma serostatus is generally unknown and thus pre-conception and antenatal education is vital. In our study cohort, only 13% of women with infected infants were aware of toxoplasmosis, 80% had identifiable risks for pregnancy seroconversion and 73% were likely to have acquired the infection from ingestion of undercooked or cured meat products. Whilst the purpose of this study was not to propose CT prevention strategies, the CT screening programme identified epidemiological data that can be targeted to prevent seroconversion in pregnant women. Primary prevention through education appears to be a potential and feasible intervention to prevent CT in Ireland.

To further assess the potential impact that education and increasing awareness of risk factors for toxoplasmosis could have, studies should be undertaken that target the general antenatal population and not just those women whose infants were infected.

In this small cohort of 15 women, 11 (73%) were estimated to have acquired infection during the third trimester, the time when transmission to the infant is highest (Dunn et al., 1999). These women possibly represent missed opportunities for primary prevention as education early in pregnancy from the first antenatal visit might have prevented maternal infection. This highlights the need for direct, accessible, language appropriate education in the antenatal clinic even for women who have had previous pregnancies.

However antenatal education may not benefit women who seroconvert early in pregnancy or prior to the first antenatal visit. Early educational awareness programmes should be targeted at secondary school curriculums and coordinated
with public health initiatives, media and advertising campaigns to reduce the burden of disease associated with this potentially preventable infection.

An important limitation of this study was the small sample size of women with infected infants. Nonetheless, recognition that there exists a vulnerable group of women who are not aware of simple measures to reduce their risk is important and should be targeted for ongoing education.

A second limitation to this study was the lack of a control group of seronegative women with uninfected infants to determine whether they were aware of or had been exposed to risk factors for seroconversion in pregnancy.

13.5.4 Future research
Recent studies on diagnostic assays for *T. gondii* infection have demonstrated new methods which can determine parasite stage-specific infection, which allows differentiation between infection from oocysts or bradyzoites (latent cysts).

Hill and colleagues in the USA identified a sporozoite specific recombinant protein (*T. gondii* embryogenesis-related protein, [TgERP]) which elicited antibody in pigs and mice infected with *T. gondii* oocysts. TgERP was applied to sera from *T. gondii* infected humans. Antibody to TgERP was detected in humans who had oocyst-acquired infection within the previous six to eight months. Of 163 individuals with acute infection demonstrable by positive IgM or low avidity, 103 (63.2%) had detectable antibodies that reacted with TgERP. Of 176 individuals with unknown infection route or chronic stage of infection (*T. gondii* IgM negative or high avidity), antibody to TgERP was detected in 31 (17.6%). Uninfected individuals had no antibody to TgERP detectable. The study suggested that TgERP may be useful in detecting exposure to sporozoites in early toxoplasma infection which implies oocysts as the source (Hill et al., 2011).

In a more recent multicentre study from Brazil, the reactivity of two recombinant proteins from oocysts was evaluated. The proteins were assessed using serum samples from infected animals, humans infected by oocysts during an outbreak and pregnant women with an unknown source of infection. The sporozoite specific
recombinant protein demonstrated reactivity for recent infection by oocysts or sporozoites in animals and humans infected by oocysts during an outbreak (Santana et al., 2015).

The identification of parasite infective stage can provide important epidemiological data on routes of infection in the pregnant population. This information can be used to determine what proportion of infection in humans and in particular the pregnant population are due to ingestion of oocysts. Such data can be used as public health initiatives to target prevention of seroconversion in pregnancy and congenital infection. Further studies in this area and its use in the pregnant population are awaited.

13.6 Conclusions
In the studied cohort of 15 women with congenitally infected infants, toxoplasma infection was likely acquired from a meat source in the majority of women who were also unaware of risks for infection and preventive measures. The presence of or domestic ownership of cats was not identified as an important risk factor.

Our results were consistent with findings from large European multi-centre studies. Overall, primary prevention seems feasible in our population with a low incidence of CT where other prevention strategies may not be cost effective to detect such small numbers of infected infants.
Figure 13.1: Geographical comparison of maternal seroprevalence of toxoplasma antibody (map a) and number of congenital toxoplasmosis cases by county (map b)
Table 13.1: Maternal data (n=15)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median maternal age [range]</strong></td>
<td>32 [23-38]</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Irish</td>
<td>10 (67%)</td>
</tr>
<tr>
<td>Eastern European</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Indian</td>
<td>1 (7%)</td>
</tr>
<tr>
<td><strong>County of residence</strong></td>
<td></td>
</tr>
<tr>
<td>Dublin</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Other counties</td>
<td>10 (67%)</td>
</tr>
<tr>
<td><strong>Trimester of seroconversion (estimated)</strong></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>1 or 2</td>
<td>2 (13%)</td>
</tr>
<tr>
<td><strong>Maternal risk factors for seroconversion</strong></td>
<td></td>
</tr>
<tr>
<td>Undercooked meat</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Undercooked meat &amp; contaminated water supply</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Undercooked meat &amp; raw cured meat product</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Raw cured meat product</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Raw cured meat product &amp; contact with cats</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Contact with soil</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>No risks identified</td>
<td>3 (20%)</td>
</tr>
<tr>
<td><strong>Total exposed to food risks</strong></td>
<td>11 (73%)</td>
</tr>
<tr>
<td><strong>Risk awareness for toxoplasma seroconversion</strong></td>
<td></td>
</tr>
<tr>
<td>Unaware</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Aware</td>
<td>2 (13%)</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
</tr>
<tr>
<td>1 (single)</td>
<td>10 (67%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>3</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>4</td>
<td>1 (7%)</td>
</tr>
<tr>
<td><strong>Gestation at delivery</strong></td>
<td></td>
</tr>
<tr>
<td>≥ 37 weeks</td>
<td>15 (100%)</td>
</tr>
</tbody>
</table>
### Table 13.2: Infected infants and corresponding maternal risk factors for pregnancy seroconversion

<table>
<thead>
<tr>
<th>Infant case number</th>
<th>Mat. county of residence</th>
<th>Mat. country of origin</th>
<th>Mat. risk factor identified</th>
<th>Risk factor awareness</th>
<th>Mat. symptoms of infection in pregnancy</th>
<th>Estimated trimester of seroconversion</th>
<th>Infant evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dublin</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Kerry</td>
<td>Pakistan</td>
<td>None</td>
<td>Unaware</td>
<td>No</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>Waterford</td>
<td>Ireland</td>
<td>Soil contact (gardening)</td>
<td>Unaware</td>
<td>Yes</td>
<td>2</td>
<td>Symptomatic in-utero. Severe signs of CT.</td>
</tr>
<tr>
<td>4</td>
<td>Dublin</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>Tipperary</td>
<td>Lithuania</td>
<td>Cured meat, contact with cats</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>Asymptomatic. Signs of CT.</td>
</tr>
<tr>
<td>6</td>
<td>Laois</td>
<td>Poland</td>
<td>Cured meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>Dublin</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>Asymptomatic. Signs of CT.</td>
</tr>
<tr>
<td>8</td>
<td>Limerick</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>Asymptomatic. Signs of CT.</td>
</tr>
<tr>
<td>9</td>
<td>Dublin</td>
<td>Russia</td>
<td>Cured meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>Cork</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>Cork</td>
<td>Ireland</td>
<td>None</td>
<td>Aware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>Donegal</td>
<td>Ireland</td>
<td>None</td>
<td>Aware</td>
<td>No</td>
<td>3</td>
<td>Asymptomatic. Sign of CT.</td>
</tr>
<tr>
<td>13</td>
<td>Galway</td>
<td>Ireland</td>
<td>Undercooked meat, contaminated water supply</td>
<td>Unaware</td>
<td>No</td>
<td>1-2</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>Dublin</td>
<td>Ireland</td>
<td>Undercooked meat</td>
<td>Unaware</td>
<td>No</td>
<td>3</td>
<td>N</td>
</tr>
</tbody>
</table>

Mat, maternal; N, normal; CT, congenital toxoplasmosis
CHAPTER 14 - Treatment of congenital toxoplasmosis with antiprotozoals and adverse effects encountered

14.1 Introduction
Available non-randomised data, both historic and recent, suggests that treatment of CT in the first year of life is associated with improved outcome (Eichenwald H F, 1959; McAuley et al., 1994). A postnatal treatment regimen with toxoplasma-specific antiprotozoals is included in the management protocol for all infants identified with CT; symptomatic or asymptomatic. Therapeutic interventions are recommended to commence as early as feasibly possible following diagnostic confirmation and evaluation. However, there is lack of international consensus regarding: optimal antiprotozoal treatment duration for CT, treatment efficacy in asymptomatic infants and whether treatment is effective in the prevention of recurrent disease. Hence practices vary globally and the duration of prescribed treatment can vary from 3 months to 24 months. The CDC recommends a 12-month treatment course with pyrimethamine and sulfadiazine for all infants with CT confirmed in infancy and most resource rich countries currently employ this regimen (available at http://www.CDC.gov accessed October 2015). Pyrimethamine and sulfadiazine act synergistically against *T. gondii* and hence use of both drugs is recommended. Treatment duration of 12-months with necessary blood monitoring for adverse effects, mainly neutropenia, can affect compliance.

14.2 Objectives
To demonstrate: 1) adverse effects encountered with a 12-month treatment regimen for CT that employed pyrimethamine and sulfadiazine and 2) compliance with the treatment protocol.

14.3 Methods

14.3.1 Population studied
Fifteen infants with CT were identified by 24 months of national newborn screening for CT. Fourteen infants entered a treatment protocol. One asymptomatic infant with persistently inconclusive serology and confirmation of CT at age nine months was not treated.
14.3.2 Treatment regimen
Therapeutic intervention for confirmed CT was employed as per current international best practice guidelines (Montoya et al., 2002; Wallon et al., 2004). Fourteen infants were prescribed a 12-month treatment regimen with pyrimethamine, sulfadiazine and calcium leucovorin. Infants were first electively admitted for clinical evaluation, following which treatment was initiated whilst in-patients.

Pyrimethamine 2 mg/kg once daily was first administered as a loading dose for two days, followed by 1 mg/kg daily for the first six months then thrice weekly for the remaining six months of therapy. Sulfadiazine was administered at 100 mg/kg daily in two divided doses for 12 months. A standard 15 mg dose of calcium leucovorin was administered Monday, Wednesday and Friday for the duration of treatment and for one week following treatment discontinuation. Prednisone (0.5 mg/kg twice per day) was added to the treatment regimen if active chorioretinitis was detected or if CSF protein was > 1 g/dL, and continued until resolution of active chorioretinitis and normalisation of CSF protein.

14.3.3 Treatment monitoring and interventions for adverse events
Infants attended their local paediatric out-patient or day-ward service for blood monitoring during the 12 months of treatment. FBCs were performed weekly or twice weekly if necessary during the first month of treatment, weekly or every second week for the remainder of the first three months, and monthly thereafter or more frequently if indicated. Infants were weighed at regular intervals during treatment for dose increments. For any queries or concerns during treatment, parents were advised to first contact the CT programme co-ordinator, and if unavailable they were to contact another member of the paediatric ID team.

Infant hematologic toxicity was graded according to the Division of AIDS Pediatric Adverse Events Scales, from the National Institutes of Health Division of AIDS U.S.A toxicity table 2004 for grading severity of adult and paediatric adverse events (available at http://rsc.tech-res.com/safetyandpharmacovigilance/). The Adverse Events grading for neutropenia was updated in 2009 and more recently in November 2014, however the infants recruited in the pilot CT screening programme were born between 2005 and 2007, hence neutropenia was classified according to the 2004 grading (table 14.1).
Specific interventions were applied when the absolute neutrophil count (ANC) decreased to < 1,000 \times 10^9/litre (L). Neutropenic events were managed according to grade until recovery of the ANC as follows: grade 1, no intervention; grade 2, doubling of prescribed leucovorin dose; grade 3, antiprotozoal dose reduction by 25% plus doubling of leucovorin for one week or longer if necessary; grade 4, antiprotozoal discontinuation for one week, with reinstatement with neutrophil recovery at a reduced dose for a further week following the week of treatment interruption.

More frequent FBCs were performed with intercurrent illness and therapy adjusted as necessary. Blood results for all infants were sent by fax to the screening programme co-ordinator who made contact with parents via telephone to discuss any necessary treatment adjustments. In situations where there was a language barrier, the programme co-ordinator liaised with the local public PHN and translators were employed if necessary.

Whilst receiving treatment, infants attended the Paediatric ID Clinic at OLCH or TCUH Dublin every three months for clinical and developmental review by the screening programme co-ordinator. At these visits FBC, liver and renal function and urinalysis were checked, in addition to the toxoplasma antibody profile.

14.4 Results

14.4.1 Summary of infant treatment

Fourteen infants commenced a 12-month antiprotozoal treatment regimen. Age at treatment initiation ranged from day 3 to week 17 of life, median 4 weeks (table 14.2). One infant with an elevated CSF protein of 1.24g/L (normal range 0.1 - 0.4g/L) was prescribed a course of oral prednisolone to be tapered following a repeat CSF sample after 8 weeks of antiprotozoal therapy (table 14.2 case number 15).

Thirteen of 14 infants completed 12 months of antiprotozoal treatment. In one case, parental consent was given for only four months of infant treatment rather than the recommended 12 months. For this infant, antiprotozoal treatment
commenced at age 9 weeks and was withdrawn by parents 16 weeks thereafter (table 14.2 case number 13).

14.4.2 Gastrointestinal side effects
Ten of 14 treated infants experienced intermittent vomiting and loose stools during the first two months of treatment. In 9 of 14 gastrointestinal side effects were self-limiting; infant feeding and weight gain were not adversely affected. In 1 of these 9 cases, a parental decision was made to temporarily discontinue treatment for two weeks despite adequate infant feeding and weight gain during the first two months of treatment (table 14.2 case number 2)

In one other case, intermittent diarrhoea and poor weight gain during the first month of treatment were subjectively reported by parents without documented record of failure to thrive; adequate weight gain was recorded by the PHN (table 14.2 case number 13).

14.4.3 Haematologic toxicity: neutropenic events
Anaemia and thrombocytopenia were not documented in the cohort. Intermittent neutropenia was the most common haematologic adverse event and was experienced by all 14 (100%) treated infants. Table 14.2 summarises grades of neutropenia experienced by each infant, number of neutropenic episodes for each grade, month of treatment during which neutropenia occurred, interventions employed and total amount of treatment completed. Neutropenia resolved in all instances following specific interventions as outlined in the methods for management of neutropenia. Temporary antiprotozoal dose reduction or interruption was not considered for one infant with unilateral blindness and vision threatening lesions in the other eye. In this case, treatment with granulocyte colony stimulating factor (GCSF) was commenced following grade 4 neutropenic events (table 14.2 case number 3).

14.4.3.1 Grades 1 and 2 neutropenic events (mild and moderate neutropenia)
Grades 1 and 2 neutropenia were the most frequent events and occurred on at least one occasion in 93% of treated infants; i.e., all but 1 of 14 treated infants (table 14.2 case number 15) experienced grade 1 or 2 neutropenia. In some infants, grade 1 neutropenic events progressed to grade 2 which was persistent in some cases but eventually responded to increased leucovorin dosage with
normalisation of the ANC in all instances. Antiprotozoal adjustments were not employed for grades 1 and 2 neutropenia.

14.4.3.2 Grades 3 and 4 neutropenic events (severe and life threatening neutropenia)

Eight of 14 treated infants (57%) experienced grade 3 neutropenia. In total, 14 grade 3 neutropenic events were recorded for the eight infants during the 12-month course of treatment. Four of 14 grade 3 episodes occurred during viral illness (table 14.2 case numbers 5, 6, 12 and 15). Three of eight infants with grade 3 neutropenic events also had grade 4 episodes (table 14.2 case numbers 5, 7 and 10). One other infant had grade 4 neutropenia (table 14.2 case number 3). Thus in total, 4 of 14 infants (29%) had grade 4 neutropenic events (table 14.2 case numbers 3, 5, 7 and 10). A collective total of six grade 4 events were documented for the treated cohort; two of six grade 4 events occurred during viral infection (table 14.2 case number 7).

Of the eight infants with grade 3 neutropenia, one experienced three episodes which responded to a 25% reduction in antiprotozoal dosage for one week on each occasion (table 14.2 case number 8).

Four infants experienced two grade 3 neutropenic events (table 14.2 case numbers 5, 6, 7 and 10), two of whom had viral illness during one of the episodes (table 14.2 case numbers 5 and 6). In one of these infants, viral infection manifested with pyrexia vomiting and diarrhoea, hence treatment interruption was necessary for one week, following which antiprotozoal treatment was successfully reinstated at full dosage (table 14.2 case number 5). A second episode of grade 3 neutropenia in this infant necessitated antiprotozoal dose reduction by 25% with increased leucovorin for one week. One episode of grade 4 neutropenia also occurred in this infant on one occasion which required temporary antiprotozoal interruption for one week with re-instatement after one week at 75% of total dose for a further week with return to full dosage thereafter.

In case number 6, both episodes of grade 3 neutropenia responded to temporary antiprotozoal dose reduction by 25% for one week on each occasion. Whilst one episode of grade 3 neutropenia was associated with viral illness, infant feeding remained adequate and treatment interruption was not necessary.
In the cohort of 14 treated infants, case number 7 experienced the most grade 3 and 4 neutropenic events that necessitated interventions. A total of four neutropenic episodes required either transient antiprotozoal adjustment or interruption. Two grade 3 events responded to dose reductions for one week on each occasion. Two grade 4 neutropenic episodes occurred during viral respiratory infections associated with reduced feeding that necessitated treatment interruptions for 2 weeks on each occasion, with reinstatement at 25% dose reduction for a further week on each occasion, with full dosage prescribed thereafter.

Case number 10 experienced two grade 3 neutropenic episodes that responded to antiprotozoal reduction by 25% for one week on each occasion. One grade 4 episode responded to antiprotozoal discontinuation for one week with reinstatement at a reduced dose for a further week prior to recommencement at full dosage.

Three infants experienced just one episode of grade 3 neutropenia (table 14.2 case numbers 12, 14 and 15), two of which occurred during viral infection. In case number 12, viral gastritis necessitated treatment discontinuation rather than dose reduction for one week, following which treatment was successfully recommenced at full doses. In case number 14, one grade 3 neutropenic event responded to 25% dose reduction for one week with normalisation of the ANC thereafter. In case number 15, a symptomatic infant with congenital hydrocephalus that required a VP shunt, one grade 3 neutropenic event occurred with viral infection during the fifth month of treatment. For this episode, antiprotozoal dose reduction by 25% was first employed following which treatment discontinuation was necessary for one week, which allowed full recovery of the ANC and reinstatement of therapy at full doses. Subsequently, no further neutropenic episodes were documented for the remainder of the treatment duration.

In the later months of treatment, a VP shunt complication occurred that required periods of fasting for further neurosurgery, in addition to a viral gastrointestinal illness albeit without neutropenia, all of which necessitated treatment interruptions until resolution of clinical symptoms. Thus whilst antiprotozoal treatment was discontinued for a total of six weeks during the 12 months of treatment, only one
week of discontinuation was for an episode of grade 3 neutropenia that occurred with viral infection at age five months.

**14.4.4 Additional interventions necessary for neutropenic events**

Interventions for grade 4 neutropenia as outlined in the methods were not applied for one infant with visual impairment at birth that manifested as right central blindness. Peri-macular chorioretinal scars were present in the left eye (table 14.2 case number 3). In this case, temporary antiprotozoal dose discontinuation was not considered appropriate for severe neutropenic events, due to the high risk for visual threat in the event of intraocular reactivation during the first year of life. The infant experienced two episodes of grade 4 neutropenia (ANC $0.1 \times 10^9/L$) in the third month of treatment that prompted initiation of GCSF to enable treatment completion at full dosage. GCSF was administered twice weekly by parents from the third to ninth month of treatment and weekly thereafter until treatment completion, during which time neutropenia did not occur. No infant required treatment with an alternative antiprotozoal regimen.

**14.4.5 Compliance with the recommended treatment regimen**

The parents of three infants had difficulty in adhering to management recommendations (table 14.2 case numbers 2, 13 and 15). Minimal non-adherence was encountered in two cases. Parents of one infant temporarily discontinued antiprotozoals on two occasions each for one week in the first two months of treatment for gastrointestinal side effects. Interruption of treatment was retrospectively communicated to the CT programme co-ordinator (table 14.2 case number 2). The infant that required adjunctive steroid therapy had prednisolone abruptly discontinued by the mother after eight weeks, rather than the recommended tapered withdrawal that first required repeat CSF sampling (table 14.2 case number 15). Discontinuation of steroid therapy was also retrospectively reported without first seeking advice. In both aforementioned cases, no other compliance issues were encountered.

In one case, parental adherence to the treatment regimen proved problematic from the initiation of the management protocol (table 14.2 case number 13). The parents were initially reluctant for their infant to embark on treatment because of concerns regarding treatment toxicity. Parental consent was subsequently given for four months of infant treatment with antiprotozoals. However treatment was
interrupted on numerous occasions during the total 16 weeks of treatment. This was primarily related to reported gastrointestinal side effects and perception of adverse impact on weight gain. Compliance with blood monitoring and clinical review was good and age appropriate weights were recorded at out-patient visits. During the 16-week treatment period, no significant neutropenic events occurred that required antiprotozoal adjustments.

14.4.6 Summary of interventions during infant treatment and percentage of treatment completed
Table 14.3 summarises interventions applied to the cohort of 14 infants during the course of antiprotozoal treatment. Following all necessary interventions for neutropenia, illness and VP shunt complications, 7 of 14 infants (50%) were on medication for 100% of the intended treatment duration of 12 months. Five of 14 infants (36%) required temporary antiprotozoal cessation and received treatment for 88% to 98% of the intended treatment duration (table 14.2 case numbers 5, 7, 10, 12 and 15). Periods of treatment cessation ranged from one to six weeks, median two weeks, as detailed for each infant in table 14.2.

Parental interventions accounted for temporary antiprotozoal discontinuation during treatment in 2 of 14 infants (14%). One of these completed 96% of the intended treatment duration (table 14.2 case number 2); the other had treatment discontinued by parents after four months and thus the infant completed approximately 19% of the intended treatment duration (table 14.2 case number 13). Thus 13 of 14 infants received treatment for 88% to 100% of the intended duration. Four infants received antiprotozoals at full dosage for 12 months. (table 14.2 case numbers 1, 3, 9 and 11). Nine infants received 84% to 98% of treatment at full dose.

14.4.7 Summary of resolution of neutropenia during treatment
Neutropenia resolved by the third month of treatment in three infants (table 14.2 case numbers 1, 3 and 9), one of who required GCSF support; and by the sixth month of treatment in seven infants. Four infants experienced neutropenic events in the latter six months of treatment, primarily grades 1 and 2 (table 14.2 case numbers 2, 5, 7, and 14).
14.4.8 Liver and renal function
Liver and renal function remained in normal range for the duration of treatment in all infants. Sulfonamide-specific adverse effects were not documented such as haematuria or crystalluria.

14.5 Discussion

14.5.1 Summary of rationale for treatment of CT during infancy
Postnatal treatment of infants with CT aims to minimise adverse developmental outcome. Infection with the type II strain of *T. gondii* that predominates in Europe has been shown to respond to treatment (McLeod et al., 2012). The recommendation for a 12-month antipROTOzoal treatment regimen for CT is based on the assumption that under the age of one year, an infant’s immune system is immature and may not provide adequate parasitic control. Without treatment in infancy, free tachyzoites can multiply and either directly target vulnerable organs, mainly the eye and brain, or sequester as latent cysts in these organs with the potential for reactivation at any time (Gomez-Marín et al., 2007).

The 12-month treatment duration for CT was initially derived from experience with treatment of AIDS patients with toxoplasmic encephalitis in the early 1980s. A 12-month treatment duration was found to be adequate to protect against CNS sequelae in an immunocompromised individual (Luft et al., 1983; Bertoli et al., 1995).

During the first year of life when the infant is not fully immunocompetent, antipROTOzoal therapy may provide crucial parasitic control. Treatment efficacy in CT has not been evaluated in RCTs, available data is largely from longitudinal follow up of treated cohorts of CT. Thus the topics of optimum treatment duration for CT and treatment of asymptomatic CT remain the subject of much debate and practices continue to vary worldwide (Wakefield et al., 2011). In the absence of evidence, some experts argue that outcome with a shorter treatment duration may be comparable to the extended 12-month duration. However comparative groups of infants studied from the NCCCTS and older collaborative studies have demonstrated that cohorts of infants with or without clinical signs treated for 12 months have less intraocular sequelae and better outcomes overall than those not treated or treated for shorter durations (Wilson et al., 1980; Phan et al., 2008b).
Whilst these studies may have incorporated selection bias, they consistently demonstrated similar results 30 years apart.

As of 2009, treatment of CT in Switzerland is offered only to infants with signs at birth (Swiss Working Group on Congenital Toxoplasmosis, 2008; Boubaker et al., 2008). This recommendation and alternative strategy for treatment of CT arose from a review of the disease burden of CT in Switzerland and was also based on suggestions from the EMSCOT study that was not randomised. Freeman and collaborators from the EMSCOT study group proposed that postnatal treatment and follow up of CT should be tailored to the child’s predicted prognosis. It was suggested that children with no clinical manifestations should be offered a short three-month course of antipROTOzoal treatment or no treatment. The rationale was that if signs were absent at birth the disease was controlled in fetal life and the probability of sequelae was too low for treatment benefits to outweigh harms (Freeman et al., 2008).

This approach to treatment of CT; i.e., risk assessment of the infant based on signs at birth and prediction of sequelae to determine the need for treatment, has not been accepted or adopted internationally in resource rich settings, given that the majority of infants with CT have no clinical symptoms or signs at birth. In the absence of RCTs to guide management of asymptomatic CT, most clinicians are reluctant to deviate from the traditional recommendations that exist for treatment of all infants with confirmed CT. In most European countries and in the U.S.A, the 12-month treatment regimen is recommended and, in some instances, is extended to 24-months for high risk infants with severe signs and favourable outcomes have been reported (McLeod et al., 2006; Phan et al., 2008a).

14.5.2 Treatment initiation in the cohort of 14 infants
The 12-month treatment regimen was accepted by parents for all but 1 of 14 infants (table 14.2 case number 13). Parents all admitted that treatment duration and necessary blood monitoring was daunting and overwhelming when first discussed. However, infant treatment initiation occurred during a time of much parental stress and anxiety, triggered by a cascade of events that began with recall for serologic confirmation, an unexpected infant diagnosis, hospital admission for evaluation and detection of infant signs where applicable.
However the majority of parents stated that after the initial months, administration of antiprotozoals and necessary monitoring did not pose challenges. One infant with inconclusive serial confirmatory serology was offered treatment when the diagnosis was confirmed at age nine months. At that point the parents opted to decline treatment initiation as following consideration of treatment vs no treatment and potential toxicity, they believed it was too late in infancy to embark on a treatment regimen (table 14.2 case number 4).

The wide range of treatment initiation from 3 days to 17 weeks represented timing of diagnostic confirmation. The infant that commenced treatment on day 3 was diagnosed with symptomatic CT in-utero (table 14.2 case number 3). The infant that commenced treatment at week 17 had inconclusive serology in the newborn period and required serial toxoplasma testing. CT was confirmed by WB from a sample taken at 13 weeks (table 14.2 case number 12). Following serological confirmation, all infants were electively admitted to a tertiary centre for full evaluation and treatment initiation, hence bed availability also contributed to timing of treatment initiation.

In one infant treatment initiation was deferred until nine weeks by parents (table 14.2 case number 13). Parental deferral also accounted for late infant treatment initiation in one other infant (table 14.2 case number 15). However in this case the newborn screening result was available on day 28 of life, infant confirmatory serology was taken on day 30 of life with confirmatory results and a positive CSF-PCR available on day 36 of life. In the interim, the infant had congenital hydrocephalus managed with a VP shunt as an isolated abnormality, as the diagnosis of CT was not suspected prior to the screening result. Hence a time delay was requested by the parents for information gathering, acceptance of the diagnosis and treatment protocol. Treatment subsequently commenced during the eighth week of life on day 54. Overall, delays with treatment initiation occurred in a minority and 13 of 14 infants had commenced treatment before age 12 weeks.

**14.5.3 Treatment adverse effects**

In the studied cohort of 14 treated infants, gastrointestinal side effects in the early stages of treatment initiation and neutropenic events during treatment were as to be expected for a 12-month course of antiprotozoals in the first year of life. For the treated cohort, neutropenia was common and can be anticipated during
antiprotozoal treatment for CT. Eleven of 14 (78%) experienced grade 3 or grade 4 events, however it was reversible in all.

Whilst the number of infants who experienced higher grades of neutropenia was more than the number who had grade 1 or 2, the frequency of occurrence of grade 3 and 4 events was less than that of lower grades. A combined total of 20 grade 3 and 4 neutropenic events were summated for 13 infants who completed 12 months of treatment; 6 of these 20 episodes occurred during viral infections. No infant experienced bacterial infection as a consequence of neutropenia. All episodes of illness during treatment were viral in nature with either respiratory or gastrointestinal symptoms that resolved with supportive care and antibiotics were not prescribed.

**14.5.4 Compliance with the treatment regimen**

The majority of parents upheld compliance with the treatment and monitoring protocol. Frequent occurrence of neutropenia during the first six months of treatment necessitated many hospital appointments for blood tests even for low grade neutropenia. During periods of illness, or following dose adjustments, additional blood monitoring posed an extra imposition. However, all parents adhered to the monitoring protocol. A minority of parents expressed that interruption of routine and infant distress that resulted from blood sampling generated parental anxiety. However these factors did not contribute to non-compliance during treatment.

Of the three cases with parental non-adherence to treatment administration, communication or language barriers in two cases initially accounted for lack of engagement with medical services to voice concerns and queries (table 14.2 case numbers 2 and 15). These issues were easily and promptly addressed which ensured no further compliance problems. Compliance issues in one other case was due to a combination of gastrointestinal side effects and parental anxiety and issues regarding antiprotozoal treatment.

**14.5.5 Comparison of toxicity encountered in the cohort with other studies.**

The rate of grade 4 events (ANC < 0.5 x 10^9/L) in the studied cohort (29%) was higher than that documented in the Danish newborn screening programme (13.8%). However in Denmark infants were treated for three months (Roser et al.,
hence the rate of toxicity is expected to differ. The overall tolerability and toxicity in our cohort was similar to that reported for other European countries and centres that employ a 12-month antiprotozoal regimen (Wallon et al., 2004).

In our cohort, change of antiprotozoal schedule was employed for grade 3 and 4 neutropenia. In some centres, interventions for neutropenia are only employed for grade 4 events, unless the child has intercurrent illness with lower grades of neutropenia. Studies on infants treated for CT demonstrate that antiprotozoals are generally well tolerated and most infants with severe neutropenic episodes do not experience complications as a result (McLeod et al., 2006). In our study, interventions were employed for grade 3 neutropenic events to avoid progression to grade 4 and hence temporary treatment cessation.

14.5.6 Future research
The rate of adverse events encountered during a 12-month treatment regimen for CT in a small cohort of 14 infants demonstrated the need for more studies on optimal treatment duration and the most efficacious dosage of antiprotozoal therapy to minimise toxicity. Whilst studies have addressed the need for alternative treatment regimens, conclusions have not been made (Petersen et al., 2003). Studies have demonstrated that antiprotozoal pharmacokinetics vary in children even when standardised for weight and the optimal serum concentration of antiprotozoals is yet unknown (Corvaisier et al., 2004). It has been proposed that short dosing intervals may be the best combination in infants and young children (Trenque et al., 2004).

New treatment regimens for CT will rely on the results of comparative studies that conclude on treatment efficacy with shorter or alternative courses of antitoxoplasma therapy. Currently one such study is underway in France; the TOSCANE study, the primary objective of which is to compare the efficacy of 3 months vs 12 months treatment with antiprotozoals in children with non-severe CT. The study began in July 2010 and recruitment is estimated to end in September 2016; the results will be eagerly awaited (Wallon et al., 2011).

14.6 Conclusions
In the cohort of 14 infants treated for CT with 12 months of combination antiprotozoals, adverse events were common. Transient gastrointestinal
symptoms occurred in 71% and neutropenic events in 100%. Grades 1 and 2 neutropenic events can be anticipated in infants treated with these agents and more than 75% of infants thus treated may experience neutropenia at a level warranting intervention. The rate of severe and life threatening neutropenia was low; viral infection contributed in one third of such events.

In general parents remained adherent to the management regimen despite the frequent occurrence of neutropenic events. Thirteen infants completed 88% to 100% of the intended 12-month duration; four at 100% dosage and nine received 84% to 98% of treatment at full dosage. Treatment toxicity in the studied cohort highlighted the need for evaluation of alternate antiprotozoal regimens.
Table 14.1: Classification of Paediatric Neutropenia*

<table>
<thead>
<tr>
<th>Absolute neutrophil count (ANC)‡</th>
<th>GRADE 1 MILD</th>
<th>GRADE 2 MODERATE</th>
<th>GRADE 3 SEVERE</th>
<th>GRADE 4 POTENTIALLY LIFE-THREATENING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paediatric &gt; 7 days</td>
<td>1,000 x 10⁹ – 1.300 x 10⁹/L</td>
<td>0.750 x 10⁹ – 0.999 x10⁹/L</td>
<td>0.500 x 10⁹ – 0.749 x10⁹/L</td>
<td>&lt; 0.500 x10⁹/L</td>
</tr>
</tbody>
</table>

‡ANC stated in standard international units

* Adapted from paediatric adverse events version 1.0, December 2004
Table 14.2: Summary of infant treatment

<table>
<thead>
<tr>
<th>Infant Case number</th>
<th>Evaluation</th>
<th>Treatment initiation</th>
<th>12-months treatment completed</th>
<th>Grade of neutropenia (n)</th>
<th>Antiprotozoal dose reduction</th>
<th>Antiprotozoal interruption</th>
<th>Treatment received at full dosage</th>
<th>% of intended treatment duration received</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Asymptomatic</td>
<td>Week 3</td>
<td>Yes</td>
<td>(4) months 1 to 3</td>
<td>(0)</td>
<td>(0)</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>Asymptomatic</td>
<td>Week 3</td>
<td>Yes</td>
<td>(5) months 1 to 8</td>
<td>(1) month 3</td>
<td>(0)</td>
<td>0</td>
<td>2 weeks‡ 96% (50 weeks)</td>
</tr>
<tr>
<td>3</td>
<td>Symptomatic</td>
<td>Day 3</td>
<td>Yes</td>
<td>(0)</td>
<td>(6) months 1 and 2</td>
<td>(0)</td>
<td>(2) month 3§ 0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Asymptomatic</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>Asymptomatic, clinical signs</td>
<td>Week 5</td>
<td>Yes</td>
<td>(&gt;5) months 1 to 10</td>
<td>(&gt;5) months 1 to 9</td>
<td>(2) month 5* and 6</td>
<td>2 weeks 2 weeks</td>
<td>92% (48 weeks) 96% (50 weeks)</td>
</tr>
<tr>
<td>6</td>
<td>Asymptomatic</td>
<td>Week 4</td>
<td>Yes</td>
<td>(3) months 1 to 6</td>
<td>(&gt;5) months 1 to 4</td>
<td>(2) month 2* and 4</td>
<td>0</td>
<td>2 weeks 0 96% (50 weeks)</td>
</tr>
<tr>
<td>7</td>
<td>Asymptomatic, clinical signs</td>
<td>Week 4</td>
<td>Yes</td>
<td>(&gt;5) months 1 to 8</td>
<td>(&gt;5) months 1 to 8</td>
<td>(2) month 3 and 7</td>
<td>4 weeks 4 weeks</td>
<td>84% (44 weeks) 92% (48 weeks)</td>
</tr>
<tr>
<td>8</td>
<td>Asymptomatic, clinical signs</td>
<td>Week 3</td>
<td>Yes</td>
<td>(4) months 1 to 5</td>
<td>(6) months 1 to 3</td>
<td>(3) months 1 to 4</td>
<td>3 weeks 0</td>
<td>94% (49 weeks) 100%</td>
</tr>
<tr>
<td>9</td>
<td>Asymptomatic</td>
<td>Week 4</td>
<td>Yes</td>
<td>(1) month 1</td>
<td>(0)</td>
<td>(0)</td>
<td>0</td>
<td>0 100%</td>
</tr>
<tr>
<td>10</td>
<td>Asymptomatic</td>
<td>Week 4</td>
<td>Yes</td>
<td>(6) months 1 to 6</td>
<td>(6) months 1 to 6</td>
<td>(2) month 3 and 5</td>
<td>3 weeks 1 week</td>
<td>92% (48 weeks) 98% (51 weeks)</td>
</tr>
<tr>
<td>11</td>
<td>Asymptomatic</td>
<td>Week 5</td>
<td>Yes</td>
<td>(1) month 4</td>
<td>(2) month 2 and 4</td>
<td>(0)</td>
<td>0</td>
<td>0 0 100%</td>
</tr>
<tr>
<td>12</td>
<td>Asymptomatic, clinical signs</td>
<td>Week 17</td>
<td>Yes</td>
<td>(7) months 1 to 6</td>
<td>(0)</td>
<td>(1) month 4*</td>
<td>0</td>
<td>1 week 98% (51 weeks) 98% (51 weeks)</td>
</tr>
<tr>
<td>13</td>
<td>Asymptomatic</td>
<td>Week 9</td>
<td>Discontinued 16 weeks after initiation§ (2) month 4</td>
<td>(1) month 2</td>
<td>(0)</td>
<td>(0)</td>
<td>0</td>
<td>6 of 16 weeks withheld‡ 19% (10 weeks)</td>
</tr>
<tr>
<td>14</td>
<td>Asymptomatic</td>
<td>Week 7</td>
<td>Yes</td>
<td>(&gt;5) months 1 to 9</td>
<td>(5) months 1 to 5</td>
<td>(1) month 4</td>
<td>0</td>
<td>1 week 0 98% (51 weeks)</td>
</tr>
<tr>
<td>15</td>
<td>Symptomatic</td>
<td>Week 8</td>
<td>Yes</td>
<td>(0)</td>
<td>(0)</td>
<td>(1) month 5*</td>
<td>1 week 6 weeks‡ 86% (45 weeks)</td>
<td>88% (46 weeks)</td>
</tr>
</tbody>
</table>

(n), number of neutropenic episodes; NT, not treated
‡ Parental decision for treatment interruptions, § GCSF commenced from third month of treatment, * Neutropenic episode occurred with viral illness, ‡ VP shunt complications, neurosurgery and viral illness accountable for total treatment interruptions

235
Table 14.3: Summary of interventions applied during antiprotozoal treatment for CT in 14 infants

<table>
<thead>
<tr>
<th>Interventions to antiprotozoal treatment</th>
<th>Number of infants affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient dose reduction only</td>
<td>3</td>
</tr>
<tr>
<td>Transient dose cessation only</td>
<td>1</td>
</tr>
<tr>
<td>Transient dose reduction and cessation</td>
<td>4</td>
</tr>
<tr>
<td>Transient cessation by parents</td>
<td>1</td>
</tr>
<tr>
<td>Transient and permanent cessation by parents</td>
<td>1</td>
</tr>
<tr>
<td>GSCF support</td>
<td>1</td>
</tr>
<tr>
<td>No interventions</td>
<td>3</td>
</tr>
</tbody>
</table>
CHAPTER 15 - Effect of treatment on intracranial signs in a cohort of children with congenital toxoplasmosis

15.1 Introduction

*T. gondii* has the ability to form cysts in multiple organs with predilection for the brain and eye. In CT, intracranial abnormalities occur less frequently than ocular signs (Remington, 2011). In Europe, symptomatic or severe CT is uncommon, the majority of infants are asymptomatic at birth with intracranial lesions detected only if specifically sought. CSF abnormality may be the only indicator of CNS involvement in the asymptomatic infants.

Calcific foci within the brain are the most common intracranial findings in CT. These can vary in size and number and can be found scattered throughout the cerebral cortex, or more commonly in the periventricular areas or basal ganglia. Each focus represents an area of destructive necrosis that occurred in fetal life. Less common findings include porencephalic cysts and encephalomalacia (Moncada et al., 2012).

In historic studies of congenitally infected children with intracranial calcification who did not receive treatment or received treatment for one month only, follow up imaging demonstrated that, in some cases, increase in number and size of calcific foci continued over months or years. This suggested that the destructive and necrotising process of infection in the brain continued long after the initial insult (Eichenwald H F, 1959). This finding was subsequently confirmed in an animal model study; within the brain, *T. gondii* parasitic growth can be inhibited by microglial cells, but neurons and astrocytes harbour bradyzoites and vacuoles with proliferating parasites (Luder et al., 1999). Studies have demonstrated that treatment of CT with antiprotozoals for one year is associated with regression of intracranial calcification (Patel et al., 1996; McLeod et al., 2006).

15.2 Objective

To demonstrate intracranial signs detected at initial evaluation and following antiprotozoal treatment in a cohort of children with CT.
15.3 Patients and methods

15.3.1 Cohort studied
Fifteen infants with CT identified during 24 months of national newborn screening in a population with an incidence of one in 10,000.

15.3.2 Methods
Children were assigned to a standard of care management protocol that included detailed clinical evaluation and antiprotozoal therapy. Intracranial imaging and CSF analysis were performed at initial assessment. Imaging by CCT scan was recommended. Infants with intracranial abnormalities at initial assessment were re-imaged following completion of 12 months of antiprotozoal therapy and as indicated thereafter. Infants with elevated CSF protein were prescribed adjunctive therapy with a short course of glucocorticoids (prednisone 1 mg/kg/day in divided doses) to be tapered depending on repeat CSF analysis eight weeks after initiation of treatment.

15.4 Results

15.4.1 Intracranial imaging at initial assessment and follow-up
Table 15.1 summarises intracranial findings in the cohort. Ten of 15 infants had a cranial ultrasound scan as first-line imaging in the newborn period. All 15 infants had a CCT scan. One of 15 infants had both CCT and MRI scans at initial assessment (table 15.1 case number 15).

Results of brain imaging in the newborn period were previously discussed in chapter 12. Four of 15 (27%) infants had intracranial signs of CT; two (13%) symptomatic (table 15.1 case numbers 3 and 15) and two asymptomatic (table 15.1 case numbers 5 and 8). All four had intracranial calcification; three had multiple foci (table 15.1 case numbers 3, 5 and 15) and one a single focus (table 15.1 case number 8). One symptomatic infant had in-utero CNS signs of CT with ventriculomegaly incidentally detected at 28 weeks gestation, thereafter maternal treatment with pyrimethamine and sulfadiazine was administered for the remainder of pregnancy. Some resolution of fetal ventriculomegaly was demonstrated prior to the end of gestation (table 15.1 case number 3). Infant CCT scans in the newborn period and following completion of treatment are demonstrated in figure 15.1.
One asymptomatic infant with multiple calcific foci in both cerebral hemispheres and the right periventricular area at initial evaluation (table 15.1 case number 5) demonstrated almost complete regression of periventricular calcification following treatment completion (figure 15.2). One infant with a single calcific focus had no change to the lesion at follow-up (table 15.1 case number 8).

MRI at age three weeks in one infant (table 15.1 case number 15) with symptoms of hydrocephalus confirmed congenital aqueduct stenosis (figure 15.3). Initial CCT scan demonstrated multiple foci of right periventricular calcification (image not shown). During the third week of life a VP shunt was inserted. Imaging was repeated at nine months, necessitated by VP shunt failure and was not repeated at treatment completion. Subsequently, CCT and MRI scans repeated at age three years of age demonstrated areas of periventricular leucomalacia (PVL) and a single calcific focus (figure 15.4).

### 15.4.2 CSF analysis

CSF abnormalities were detected in one infant who had congenital aqueductal stenosis and non-communicating hydrocephalus. CSF protein obtained by lumbar puncture was elevated at 1.26g/L (range 0.1-0.4g/L) and *T.gondii* PCR negative. CSF-PCR was *T.gondii* positive in a sample obtained directly from the periventricular area at VP shunt placement (table 15.1 case number 15). Adjunctive glucocorticoid treatment was prescribed for eight weeks or until normalisation of CSF protein. Repeat CSF by lumbar puncture eight weeks after initiation of treatment demonstrated protein in normal range and a negative *T.gondii* PCR.

### 15.5 Discussion

Treatment of CT in the first year of life aims to protect the CNS from parasite activity and disease progression or subsequent recurrence, particularly in the higher risk infants with CNS symptoms or signs detected at birth (McLeod et al., 2009).

A recent report has demonstrated that the fetal brain is vulnerable to continued parasite replication even when replication in other organs has ceased. Ferguson and colleagues (Ferguson et al., 2013) reported on two fetuses with confirmed CT aborted by induction at 21 and 30 weeks gestation. In both cases maternal
seroconversion was confirmed in trimester 1 with demonstrable IgM only at that point and IgG detected at abortion. At autopsy, detailed examination of the placenta, fetal adrenals and heart demonstrated tissue cysts (bradyzoites) but few tachyzoites, consistent with a chronic or largely controlled infection in these organs. However areas of the fetal brain contained large numbers of actively dividing tachyzoites with associated tissue destruction and necrosis. This finding suggests that treatment could be beneficial in infancy.

Even in immunocompetent adults, acute and latent toxoplasma infection has been associated with development of neuropsychiatric sequelae which is thought to be the consequence of continued parasite replication in the brain (Holliman, 1997; Kirby, 2012; Wong et al., 2013). Hence it is hoped that antiprotozoal therapy might achieve parasitic control in the brain of infants until immunologic maturation occurs to enable control of infection.

In the studied cohort of 15 infants, a low incidence of intracranial signs was found (27%). Two of four infants with intracranial calcification were asymptomatic and would not have been identified as having CT without the screening programme, one of whom had multiple calcification and by definition severe CT (Table 15.1 case number 5).

All four infants had calcification in the periventricular area, which is the most common location for intracranial calcification in CT (Remington, 2011). The presence of multiple calcific foci do not necessarily indicate a poor neurological prognosis as they are usually not associated with extensive white matter damage, unless progression in size occurs (Roizen et al., 1995). Patel et al reported on a cohort 40 infants with intracranial calcification recruited between 1982 and 1994. The majority of children (75%) who had one year of treatment demonstrated resolution of calcification. In children who received no treatment or treatment for one month, calcification increased in size or remained stable during the first year of life (Patel et al., 1996).

In our cohort, after 12 months of antiprotozoal treatment, calcification regressed in three children with multiple foci and remained stable in one with a single focus. Hydrocephalus is one of the most common findings in severe CT. Anatomical patterns of hydrocephalus in CT vary and are thought to be related to parasite
genetics (Hutson et al., 2015). The infant in this study had hydrocephalus secondary to aqueduct stenosis. Hydrocephalus in CT is secondary to a *T. gondii* antigen-antibody reaction which causes ventriculitis and blockage of CSF flow in the developing fetal brain (Frenkel, 1988).

Positive CSF-PCR with elevated protein levels indicating intracranial infection were found in only one severely symptomatic infant (table 15.1 case number 15). The findings resolved during therapy with antiprotozoals and glucocorticoids. Adjunctive low dose glucocorticoid treatment is recommended for infants with CSF protein > 1g/L until normalisation. Glucocorticoid treatment is directed at the inflammatory process in the brain. The recommendation for glucocorticoid treatment is not evidence based nor has it been studied in randomised trials. However it’s use for this purpose in CT has not been associated with harm (McAuley et al., 1994)

Whilst this study focused on postnatal treatment, one symptomatic infant with CNS signs detected at 28 weeks gestation potentially benefited also from maternal antiprotozoal treatment initiated antenatally (table 15.1 case number 3). Arrest of ventricular enlargement was demonstrated following treatment initiation in trimester 3. It is reasonable to speculate that without maternal treatment, progressive ventricular enlargement would have occurred with more severe intracranial signs demonstrated at birth. Ventricular dilatation present in the newborn period had fully regressed following 12 months of treatment.

A limitation to this study was the small number of children with intracranial signs of CT. However, significant regression of intracranial calcification was demonstrated in all children with multiple lesions following treatment completion.

A second limitation to this study was that children with absence of intracranial signs at initial evaluation were not re-imaged following treatment completion. Thus it is not possible to measure the effect of treatment on the development of intracranial signs.

Children with normal imaging at initial evaluation were not re-imaged following treatment completion as this is not recommended as best practice due to the potential harms of radiation exposure outweighing the benefits. In such cases
further imaging is recommended only if neurological abnormalities subsequently manifest that warrant CNS imaging. For one of 11 children without intracranial signs this was indicated following a generalised seizure associated with febrile illness at age 3 years and repeat CCT scan was normal (case number 7).

15.6 Conclusions
A low incidence of intracranial signs was demonstrated in the studied cohort at initial imaging (27%). The most frequent intracranial sign was calcification. Post-natal antiprotozoal treatment was successful and lead to the regression of intracranial calcification and ventricular dilatation. Due to the ethical contraindication of an untreated control group, it is not possible to establish whether these signs can regress spontaneously.
**CCT scan of the brain at birth**

a) multiple foci of calcification (red arrows) in both frontal lobes with loss of cortical volume in the frontal lobe noted.

b) foci of calcification also noted bilaterally in the peri-ventricular regions adjacent to both frontal horns. Bilateral prominent ventricular system and diffuse hypo-density involving the cerebral white matter also noted.

**CCT scan following treatment completion**

c) Almost complete resolution of intracranial calcification. One focus of calcification is seen anterior to the anterior horn of the left lateral ventricle. Normal ventricular size.
Figure 15.2: Cranial imaging for case number 5

a) Neonatal CCT scan
Multiple linear calcifications (red arrows) in the right lateral periventricular area. Calcification size and number were computed. Repeat cranial scans obtained at follow-up were reviewed by the same radiologist.

b) CCT scan following antiprotozoal treatment
Diminution in size and almost complete resolution of calcifications in the right lateral periventricular area.
Figure 15.3: Intra-cranial imaging for case number 15 at age three weeks

**MRI scan at age three weeks**

a) Axial T2 image shows hugely dilated lateral ventricles and dilated third ventricle.
b) Sagittal T2 image shows that the aqueduct is dilated superiorly (black arrow) and occluded inferiorly consistent with aqueduct stenosis. Fourth ventricle has a normal size. The midbrain is slightly compressed. The cerebellum and brain stem are normal. The cortex is thin in the posterior parietal regions.
Figure 15.4: Follow-up imaging for case number 15

**a) CCT scan at 3 years old**
Ventricles are normal size with shunt in place (green arrow). Focus of calcification is seen in the right periventricular area (white arrow). Shunt tubing outside skull (red arrow).

**b) MRI scan at 3 years old**
Some residual high signal is seen in the deep white matter bilaterally (white arrows) and represents periventricular leukomalacia confined to the margins of the posterior ventricles. The brain parenchyma is otherwise normal. The ventricles have a normal size and configuration.
Table 15.1: Intracranial imaging and effect of treatment on intracranial signs in a cohort with CT

<table>
<thead>
<tr>
<th>Infant case number</th>
<th>Imaging in newborn period</th>
<th>Treatment Duration</th>
<th>Repeat imaging following treatment completion (where applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cranial Ultrasound</td>
<td>CCT scan</td>
<td>MRI scan</td>
</tr>
<tr>
<td>1</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>3</td>
<td>Mild ventricular dilatation</td>
<td>Multiple areas of frontal lobe calcification and B/L periventricular calcification</td>
<td>NP</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>5</td>
<td>B/L multiple foci intracranial calcification</td>
<td>Multiple foci of punctate calcification both hemispheres and RT periventricular calcification</td>
<td>NP</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>8</td>
<td>NP</td>
<td>Single focus of calcification left periventricular area adjacent to frontal horn of left lateral ventricle</td>
<td>NP</td>
</tr>
<tr>
<td>9</td>
<td>NP</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>10</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>11</td>
<td>NP</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>13</td>
<td>NP</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>14</td>
<td>NP</td>
<td>N</td>
<td>NP</td>
</tr>
<tr>
<td>15</td>
<td>Severe ventriculomegaly lateral and 3(^{rd}) ventricles</td>
<td>Compressed midbrain. Thin cerebral cortex posterior parietal regions. Multiple foci RT periventricular calcification</td>
<td>Aqueduct stenosis. Periventricular cystic changes</td>
</tr>
</tbody>
</table>

N, normal; NP, not performed; NA, not applicable; B/L, bilateral; RT, right; VP, ventriculoperitoneal; PVL, periventricular leukomalacia
CHAPTER 16 - Ophthalmic outcome in a cohort of children with CT

16.1 Introduction
Congenital toxoplasmosis is the result of maternal primary *T. gondii* infection in pregnancy with transmission to the developing fetus. The majority of congenitally infected infants are asymptomatic at birth, some of who have clinical signs which will be detected only if specifically sought.

The most common clinical sequel of congenital toxoplasmosis is inflammation of the choroid and retina, known as chorioretinitis or retinochoroiditis, which can cause visual impairment. Early diagnosis and treatment in infancy are believed to improve visual and neurological outcome (Bosch-Driessen et al., 2002; Phan et al., 2008a). In addition to retinochoroiditis, other ocular manifestations of CT are described including microphthalmia, optic nerve atrophy, cataract, strabismus, nystagmus, anisometropia and abnormalities of the iris (Melamed et al., 2010).

Congenitally infected infants can have a normal initial ocular examination yet subsequently develop chorioretinal lesions at any stage following diagnosis. If untreated in infancy, 70% to 80% of congenitally infected individuals will have a chorioretinal lesion by the second or third decade of life. Based on observational and non-randomised studies, the risk of recurrent chorioretinitis is reduced albeit not eliminated in treated cohorts of CT (Faucher et al., 2012). The risk of chorioretinitis is highest in the first two years of life but also peaks during the adolescent years. Thereafter the recurrence rate is less frequent later in the second decade and beyond the age of 20 years. (Kieffer et al., 2008).

Worldwide, the variability in reported rates and severity of ocular disease of CT has been attributed to variation in parasite characteristics and the immune response of the host (Holland, 2003; Vallochi et al., 2005). Compared with Europe, children with CT in South America demonstrate a significantly higher frequency of severe ocular disease and visual impairment. Ocular disease in CT is more benign in Europe (Peyron et al., 2006; Gilbert et al., 2008).

16.2 Objective
To demonstrate ocular outcome in a prospectively studied cohort of children with CT.
16.3 Patients and methods

16.3.1 Cohort studied
Fifteen children with CT identified by 24 months of national newborn screening.

16.3.2 Methods
Children were assigned to a standard of care management protocol which was divided into three major categories: 1) clinical evaluation at diagnosis 2) treatment with toxoplasma specific antiprotozoal medication for 12 months and 3) clinical monitoring following completion of treatment.

Retinal examinations were performed in the newborn period, dilated funduscopy was carried out by a paediatric ophthalmologist. Subsequent ocular examinations were performed three to six monthly for the first 18 months, six monthly thereafter up to age three years, annually thereafter and more frequently if necessary. Recurrence or reactivation was defined as a new focus of retinochoroiditis either adjacent to or remote from pre-existing retinochoroidal scars, or in a previously healthy portion of the retina with no surrounding lesions. A relapse was defined as new onset of active inflammation in a pre-existing inactive scar (Silveira et al., 2002).

The median ocular follow up at the time of this report was 9 years (range 2 to 10 years) and ophthalmology surveillance remains ongoing for the cohort.

16.4 Results
In the cohort of 15 children, 2 of 15 (13%) were symptomatic and 13 (87%) were asymptomatic at birth. One of the 15 identified in the screening programme had been previously diagnosed with CT following presentation with ventriculomegaly, ascites and intracranial calcification at 28 weeks gestation. Maternal treatment with antiprotozoals had been employed for the remainder of trimester 3.

Age at initial ophthalmology examination ranged from 3 days to 17 weeks of life, median 4 weeks (table 16.1). Six of 15 (40%) infants had inactive chorioretinal lesions at initial evaluation; 3 had a unilateral lesion (table 16.1 case numbers 7, 12 and 15) and 3 had bilateral lesions (table 16.1 case numbers 3, 5 and 8).
Four of the six infants (67%) who were found to have inactive chorioretinal lesions were clinically asymptomatic at birth; two had bilateral and two unilateral lesions (table 16.1 case numbers 5, 7, 8 and 12). Only two of six infants with ocular lesions, one with an antenatal diagnosis (table 16.1 case number 3) and one with hydrocephalus (table 16.1 case number 15), were symptomatic. Four of six infants with eye lesions also had intracranial lesions (table 16.1 case numbers 3, 5, 8, and 15).

Intra-ocular lesions were located in the peripheral retina in five of the six infants, none of whom had evidence of visual impairment on their initial evaluation. The one infant with an antenatal diagnosis of CT was found to have a central macular scar with absence of central visual potential was in the right eye, i.e., unilateral central blindness (table 16.1 case number 3). The left eye had an inactive lesion in the posterior pole, i.e., the portion of the retina that lies between the macula and the optic disc, in close proximity to the macula. Hence significant visual impairment was detected in 1 of 15 (7%) congenitally infected infants on their initial evaluation. The remaining 5 children with ocular lesions had no discernible visual impairment on initial examination.

Thirteen infants completed one year of pyrimethamine and sulfadiazine therapy. One asymptomatic infant, for whom persistently indeterminate serology resulted in a delayed diagnosis of CT, was not treated (table 16.1 case number 4) and treatment was discontinued after four months by parental request for another (table 16.1 case number 13).

Children were monitored with scheduled ophthalmology examinations for a median of 9 years (range 2 to 10 years). Two children were lost to follow up between age two and three years; both had normal retinal examinations at age two (table 16.1 case numbers 2 and 9). The parents of one other child chose to discontinue clinical monitoring and follow-up at age five years; ophthalmology examinations were normal up to that time (table 16.1 case number 1).

One child with a right unilateral lesion at birth (figure 16.1 a), treated for 12 months in infancy, developed three new retinochoroidal lesions between 24 and 33 months of age (table 16.1 case number 7). The first of these was detected by ophthalmology surveillance at 24 months of age in the equatorial periphery of the
left retina; spontaneous regression without retreatment occurred (lesion not shown). The second was noted at 30 months, on this occasion in the posterior pole of the left retina in proximity to the optic disc (figure 16.1 b). On that occasion, the infant received oral antiprotozoals and steroids for three months with a good response (figure 16.1 c). The third recurrence in the right eye at age 33 months, developed almost immediately following completion of treatment for the earlier episode. The right lesion was also in close proximity to the optic disc with adjacent vasculitis (figure 16.1 d). Retreatment was initiated with six months of antiprotozoals and a short course of tapering steroids, following which lesions remained quiescent. Visual function remains normal.

Three children in the cohort were treated for strabismus in childhood. Two symptomatic who had been treated and one asymptomatic untreated infant (table 16.1 case numbers 3, 4 and 15).

Five of six children with chorioretinal lesions had normal vision in both eyes. One with a central macular scar had unilateral visual impairment from the first assessment. No child had bilateral impairment. New chorioretinal lesions occurred between the second and third year of life in one of 15 (7%) children (table 16.1 case number 7).

After a median follow-up of 9 years (range 2 to 10 years), 6 of 15 prospectively monitored children with CT had ocular lesions, all six had evidence of retinal involvement in the neonatal period. In this cohort of treated infants, ocular reactivation or recurrence was rare, affecting only one child, with no increase in the number of children with central macular lesions or visual impairment during the study.

16.5 Discussion
In Europe, CT is associated with a low rate of severe disease, due to predominance of the less aggressive type II strain of *T. gondii*. The majority of infants with CT are asymptomatic at birth. Some European countries employ linked antenatal and postnatal screening programmes to facilitate early identification and treatment of infants with CT. Favourable visual outcomes, demonstrated by a low rate of visual impairment has been reported in most European populations studied (Brezin et al., 2003; Faucher et al., 2012).
Whilst the total number of congenitally infected infants in our cohort was small, the rate of ocular involvement was high (40%) and in the absence of a newborn screening programme, would not have been detected as the majority were asymptomatic. The rate of severe ocular disease with macular scarring and visual impairment was low, as was recurrent chorioretinitis over the study period. The prognosis for children with congenital ocular toxoplasmosis has been extensively studied in France, where the incidence of ocular lesions at initial evaluation (approximately 8% of those infected) is lower than reported in this study (40%). However, in France prenatal screening, active management of maternal primary toxoplasmosis and CT treatment in-utero is routine, hence it is expected that infant sequelae may be prevented or arrested, with a lower incidence of anomalies at birth.

Studies of French cohorts have demonstrated that approximately 29% of children develop at least one new eye lesion in the first 10 to 12 years of life (Wallon et al., 2004; Peyron et al., 2011). In these studies, ocular recurrences whilst common, were mostly peripheral, did not lead to visual impairment or impact on quality of life. The overall prognosis was good when early treatment of ocular recurrences was initiated.

In our cohort although 40% had ocular signs at first assessment, only 7% developed at least one new eye lesion in the subsequent 8 to 10 years.

A 9-year postnatal screening programme for CT was carried out in Denmark, during which 100 children with CT were identified in a population with an incidence of 1.6 per 10,000 live-born infants. Children were treated with antiprotozoals for three months and outcome at 3-year follow-up was published. The results of the Danish programme were the subject of two reports; the initial report detailed results based on the first four years of screening and the second and final report summarised the total 9-year experience (Roser et al., 2010).

In the initial four years of screening, 4% of the cohort were found to have chorioretinal signs at their initial evaluation and 18% by age three years. Progression of eye lesions was observed in children with and without lesions at the initial assessment. A higher occurrence of central macular lesions was also found at follow-up compared with the initial evaluation. The Danish study group
concluded that screening for CT had no effect on recurrence of chorioretinitis, development of macular scars or visual impairment.

These results contrast with the findings in our cohort, where a higher percentage of lesions at initial assessment, but a lower rate of recurrence and no increase in macular lesions at follow up or progression to visual impairment was demonstrated. A notable difference between the cohorts however was the duration of antiprotozoal therapy; three months for the Danish cohort and 12 months for the Irish cohort which possibly accounts for the better ocular outcome observed in our cohort. Given the similarities in populations, the similar incidence of CT, albeit with a higher incidence of ocular abnormality detected at baseline in our cohort, it is likely that treatment duration was a contributory factor in the better ocular outcome observed in the Irish cohort. The question as to whether 12 months of antiprotozoal therapy is significantly better than three months has not yet been definitively answered although results from this study are supportive of this concept and further study is warranted. Definitive answers will only be obtained through larger randomised controlled trials, potentially in those areas of the world with higher incidence of infection with the more aggressive and virulent serotypes. A major strength of the Irish study is the duration of ophthalmology follow-up, median nine years and on-going for 12 children, co-ordinated by a single investigator thus ensuring robust and accurate data collection.

A limitation of this study is the small number of infected infants. Thus, lost to follow up of even a small minority of infants could potentially confound the results. In this study two children were lost to follow up after age two and one after age five years. While all three had normal ocular examinations at their final assessments (table 16.1 case numbers 1, 2 and 9) the possibility of a retinal lesion developing at a later stage cannot be excluded. However, the low rate of progression of the ocular lesions in our cohort is noteworthy. Despite three children lost to follow-up, more importantly the 40% with chorioretinal lesions all remained in the cohort. The total number of six children with ocular lesions at birth represented the same six with lesions at median nine year follow-up (table 16.1). De-novo lesions did not arise in children with initially normal ophthalmology examinations.

The risk of severe eye disease in our cohort was low. There was only one child with a central macula scar and unilateral blindness, present at the initial evaluation
and in whom the findings remained stable throughout. No other child developed macular scarring or visual impairment.

The importance of diagnosis of CT and continued ophthalmic surveillance was highlighted by case number 7. This asymptomatic infant had CT detected only by virtue of the screening programme. Potentially sight threatening recurrences were aborted by early intervention as a result of regular ocular examinations. In particular, this demonstrated that all children with CT should receive regular assessment for early detection of ocular recurrences and not just those with severe symptoms or signs at initial evaluation as was suggested by the EMSCOT researchers (Freeman et al., 2008).

Whilst most lesions in ocular toxoplasmosis will regress spontaneously, it is recommended that sight threatening lesions are promptly treated. The best therapy for recurrences of ocular toxoplasmosis remains debatable (Bosch-Driessen et al., 1998). Some experts advise that intra-ocular therapy can suffice in immunocompetent individuals, and courses of oral antiprotozoals may not be effective in preventing future recurrences (Commodaro et al., 2009; de-la-Torre et al., 2011).

However, for the case with recurrences in our cohort, given the age of the child and the fact that episodes had occurred within a short time frame, systemic treatment was prescribed and other treatment options were not considered. It has been predicted that in both adults and children, after each episode of active chorioretinitis, two-thirds of all patients will experience a further relapse (Garweg et al., 2008). The recurrence pattern in the described patient demonstrated this.

Predictors of retinochoroiditis and intraocular recurrence have been studied in European cohorts. Freeman and investigators from the EMSCOT cohort predicted that: 1) children who had no retinochoroidal lesions by the age of four months had a low probability of developing retinochoroiditis by four years, whether other clinical manifestations were present or not and 2) if children had no chorioretinitis in early infancy, the risk of subsequent ocular disease was low (Freeman et al., 2008).

French studies on predictors of retinochoroiditis concluded that overall the risks of a child developing ocular disease were higher when mothers were infected early in gestation, when birth was premature, when symptoms of CT were apparent prior
to or at birth and when non-ocular and systemic symptoms were present at initial evaluation (Binquet et al., 2003). Female gender, and cerebral calcifications were identified as risk factors for retinochoroiditis during the first 2 years of life in infants treated for CT in France (Kieffer et al., 2008).

Two infants in our cohort had severe symptoms of CT (table 16.1 case numbers 3 and 15). One was symptomatic before birth and one other with hydrocephalus at birth was born to a mother with trimester 1 or early trimester 2 seroconversion. One of these infants was female (table 16.1 case number 3), both had multifocal cerebral calcifications, but neither had progression of eye lesions. In addition, one other infant born to a mother who seroconverted during a high-risk period in gestation had an inactive lesion at birth with unchanged intraocular findings at eight years (table 16.1 case number 12).

It is noteworthy that in our cohort, intraocular reactivation occurred in a female child with mild disease born to a mother with trimester 3 seroconversion (table 16.1 case number 7). Hence in the Irish cohort, with the exception of female gender, the cited predictors for recurrent retinochoroiditis were not observed, however the small numbers involved prevent any firm conclusions being drawn. The number and location of existing chorioretinal scars at initial evaluation has not been reported to predict recurrences in CT. Reactivation can occur with existing lesions but the majority of reactivations occur in a previously health location of the retina (Wallon et al., 2004).

Three children in this cohort required treatment for strabismus in childhood (table 16.1 case numbers 3, 4 and 15). Two had severe disease at birth and one was an asymptomatic child who did not receive treatment due to late serologic confirmation of CT. Ocular abnormalities, apart from retinochoroiditis, have been reported in children with CT including strabismus, cataract and microphthalmia. Studies have demonstrated that these manifestations are less frequent than retinochoroiditis and whilst they occur more frequently in children with macular lesions, they are also present in children with non-severe CT and represent a late manifestation of ocular sequelae of CT. Nineteen percent of children in a French cohort and 50% of children in a Brazilian cohort demonstrated these manifestations which are referred to as associated ocular alterations in CT (Kodjikian et al., 2006; Melamed et al., 2010). Hence based on these reports it was
assumed that three children in our cohort demonstrated late ocular associations of CT. Results of continued ophthalmology evaluations as the children in our cohort progress to adolescence are awaited with interest.

16.6 Conclusions
The studied cohort of 15 children with CT had a 40% rate of initial intraocular signs at birth but a low rate of macular scars, visual impairment and recurrence.

A favourable visual outcome was demonstrated at median 9-year follow-up of the cohort postnatally screened and treated for CT. Regular ophthalmology evaluation is essential for all children with CT.
Figure 16.1: Eye fundus photographs of case number 7

a) Right eye
Sharply demarcated pigmented area represents a peripherally located inactive chorioretinal lesion (black arrow) present from birth. Infant treated for 1 year with antiprotozoals. Retinal examinations performed by a single ophthalmologist at specific intervals.

b) Left eye
Age 30 months, second recurrence. Active chorioretinal lesion in close proximity to optic disc (black arrow). Fluffy appearance and poor demarcation of the lesion indicates active inflammation. Patient treated with antiprotozoals and steroids.

c) Left eye
Healing focus of chorioretinitis. Cicatrisation occurring from the periphery towards the centre with pigmented changes at the edges indicates regression.

d) Right eye
Age 33 months. One month following completion of three months of therapy for reactivation, new focus of chorioretinitis (black arrow) presented at temporal aspect of right macula with adjacent vasculitis. Patient retreated with six months antiprotozoals and tapering steroids. Therapy was successful and retinal lesion became dormant. All intraocular lesions subsequently remained inactive. Patient has normal visual acuity.
Table 16.1: Clinical findings and ophthalmic outcome of 15 children with CT

<table>
<thead>
<tr>
<th>Infant Case Number</th>
<th>Trimester of maternal seroconversion (est)</th>
<th>Intracranial signs</th>
<th>Initial ocular evaluation</th>
<th>Follow-up ocular evaluation</th>
<th>Age at last evaluation (yrs)</th>
<th>Visual Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>ND</td>
<td>N</td>
<td>N</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>ND</td>
<td>N</td>
<td>N</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Multiple calcification</td>
<td>RT central macular scar and absence of RT central vision. LT posterior pole scar</td>
<td>RT central macular scar and absence of RT central vision. LT posterior pole scar. RT strabismus</td>
<td>10</td>
<td>RT central blindness</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>ND</td>
<td>N</td>
<td>LT strabismus</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Multiple calcification</td>
<td>B/L peripheral scars</td>
<td>B/L peripheral scars</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>ND</td>
<td>RT peripheral scar</td>
<td>B/L posterior pole and peripheral scars</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>Single calcific focus</td>
<td>B/L peripheral scars</td>
<td>B/L peripheral scars</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>1 or 2</td>
<td>ND</td>
<td>RT peripheral scar</td>
<td>RT peripheral scar</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>N</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>N</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>1 or 2</td>
<td>Hydrocephalus and multiple calcification</td>
<td>RT peripheral scar</td>
<td>RT peripheral scar. LT strabismus</td>
<td>8</td>
<td>ND</td>
</tr>
</tbody>
</table>

est, estimated; yrs, years; ND, not detected; N, normal; RT, right; LT, left; B/L, bilateral
Chapter 17 - Serological profile during and after treatment for congenital toxoplasmosis

17.1 Introduction
Serological profiles of infants treated for CT during the first year of life with toxoplasma specific antiprotozoals usually demonstrate a reduction in quantifiable IgG (dye test) and suppression of specific IgM and IgA, particularly in the latter months of treatment. Serological rebound, also referred to as serological reactivation, is common following discontinuation of treatment and is demonstrated by an increase in the DT or rate of detection of IgM and IgA. The significance of rebound is not clearly understood. The explanation proposed is that although antimicrobials can control active toxoplasma infection and inhibit tachyzoite replication, not all organisms are eliminated despite one year of treatment (Remington, 2011). The ability of T. gondii to survive in cysts during treatment and subsequently reactivate explains this phenomenon, hence an increase in antibody production can occur months or years after treatment discontinuation (Sibalic et al., 1990; Fortier et al., 1997).

Serologic rebound has been demonstrated in children with and without manifestations of disease at birth and are most common two to six months after treatment completion (Wallon et al., 2001).

17.2 Objectives
To demonstrate: 1) the serological response during 12 months of treatment for CT; 2) the incidence of serological rebound following treatment completion and 3) the clinical significance of serological rebounds.

17.3 Patients and methods

17.3.1 Cohort studied
Fifteen infants with confirmed CT identified by 24 months of national newborn screening for CT.

17.3.2 Methods
Prospective observation of the serological profile of 15 children with CT.
Children were assigned to 12 months of treatment with pyrimethamine and sulfadiazine.

The serological profile was monitored three-monthly during treatment and up to 24 months. Thereafter testing was offered six-monthly until age 48 to 60 months. Clinical evaluation was performed in conjunction with serological testing.

17.4 Results

17.4.1 Treatment period
Fourteen of 15 infants received antiprotozoal treatment. The median age at treatment initiation was 4 weeks (range 3 days to 17 weeks). One infant with late serologic confirmation of CT was not treated (table 17.1 case number 4). Thirteen of 14 infants completed 12 months of treatment. One infant (at parental request) received treatment for just four months in total, from two to six months of age (table 17.1 case number 13).

17.4.2 Serology profile during treatment
Thirteen infants who completed 12 months of treatment demonstrated suppression of the DT; some from the early half of treatment (table 17.1 case numbers 1, 3, 8 and 10) and the majority from the ninth to twelfth month, by which time the DT showed a > fourfold reduction from the level quantified in the initial specimen taken shortly after birth. In addition, for all but 1 of 13 infants (table 17.1 case number 14) IgM and IgA were not detectable by ISAGA at the end of treatment. One infant who received treatment from two to six months of age had one nadir in the DT at six months (table 17.1 case number 13).

17.4.3 Duration of serology monitoring beyond the first year of life
All 15 children in the cohort, including one untreated child (table 17.1 case number 4), had serial monitoring of the antibody profile up to the 24th month and in 13 cases serological monitoring continued beyond the 24th month. For the children who had serology profiles beyond 24 months of age, the frequency of testing up to the age of 48 to 60 months was dependent on parental uptake on behalf of the child.
Of the 14 treated children, 12 had antibody profiles after age 24 months. Two treated children did not have serology testing after 24 months; the parents of one child opted out from further serological monitoring (table 17.1 case number 1) and one child was lost to follow up after age 24 months (table 17.1 case number 9). Of the remaining 12 treated children who had serological testing after 24 months, 11 were tested up to or beyond 36 months of age. The family of one child migrated shortly after the child was 34 months of age (table 17.1 case number 2).

One untreated child had serological monitoring until age 60 months (table 17.1 case number 4). Hence in the total cohort of 15 children, 13 had serological testing after the second year of life. The age at final testing ranged from 34 to 60 months, median 48 months.

17.4.4 Rebound of the dye test in treated children
Following completion of treatment, all 14 children (100%) demonstrated serological rebound of the DT between 15 and 24 months of age including the one child in whom treatment was terminated at six months of age (table 17.1 case number 13). Twelve treated children were tested beyond 24 months. The occurrence of DT rebound was less common between ages 24 and 36 months, but persistently high dye tests quantifiable at ≥ 2,000 IU/mL between the second and third year of life were demonstrated in 4 of 12 children (table 17.1 case numbers 2, 3, 8 and 13). Dye test rebound was rare after the age of 36 months and was demonstrated in just one treated child who had an increase in the DT from 16 IU/mL at 36 months to 2,000 IU/mL at 42 months (table 17.1 case number 3). One untreated child had no significant change in the DT during the first 24 months of follow-up (table 17.1 case number 4).

17.4.5 Reappearance of IgM and IgA following treatment completion
Complete suppression of IgM and IgA at the end of treatment was demonstrated in 13 of 14 (93%) treated children, following which 10 of 14 (71%) subsequently had re-emergence of IgM and/or IgA detectable by ISAGA. In nine children reappearance of IgM or IgA occurred in the second year of life and in one IgA first reappeared after 24 months (table 17.1 case number 6).
In 9 of the 10 children, IgA reappeared, and in 2 of these children IgM was also detected (table 17.1 case numbers 2 and 3). One child had reappearance of IgM only (table 17.1 case number 15).

Four of 14 treated children (29%) had no demonstrable IgM or IgA at any point following treatment completion; 1 up to age 24 months (table 17.1 case number 1), 1 up to 39 months (table 17.1 case number 14), and 2 up to 48 months (table 17.1 case numbers 12 and 13).

The rate of IgM and IgA detection decreased after the 24th month of life. Twelve of 14 treated children were tested between 24 to 36 months; 6 had IgA detected and 1 also had IgM detected (table 17.1 case numbers 2, 3, 5, 6, 7 and 8).

In the 11 treated children who were tested at or beyond 36 months, neither IgM nor IgA was demonstrable by ISAGA. One infant with equivocal serology at birth who was not treated demonstrated no significant change in the serological profile up to age 24 months. Thereafter IgA was undetectable and a decrease in the DT occurred after 36 months (table 17.1 case number 4).

**17.4.6 Serological rebound and clinical correlation**

Episodes of high DT rebound ≥ 2,000 IU/mL or reappearance of IgM and IgA were not associated with clinical significance in the studied cohort.

**17.4.7 Reactivation and the serological profile**

One child who had recurrent chorioretinitis at 24, 30 and 33 months did not demonstrate correlation between clinical signs of reactivation and the serological profile which remained unchanged for all three episodes of reactivation (table 17.1 case number 7). The highest rebound occurred at 18 months when the serological profile demonstrated a DT of 4,000 IU/mL which had no detectable clinical association. Suppression of the DT was demonstrated during two courses of retreatment for recurrences, following which the DT remained stable.

**17.5 Discussion**

In the cohort of 14 children treated for CT during the first year of life, antipROTOZOAL effect was demonstrated by suppression of all classes of antibody tested,
particularly during the second six months of treatment and most notably by the end of the 12-month treatment regimen. A feasible explanation is that as treatment progressed, antiprotozoals decreased the parasite load, which in turn progressively reduced the stimulus for antibody production.

In addition to the antiprotozoal treatment effect on antibody production, the impact of passively acquired maternal antibody should also be considered. Infants with CT will have both actively produced and passively acquired antibody in their circulation. Passively acquired maternal IgG will decay at a rate of approximately one half its value every 30 days. Thus the maternally derived contribution to the total antibody in the child’s circulation will be reduced progressively during the first year of life. Hence, total antibody levels during the first year of life of a child with CT will reflect the kinetic interaction of active antibody production (with or without suppression due to antiprotozoal treatment) plus a progressively declining level of passively acquired maternal antibody.

Following discontinuation of treatment, all 14 children (100%) subsequently demonstrated at least one episode of serological rebound with an increase in quantifiable DT and or re-emergence of IgM or IgA up to final testing at median age 48 months. Serological rebound following one year of treatment for CT is common and represents a secondary increase in antibody production which is thought to result from re-exposure of the child’s immune system to the parasite. At this point antiprotozoal suppressive effect no longer plays a role in parasite control, hence antibody production is stimulated with a demonstrable increase in all classes of toxoplasma antibody (Kahi et al., 1999).

The serological profile of large cohorts of children treated for 12 months with antiprotozoals have shown that the occurrence of rebounds months or years after initial treatment generally do not correlate with new clinical signs or evidence of disease progression (Djurkovic-Djakovic et al., 2000; McLeod et al., 2006). In a study by Wallon et al of 133 children with CT treated for one year, 93 (70%) had at least one rebound at mean follow-up of 95 months. One sub-group received an additional three months of treatment with antiprotozoals for rebound and another sub-group were not treated. There was no difference in the incidence of secondary eye lesions in those with and without rebound and those who were or were not retreated for rebound. The presence of intracranial calcification was associated
with an increased relative risk for rebound, and treatment with antiprotozoals between two and 12 months with a decreased risk. Conclusions were that serological rebounds did not warrant increased ophthalmology surveillance or further courses of treatment (Wallon et al., 2001).

The highest rate of serological rebound in our cohort of 14 treated children was in the second year of life and likely indicates that during this time, either free tachyzoites or parasites that have re-emerged from latent cysts replicate or reactivate after treatment is discontinued. This equates with the fact that in CT, the first two years of life represent the highest risk period for secondary eye lesions (Kieffer et al., 2008) and also provides the rationale for the 24-month treatment regimen for CT employed in some centres in France and the USA (McAuley et al., 1994; Wallon et al., 2004).

However, after the age of one year, it is expected that the child's immune response can effectively control the parasite without the need for an extended period of treatment. The increase in detectable antibody demonstrated in our cohort after treatment cessation indicated an appropriate immune response to parasite activity which was sustained for months or years in some children in the cohort.

Hence whilst serological rebounds may raise concerns regarding parasite activity and consequent disease progression, it provides reassurance that the increase in antibody represents the child’s ability to combat the parasite and avoid sequelae. This was proven in our cohort as rebounds were not associated with the discovery of new clinical signs. In addition, for those who had more than one rebound, the number of rebound episodes following treatment was not of any detectable clinical significance. Persistently high dye tests and detectable IgM or IgA between 24 to 36 months demonstrated in four children were of no clinical significance (table 17.1 case numbers 2, 3, 8 and 13).

After the high risk period for reactivation in the first two years of life, it may be possible that either the parasite enters a latent phase from the child's third year of life until adolescence, or full immunologic control of parasite activity is accomplished by the host from the third year of life. This may account for the decrease in the incidence of rebound from the age of 36 months.
In the one child with intraocular reactivations (table 17.1 case number 7), the serological profile did not predict recurrences, nor did it differ from the profile of others in the cohort.

The presence of intracranial calcification was not found to be a risk for serological rebound in our cohort; 4 of 14 (29%) treated children had intracranial calcification (table 17.1 case numbers 3, 5, 7 and 15) and 100% of the treated cohort demonstrated rebound. However the numbers are too small to draw accurate conclusions in this regard.

Despite the limitation of small numbers, the findings in our study reflect the results from other studies on serological rebound which demonstrate that rebound episodes are of no clinical significance.

17.6 Conclusions
In the studied cohort of 15 children with CT, 14 treated children demonstrated a serological response to treatment with a reduction in quantifiable or detectable specific antibody during the first year of life. Following cessation of treatment, all children had at least one rebound of IgG, IgM or IgA, most frequently in the second year of life. Reappearance of IgA was more common than IgM. The rate of serological rebound decreased after the second year of life. Rebound episodes at median follow up of 48 months did not precede or indicate intraocular recurrences and should not necessitate more frequent ophthalmology surveillance or further treatment.
Table 17.1: Serological profile during treatment and rebound post treatment in a cohort of infants with CT

<table>
<thead>
<tr>
<th>Infant Case No.</th>
<th>Dye test IU/mL (ISAGA IgM/IgA); [month of test]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 months</td>
</tr>
<tr>
<td>1</td>
<td>2000 (+/+)</td>
</tr>
<tr>
<td>2</td>
<td>4000 (+/+)</td>
</tr>
<tr>
<td>3</td>
<td>4000 (+/+)</td>
</tr>
<tr>
<td>4</td>
<td>125 (BD/+)</td>
</tr>
<tr>
<td>5</td>
<td>4000 (+/+)</td>
</tr>
<tr>
<td>6</td>
<td>2000 (+/+)</td>
</tr>
<tr>
<td>7</td>
<td>2000 (+/+)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>12†</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

+, positive; -, negative; NA, not available; BD, borderline

*Infant not treated with antiprotozoals; †Infant treated from 4 months of age; ††Infant treated from 2 to 6 months of age

266
CHAPTER 18 - Clinical, neurodevelopmental and educational progress summary in a cohort of infants with congenital toxoplasmosis identified by newborn screening

18.1 Introduction
Newborn screening for CT enables early identification of infants, the vast majority of who are asymptomatic in Europe. Infants with CT who have CNS symptoms apparent before or at birth are at higher risk for neurologic progression and developmental delay. Asymptomatic children may demonstrate untoward neurological sequelae later in childhood such as delayed psychomotor development, cognitive dysfunction or intellectual impairment of minor degrees which may not be apparent until school age. The prevalence of subtle neurodevelopmental sequelae amongst asymptomatic infants is not known due to paucity of long term follow up studies in children with subclinical CT. The potential benefits of screening allows not only early antiprotozoal treatment, but facilitates monitoring of those detected, which enables prompt interventions where necessary to maximise outcome long term.

18.2 Objectives
To demonstrate clinical, developmental and educational outcome in a cohort of infants with CT.

18.3 Patients and methods

18.3.1 Cohort studied
Fifteen children with CT identified by newborn screening over a 24-month period were enrolled at diagnosis in a prospective observational study. Specific outcome measures were clinical, developmental and educational progress during the first decade of life.

18.3.2 Methods

18.3.2.1 Clinical follow-up
Following completion of treatment in the first year of life, children continued with ophthalmology and audiology examinations at regular intervals. Intracranial
imaging was repeated after treatment completion where indicated and as necessary thereafter.

18.3.2.2 Developmental follow-up during the first decade

Developmental examinations were performed 3-monthly during the first year of life whilst on antiprotozoal treatment and 6-monthly following treatment completion up to age 42 months using Bayley scales for infant and toddler development third edition (Bayley-III). Subsequently, between the age of 42 months and school entry, achievement of milestones were assessed and documented by 6-monthly paediatric examinations for all main areas of development; gross and fine motor, speech, language and social skills. Developmental examinations were performed by a single paediatrician, the CT screening programme co-ordinator.

Children with deficits or abnormalities identified at any point during the pre-school years were referred to interventional services and psychology assessment was also sought. Psychological evaluation included standardised assessment of cognitive ability using the Wechsler Intelligence Scale for Children–Third Edition (WISC-III) and IQ was measured to determine general intellectual ability and whether the child was a candidate for main-stream schooling. During primary school years, children were offered annual paediatric review that took place at an ID out-patient clinic. At these visits children had a physical examination and school progress reports were reviewed with parents to determine if the child required further specialist input or referral.

18.3.2.3 Educational and cognitive assessment during the first decade

Following primary school entry, educational and behavioural progress were assessed and documented by teachers according to the National Council for Curriculum and Assessment (NCCA) guidelines from the department of education (available at http://www.ncca.ie/en/Curriculum_and_Assessment/Early_Childhood_and_Primary_Education/Primary-Education/Assessment/ accessed November 2015). Overall progress through primary school was determined using a standard range of methods; mainly national standardised tests and teacher designed tests and observation (figure 18.1). Academic progress was assessed using the standardised academic testing out of ten (STEN) for scoring in English reading, spelling and Maths applied to primary school children from the third year in school. STEN results were graded according to a standard scale as follows: 8-
10; well above average; 7, high average; 5-6, average; 4, low average; 1-3; well below average. In addition, STEN scores for each child in English, spelling and Maths were routinely categorised into percentiles according to age matched children nationally. STEN scores were displayed in the child’s end of school year report, primarily for a parent to understand the child’s academic progress. Teachers also routinely used other standardised academic tests during the school year to screen children’s academic ability, but these scores were kept for professional use only. The teacher’s standardised scores were graded as follows: 100, average; >130, academically gifted; <85, very weak.

Non-reading intelligence tests were used to assess overall practical intelligence in non-academic areas. These tests were also for teacher assessment only and the results were not shared with the parent or pupil unless a low score was obtained which indicated a potential problem area that required targeting. Scores of > 85% < 95% are acceptable and scores of > 95% indicate a high level of practical intelligence.

Teacher observation is the method routinely employed in primary schools to screen for a wide range of problems in the areas of coordination, concentration, sequencing and fine-motor skills. Teacher observation is first informal or intuitive, and if concerns are generated, planned observation takes place which focuses on a specific issue to identify whether the child need assistance.

If any observational or formal tests in academic or non-academic areas identified weaknesses or issues for concern at school, parental assessment of the child was sought along with the child’s self assessment and a referral was then made to an educational psychologist for a formal report. Particular attention was given to identification of cognitive difficulties such as problems with auditory discrimination or visual memory.

18.4 Results

18.4.1 Clinical outcome
Infant clinical and developmental outcome to date is summarised in table 18.1 and figure 18.2. Median follow-up was 9 years; range 2 to 10 years. Ocular outcome and follow-up intracranial imaging has been described for the cohort in previous
chapters. Audiological examinations were normal for the cohort when last assessed.

18.4.2 Developmental progress

In the cohort of 15 children, two symptomatic infants with abnormalities of tone and power at birth had neurological abnormalities and delayed milestones identified during the first year of life and were referred for multidisciplinary early interventional services. Both children received necessary interventions from infancy for four years (table 18.1 case numbers 3 and 15). Psychology assessments were performed for these two children, who at the age of four years were deemed fit for main stream primary school. At that point one child had a full scale IQ score of 100 (table 18.1 case number 3) and the other a score of 84 (table 18.1 case number 15). One symptomatic child has had a normal neurodevelopmental outcome (table 18.1 case number 3).

One symptomatic child with severe hydrocephalus who had VP shunt placement at age three weeks had global developmental delay targeted during infancy and the preschool years. The child to date has had a number of VP shunt complications and brain imaging demonstrates linear areas of PVL bilaterally. The child at age eight years has mild residual motor delay which manifests in the areas of co-ordination and fine-motor skills. The co-ordination problems which remain affect the ability to hop and skip effectively, and fine motor delay affects writing skills. However daily life and the ability to attend school are not hindered.

Thirteen asymptomatic children had no developmental deficits identified by Bayley-III scores and thus did not require referral for adjunctive therapies. In the cohort of 15 children, all (100%) were assessed up to the age of 2 years, 13 (87%) up to the age of 5 years and 12 (80%) remained for regular evaluation who at the time of this report were between the ages of 8 and 10 years, median 9 years (table 18.1).

Three asymptomatic children with no signs of CT were lost to follow up. Growth and development were normal when last assessed at age two years in two of these children and at age five in one child (table 18.1 case numbers 1, 2, and 9). Eleven of 12 children at the most recent evaluation between the ages of 8 and 10 years had normal development in the areas of gross motor, fine motor, language
and social skills. Clinical and developmental outcome for the cohort to date is summarised in figure 18.2.

**18.4.3 Educational progress**

The 12 children who remained in the cohort for follow-up entered main-stream primary school between the age of four and five years. School progress reports and teacher assessments were reviewed at annual visits when the child attended the paediatric ID clinic for medical examination. For the 12 children followed through school thus far, none were identified as at risk for adaptive, behavioural or attention problems. In addition, teacher screening, observation and academic tests did not identify deficits or weaknesses in any areas of cognitive ability for the cohort and no child to date has been referred to an educational psychologist for assessment. STEN scores were high average and above average for all 12 children, i.e., 7-10. Eight children had STEN scores of 8-10 for English, spelling and Maths and four children had scores of 7 and 8 thus far. Collectively, STEN scores for the cohort ranked between the 84th and 100th percentile compared to the national average for age matched peers.

One asymptomatic child with multiple intracranial calcification at birth and thus by definition severe CT, had STEN scores of 8-10 throughout school years (table 18.1 case number 5).

One child in the cohort with symptomatic CT *in-utero* had STEN scores persistently above average through school, in addition to scores persistently > 150 in teacher-designed tests which indicated the child was academically gifted (table 18.1 case number 3). Due to exceptional academic performance, at the age of 10 years the child was formally identified as having superior intellectual ability compared with age matched peers nationwide. Subsequently, the child was selected for assessment and granted a place in the Centre for Talented Youth of Ireland (CTYI), an academy which provides adjunctive education for academically gifted youth. Apart from this child, it was not possible to acquire teacher-based academic test results for the other 11 children as these results are confidential and not routinely shared with parents or pupils. The high scores documented for case number 3 were only available as parental consent was required for the child’s referral to CTYI.
One other symptomatic child with hydrocephalus at birth had mild fine motor problems identified at school which affected writing skills. However, resources were provided to maximise development of writing skills and progressive improvement has been reported. No other intellectual or behavioural deficits were identified at age eight years. The child's STEN scores remain persistently $\geq 7$ in primary school thus far.

18.5 Discussion

18.5.1 Clinical outcome

The outcomes of three specific clinical evaluations were documented at the final assessments prior to this report: ocular, intracranial and audiological. Ocular reactivation occurred in one child (7%) in the cohort, intracranial signs regressed in all children with multiple signs. Both ocular and intracranial outcomes for the cohort have been detailed in previous chapters.

Audiological abnormalities were not demonstrated in the cohort at initial and final sensorineural hearing tests. Brown et al performed a database review of longitudinal studies on audiologic findings in cohorts of children with CT identified at birth and reported that the overall incidence of hearing loss was 0% to 26%. The highest percentage of sensorineural hearing loss (SNHL) was found in children from historic cohorts who received no treatment or treatment for one month. The prevalence of SNHL was 12% in children who had treatment initiated beyond age two and a half months and in whom treatment compliance was uncertain. The prevalence of SNHL was 0% in children who had treatment initiated before age two and a half months for 12 months, with serological compliance confirmed (Brown et al., 2009).

18.5.2 Neurodevelopmental and educational outcome to date

All children in the studied cohort had a favourable neurodevelopmental outcome when last assessed, including two symptomatic children with poor prognoses initially predicted based on findings at diagnosis. Both symptomatic children exemplified the benefit of early diagnosis, antiprotozoal treatment and additional interventions employed from infancy to maximise outcome (table 18.1 case numbers 3 and 15).
Early interventional programmes for children with CNS symptoms secondary to various aetiologies have proven benefit, in particular for prevention of motor and cognitive impairments. Studies have demonstrated that outcome following early intervention is best if commenced under the age of 12 months and if interventions are focused on infant development and the parent/infant relationship (Anderson et al., 2003; Spittle et al., 2007).

The diagnosis of CT and early referral for intervention maximised outcome for two symptomatic children with severe signs of CT, one of who had unilateral blindness and frontal lobe destruction, and the other significant hydrocephalus at initial evaluation with signs of PVL at follow-up. The latter infant demonstrated that VP shunt, antiprotozoal therapy and adjunctive therapies for developmental delay in early childhood enabled main-stream schooling (table 18.1 case number 15). Without early treatment and intervention, it can be concluded beyond doubt that both symptomatic infants would have had less favourable developmental outcomes than that demonstrated at final assessment. In the studied cohort where 80% have remained for follow-up assessments, academic and other educational scores did not identify areas of concern or issues that required intervention.

A uniform and thorough follow-up evaluation programme was provided for the children in the cohort by the CT screening programme co-ordinator which encouraged and facilitated compliance with serial clinical and developmental assessments in order to draw conclusions on outcome. The majority of parents welcomed clinical and developmental follow-up after treatment completion as it provided reassurance. In general compliance issues were not encountered with the follow-up protocol, as out-patient visits were less frequent than that necessary for blood monitoring during treatment. Clinical appointments were tailored to suit the families’ schedule, particularly after children entered primary school.

Despite the provision of accessible follow-up, 3 of 15 children (20%) were lost to follow-up. However the three children had no identifiable deficits when last reviewed at or beyond the age of two years (table 18.1 case numbers 1, 2 and 9). The families of two of these children emigrated for employment reasons (table 18.1 case numbers 2 and 9) and the parents of one child chose to disengage from clinical follow up after five years (table 18.1 case number 1). Thus in effect, there was only one child in the cohort who was lost to follow up as a result of parental
choice or true non-compliance as the other two families left Ireland for employment opportunities. Even in one other case where parental non-compliance with the treatment protocol resulted in treatment discontinuation after four months of therapy (table 18.1 case number 13), the family were adherent to the clinical follow-up protocol. The monitoring period attained for the children who remained in the cohort enabled the conclusion that good compliance was achieved for follow-up during the first decade of life.

A limitation to this study was the small number of children with CT. However, clinical, developmental and educational progress was available for 12 of 15 (80%) at median age nine years. More importantly, the symptomatic infants at higher risk of neurological sequelae remained in the cohort.

A second limitation to this study was lack of controls for comparative analysis of development and educational progress. As previously discussed, it is not ethically possible to randomise children with CT to a non-treatment group or withhold interventions. Hence for this study, judgements on outcome were purely based on longitudinal observations of the children as they progressed through childhood and school. However, academic comparison between the children in the cohort and the national average was available as STEN scores were plotted on a graph to determine the child’s percentile ranked with age-matched results. The striking outcome for the 12 children followed through school is that they all consistently demonstrated high average and above average academic scores which plotted at or above the 84th percentile. Outcome for the cohort is summarised in figure 18.2. Detailed individual case histories for each infant are provided in the subsequent chapter.

18.6 Conclusion
At median 9-year follow-up, a favourable clinical, developmental and educational outcome predominated in the studied cohort of 15 children that included two symptomatic children and four with clinical signs of CT; one with multiple intracranial calcification at birth and one of whom had intraocular reactivation.

The most severely affected child with hydrocephalus at birth had minor motor deficits at eight years that did not alter quality of life or ability to attend primary school.
Figure 18.1: A continuum of primary school assessment methods

(Source http://www.curriculumonline.ie/)
Figure 18.2: Outcome of a cohort screened for CT in Ireland at median 9-year follow-up
Table 18.1: Clinical, developmental and educational progress summary for 15 congenitally infected infants

<table>
<thead>
<tr>
<th>Infant Case No.</th>
<th>Ophthalmology</th>
<th>Brain imaging</th>
<th>Audiology</th>
<th>Developmental progress (age at last evaluation)</th>
<th>STEN scores (0-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (5 yrs)</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (2 yrs)</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>B/L inactive chorioretinal scars</td>
<td>Ventriculomegaly at birth, regressed at 1 year Multiple frontal lobe and periventricular calcifications, all but one focus resolved at 1 year</td>
<td>N</td>
<td>N (10 yrs)</td>
<td>9-10</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (10 yrs)</td>
<td>8-10</td>
</tr>
<tr>
<td>5</td>
<td>B/L inactive chorioretinal scars</td>
<td>Multiple foci of intracranial and RT periventricular calcification, regression at one year</td>
<td>N</td>
<td>N (10 yrs)</td>
<td>8-10</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>7-8</td>
</tr>
<tr>
<td>7</td>
<td>Unilateral inactive chorioretinal scar at birth Bilateral recurrences at age 2 years</td>
<td>N</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>8-9</td>
</tr>
<tr>
<td>8</td>
<td>B/L inactive chorioretinal scars</td>
<td>Focus of intracranial calcification</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>8-10</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (2 yrs)</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>7-8</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>8-9</td>
</tr>
<tr>
<td>12</td>
<td>Unilateral inactive chorioretinal scar, no progression to date</td>
<td>N</td>
<td>N</td>
<td>N (9 yrs)</td>
<td>8-9</td>
</tr>
<tr>
<td>13</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (8 yrs)</td>
<td>7-8</td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N (8 yrs)</td>
<td>8-9</td>
</tr>
<tr>
<td>15</td>
<td>Unilateral inactive chorioretinal scar</td>
<td>Congenital hydrocephalus, resolved with VP shunt Periventricular calcification, all but one focus resolved at one year.</td>
<td>N</td>
<td>Mild fine motor delay (8 yrs)</td>
<td>7-8</td>
</tr>
</tbody>
</table>

N, normal; yrs, years; NA, not available; B/L, bilateral; RT, right; VP, ventriculoperitoneal
CHAPTER 19 - Congenital Toxoplasmosis: 15 individual case histories

19.1 Case number 1

19.1.1 Screening and confirmation
The first confirmed case of CT was born by instrumental delivery at term following an uncomplicated pregnancy. The mother had a history of three previous miscarriages. The parents were Irish born and resided in Dublin. Heel blood was collected on day four of life, results were available on day 11 and confirmatory mother-infant samples were taken on the same day for dispatch to the TRL Swansea.

Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from three months gestation confirmed pregnancy seroconversion, likely during trimester 3, with a strongly positive ELISA IgM detected postnatally (table 19.1 case number 1). The only identified maternal risk factor for toxoplasma infection during pregnancy was ingestion of rare meat and lack of maternal knowledge regarding the associated risk.

19.1.2 Infant evaluation and treatment
The infant was asymptomatic with no signs of CT on evaluation. *T. gondii* PCR tests of infant blood and CSF were negative. Treatment commenced on day 21 of life and continued for 12 months. The treatment course was uneventful with four episodes of transient grade 1 neutropenia (ANC 1.0 x 10⁹ – 1.2 x 10⁹/L) during the first three months of treatment that required no intervention. The full course of antiprotozoal treatment was completed. Liver function and urinalysis remained normal throughout treatment.

19.1.3 Compliance with the management protocol and challenges encountered
The infant was a firstborn child after three early pregnancy losses. The parents were initially shocked and overwhelmed by the diagnosis and the one-year treatment recommendation. As time progressed they were more accepting of the diagnosis and were reassured by the normal evaluation. However, they resented their child being 'labelled' as they perceived her with a diagnosis and preferred to view her 'as normal' and not as having 'a condition'. Efforts were made to explain
that CT was a treatable condition and as the infant was asymptomatic long-term prognosis was favourable.

The infant's father, a medical professional, had issues with potentially toxic medication being administered for one year for what he perceived to be prophylaxis, as there were no toxoplasma-specific lesions to target. However, the parents were compliant with administering medication and attending hospital appointments for toxicity monitoring and evaluation during the 12 months of treatment.

19.1.4 Clinical follow up and outcome to date
Attendance for follow-up evaluation after treatment completion was poor and many appointments were rescheduled to encourage attendance. The parents were generally disinterested regarding information about CT and the need for clinical monitoring in the first decade of life.

Audiology assessment was normal up to the final evaluation at age four. Neurodevelopmental and ophthalmology examinations were normal up to the age of five years when the infant was last assessed. Numerous appointments for ophthalmology surveillance were not attended after age five years. The infant’s mother stated that she did not wish to engage with services for further follow up as she felt it was an unnecessary disruption to the routine of a child who appeared healthy with normal development. Thus the child was not rescheduled for follow up thereafter. The parents were advised to engage with services if there were ever concerns related to CT and disease reactivation such as symptoms of visual dysfunction. No further information was available on the child after the age of five.
19.2 Case number 2

19.2.1 Screening and confirmation
The second confirmed case of CT was born by emergency caesarean section (EMCS) at term to a primigravida following an uncomplicated pregnancy. The parents were Pakistani and had been residing in Ireland in county Kerry for two years. Heel blood was collected on day four of life, results were available on day 11 and confirmatory mother-infant samples were taken on day 12 for dispatch to the TRL.

Infant serology confirmed CT. Maternal postnatal serology demonstrated a high titre DT, positive IgM and IgA and very low avidity (table 19.1 case number 2). Stored serology from the first antenatal visit at 20 weeks gestation demonstrated a low titre DT and a reactive IgM which suggested recently acquired infection, consistent with trimester 2 seroconversion. Maternal risk factors for toxoplasmosis during pregnancy were not identified and the mother was unaware of toxoplasmosis and risks for acquisition.

19.2.2 Infant evaluation and treatment
The infant was asymptomatic with a normal evaluation. Blood and CSF were T. gondii PCR negative. Following in-patient evaluation, antiprotozoal treatment was commenced on day 20 of life for 12 months. Transient gastrointestinal side effects occurred during the initial weeks of treatment. Five episodes of grade 1 neutropenia (ANC 1.0 x 10^9 – 1.18 x 10^9/L) occurred during the first eight months of treatment and required no interventions. One episode of grade 2 neutropenia (ANC 0.8 x 10^9/L) occurred in the third month of treatment that responded to increased dosage of calcium leucovorin. Liver function and urinalysis remained normal throughout treatment.

19.2.3 Compliance with the management protocol and challenges encountered
The infant was a firstborn child and initially parents disbelieved the diagnosis. The father, a medical professional, was not convinced the infant had CT based on confirmatory serology and argued that the results were not conclusive. Despite this, the parents agreed to infant treatment.
The parents admitted they were initially not fully compliant with administering medication and discontinued antiprotozoals on two occasions for one week each in the first two months for gastrointestinal adverse effects. Interruptions were retrospectively communicated to the CT programme co-ordinator. The infant received 50 weeks (96%) of the treatment course. In addition, it became apparent that occasionally the infant received incorrect drug doses due to the parents' reluctance to communicate queries with the team. Efforts were made to stress the importance of compliance and upkeep of regular communication with the CT programme co-ordinator.

19.2.4 Clinical follow up and outcome to date
The family migrated to the UK when the child was two years and 10 months as a result of the father's employment. Audiology, ophthalmology and neurodevelopmental examinations were normal up to this point. Letters detailing the child's medical history were given to the parents and they were advised to avail of paediatric services, and in particular ophthalmology services in the UK for necessary follow up. The parents indicated they intended to do so as they had medical contacts in the UK. No further information on the child's progress is currently available.
19.3 Case number 3

19.3.1 Screening and confirmation: antenatal confirmation of CT
The third confirmed case of CT was born by planned caesarean section (PLCS) at 37 weeks gestation to a primigravida. The parents were Irish born and resided in county Kilkenny. CT was diagnosed antenatally when a routine ultrasound scan at 28 weeks gestation demonstrated fetal ascites and ventriculomegaly; fetal anomaly scan at 22 weeks gestation was unremarkable. The maternal toxoplasma antibody profile at 28 weeks demonstrated a DT of 4,000 IU/ml, a positive ELISA IgM and an avidity of 0.03 consistent with acute or recent infection (table 19.1 case number 3). Retrospective analysis of earlier serology from 22 weeks gestation was negative for toxoplasma antibody. Infant congenital infection was confirmed by *T. gondii* PCR detected in amniotic fluid.

Retrospectively the mother reported symptoms of a flu-like illness at 24 weeks gestation but medical attention was not sought. Maternal treatment with pyrimethamine and sulfadiazine was initiated from 30 weeks gestation and continued for the remainder of the pregnancy. Serial fetal ultrasound scans were performed thereafter and resolution of fetal ascites and arrest of ventriculomegaly were demonstrated by 37 weeks. The maternal serological response to treatment was demonstrated by a rapid decline in DT titres to 125 IU/mL at delivery. The mother had been aware of the risk of toxoplasmosis posed by ingestion of undercooked meats in pregnancy, which she avoided. She was unaware of any risk associated with soil contact, which she had had while gardening without gloves and without attention to hand hygiene.

Heel blood was collected from the infant on day three of life and results were available on day nine. Infant confirmatory serology was consistent with CT.

19.3.2 Infant evaluation and treatment
The infant was initially evaluated at the regional hospital of birth then transferred to the paediatric ID service at OLCH Dublin to commence the treatment protocol. As the diagnosis of CT had been confirmed antenatally, infant treatment with pyrimethamine and sulfadiazine was commenced on day three of life, prior to acquisition of the DBS result or confirmatory serology, which were both subsequently positive. Infant physical examination in the newborn period
demonstrated central hypotonia. Blood and CSF were *T. gondii* PCR negative. Infant evaluation demonstrated signs consistent with CT. Cranial ultrasound demonstrated mild ventricular dilatation and CCT brain scan revealed multiple areas of frontal lobe and periventricular calcification with some destruction of the frontal lobe cortex (figure 19.1 a and b). Ophthalmology examination demonstrated bilateral inactive chorioretinal scars and a large scar at the right fovea with no central visual potential in the right eye indicative of right central blindness. Scarring in the left eye spared the macula but an inactive chorioretinal lesion was located in close proximity. Audiology testing was normal.

Six episodes of grade 2 neutropenia occurred during the first two months of treatment (ANC > 0.75 x 10^9 < 1.0 x 10^9/L) that responded to increased dosage of leucovorin. At three months of age two episodes of grade 4 neutropenia occurred with ANC of 0.3 x 10^9 and 0.1 x 10^9/L. Due to right visual impairment, plus potential for visual loss in the left eye; temporary drug withdrawal or dose reduction was not deemed appropriate, thus GCSF support was commenced at age three months and continued for the duration of treatment. This was administered subcutaneously by the child’s parents twice weekly until the ninth month of treatment and weekly for the last three months. Neutrophil counts were sustained above 1.0 x 10^9/L. The infant completed 12 months of antiprotozoal treatment at full dosage. Liver function and urinalysis remained normal throughout treatment.

19.3.3 Compliance with the management protocol and challenges encountered

The infant was a firstborn child. When CT was confirmed antenatally the parents received counselling in preparation for a poor outcome. They were told that fetal signs present at 28 weeks were likely to be associated with neurological consequences and if further progression occurred *in-utero*, fetal survival could be threatened. Whilst the parents were totally devastated by the grim prognosis predicted, the diagnosis was fully accepted. They were given hope by resolution of fetal signs during maternal treatment and they were determined to do whatever possible to optimise outcome once the child was born.

Infant evaluation in the newborn period further reinforced a guarded prognosis.
Hypotonia and intracranial abnormalities detected in the newborn period indicated that neurological consequences were likely, and whilst the precise effects could not be accurately predicted, sequelae could become evident in the future. Right central blindness and a left inactive scar in close proximity to the fovea signified the potential for bilateral central blindness if reactivation in the left eye ever occurred.

Neutropenia requiring GCSF support necessitated twice weekly or weekly blood counts for the entire year of treatment and this imposed an extra stress on the family. Despite this, parents engaged well with the various services, were fully compliant with the treatment protocol and infant follow up.

19.3.4 Clinical follow up and outcome to date
The chorioretinal scars have remained inactive since birth. Other ocular signs that subsequently became apparent included a right convergent strabismus, bilateral myopia and astigmatism. Patch therapy successfully corrected the strabismus, hence surgical correction, initially scheduled for when the child reached eight years of age, was unnecessary. Corrective lenses were prescribed for myopia and astigmatism. Visual function in the right eye was 25% and was representative of peripheral vision only. Visual function was 75% in the left eye, however with corrective lenses almost 100% visual function was achieved in the left eye. There was no change in visual function over time. Audiology assessments were normal up to the age of six years at which point no further testing was scheduled.

During the first year of life there were concerns regarding an evolving left hemiplegia. Signs of mild left side weakness were observed involving the upper and lower limb. The child was referred to early intervention services and developmental physiotherapy was commenced at three months of age. Other intense multidisciplinary services were employed during the first year of life to optimise global developmental potential. The infant walked at 13 months and from approximately 19 months onward unilateral weakness was no longer demonstrable. Repeat CCT scan after one year of treatment demonstrated almost complete resolution of signs (figure 19.1c).

The child attended Montessori school between the ages of three and five years. Problems with fine co-ordination and perceptual motor skills were apparent during
these pre-school years and were attributed to unilateral visual impairment. The parents enrolled the child with The National Council for the Blind in Ireland (NCBI). Throughout the pre-school years the family attended sessions that specifically targeted everyday challenges encountered by a child with visual impairment and they were instructed on practical methods to maximise the child’s confidence and coping skills in preparation for primary school.

The child continued to receive developmental, occupational and speech therapy for a total of four years with excellent progress reported. Intervention services were reduced and gradually withdrawn at four and a half years of age by which time the child had skills within range for all areas of neurodevelopment. Psychological evaluation continued up to age five, at which point WISC-III evaluation demonstrated that the child had normal IQ and cognitive function and was deemed fit for main-stream primary school.

The child entered primary school at age five years. The local primary school funded a desktop visual aid reader to enhance reading of books and the teaching board. The child integrated well into primary school and excellent performance in all areas of the school curriculum was recorded from the beginning. No learning deficits were identified.

At age seven and a half years, the child sustained severe burns when her dress accidentally caught flames from a domestic fireplace. She sustained significant burns affecting 19% of the body, primarily the chest, back, abdomen and left axilla. She received intensive care and a series of skin grafts over a 3-month period at a local burns unit. During this time of physical and emotional stress, the child underwent more frequent ophthalmologic examinations for early detection of intraocular reactivation but chorioretinal scars remained quiescent. The child required rehabilitation in the form of physiotherapy and occupational therapy to regain full mobility of the left upper limb. Initially the grafted left axilla restricted shoulder movements and there were concerns of contracture formation. However physiotherapy regained full range of movement of the left shoulder. Psychotherapy and counselling were also employed to help deal with altered body image. Skin grafts healed well and there were no complications. It was necessary for the child to continuously wear a pressure vest and underwear specifically designed to gradually shrink grafted skin and minimise their appearance over time. The child
attended plastic surgery services regularly for review of skin grafts and there were no concerns.

Despite this major drawback, the child continued to function well at school with exceptional progress academically and socially; STEN scores were consistently ≥ 9 from the beginning of primary school. At the age of 10 years, having consistently demonstrated ability above the 97th percentile in all areas of academic performance throughout primary school thus far, the child was identified as a gifted individual with superior intelligence and mental ability compared with national standards for age matched children throughout Ireland and was ranked in the top 2% for academic ability. The child was referred to the Irish Department of Education for an assessment and was selected to participate in a programme with the CTYI; a centre that provides supplementary courses to students between the age of six and 13 who demonstrate exceptionally high academic ability.

Thus, despite many challenges from birth, the child made remarkable progress through the first decade of life, in all areas of development, most notably and unexpectedly in the academic area. The outcome to date can be attributed to early identification, treatment and interventions for symptomatic CT.
19.4 Case number 4

19.4.1 Screening and confirmation
The fourth confirmed case of CT was born by spontaneous vaginal delivery (SVD) at term following an uncomplicated pregnancy. The parents were Irish born, resided in Dublin and this was their third child. Heel blood was collected on day four of life, results were available on day eight and confirmatory mother-infant samples were taken on day nine for dispatch to the TRL Swansea. Stored maternal antenatal serology from trimester 1 tested negative for toxoplasma antibody, postnatal serology confirmed pregnancy seroconversion likely during trimester 3 (table 19.1 case number 4). The maternal risk factor for primary toxoplasma infection during pregnancy was ingestion of rare meat with unawareness of the associated risk.

19.4.2 Infant evaluation and confirmatory serology

The infant’s first confirmatory toxoplasma antibody profile at age two weeks was equivocal and serial samples up to the age of 12 weeks were also inconclusive (table 19.2). Blood PCR was negative at age six weeks. The infant was fully evaluated at age six weeks and there were no detectable signs of CT. Cranial ultrasound, CCT, audiology and ophthalmology examinations were normal.

Serology at 12 weeks was analysed by WB in conjunction with maternal blood and the result was negative. Comparative electrophoresis demonstrated that only maternal IgG antibody proteins were seen in infant’s blood and the quantity of antibody in infant blood was sparser than the quantity of antibody in maternal blood. The conclusion from WB analysis was that there was no evidence of infant antibody production and hence this was not a case of congenital toxoplasmosis. The infant had standard immunoglobulins in normal range for age and hence the capacity to make an immune response was proven.

Based solely on the results accumulated thus far, the conclusion drawn at 12 weeks was that the infant was not congenitally infected. As a result, treatment was not offered at that point. The Director of the TRL liaised with other European experts for interpretation of results and management advice for this case. Congenital infection was subsequently confirmed by a persistently positive IgA at
and beyond nine months, with demonstrable antibodies at 12 months further confirming the diagnosis (table 19.2).

Treatment was offered at the age of nine months, at which point the parents opted not to treat the infant but to remain in the follow-up protocol. The parents’ decision to decline treatment was based on their reasoning that: 1) intervention at that point may not be beneficial to the infant 2) adverse effects of treatment commenced late in infancy possibly outweighed the long-term benefits and 3) a conservative approach with regular clinical monitoring was their preferred option at that point.

19.4.3 Challenges encountered during serial testing and following confirmation of CT

Tremendous anxiety was generated by the possible diagnosis, the need for serial confirmatory blood tests and the inability to confirm the diagnosis throughout almost the entire first year of life. Initial confirmatory serology during the first six weeks coincided with the Christmas and New Year period. Due to overwhelmed courier services and provision of on-call services only at the TRL in Swansea, there was a delay in the processing of toxoplasma serology samples. Samples received after December 21st were not tested until after January 2nd. The infant's parents awaited each test result anxiously only to be told that the diagnosis was not confirmed and repeat testing was necessary. The parents were aware of the possibility of treatment for toxoplasmosis and were initially keen for treatment initiation in the event that the infection was confirmed. They expressed concerns re the potential impact of treatment delay and possible sequelae as a result. During the months of serial testing, regular meetings were held with the parents to update them on serology results and to stress the importance of confirming the diagnosis prior to embarking on a treatment regimen. The parents were delighted and relieved when the WB results were negative. From the age of six months onwards, the parents were prepared for the possibility of the infant having CT, based on the persistently positive DT and IgA, nonetheless they were devastated when infection was later confirmed. Renewed anxiety occurred and this persisted for a number of years. Their main concern was that their child could be at increased risk for long-term sequelae due to non-treatment within the first year of life. Anxiety abated somewhat when the child attained school age and remained healthy with normal neurodevelopmental progress.
19.4.4 Clinical follow up and outcome to date

Retinal examinations were normal up to the age of 10 years and monitoring continues. At age two years, mild anisometropic amblyopia with hypermetropia and strabismus of the left eye was diagnosed. Occlusion therapy and corrective lenses were prescribed which achieved good results. The child continues to wear corrective lenses. Strabismus is referred to as an associated ocular alteration in CT, which is thought to represent a late ocular manifestation of CT. Thus it is likely the child developed an ocular association of CT at age two years. Audiology examinations were normal up to the age of six years when the final assessment was performed.

At the age of six years, isolated growth of pubic hair was noted without other symptoms of precocious puberty. The child was fully evaluated by a consultant endocrinologist. Central precocious puberty was out ruled. Bone age was within standard deviations for age. Adrenal androgens and urinary levels of 17-ketosteroids were in normal range, which confirmed benign isolated premature adrenarche. To date there has been no progression of the development of pubic hair or other secondary sexual characteristics.

Precocious puberty and other endocrine disorders have been reported in CT (Setian et al., 2002; Remington, 2011). It has been suggested that as CT and endocrine dysfunction are two relatively uncommon disorders, concurrent appearance suggests more than mere coincidence. Endocrine disorders such as hypothyroidism have been reported in children with severe intracranial manifestations of CT and also in children with only calcifications. However reports to date of children with CT and signs of precocious puberty were cases with severe ocular and intracranial manifestations that had true precocity proven by hormone assays. Thus true precocious puberty in CT has been attributed to hypothalamic/pituitary dysfunction as a result of intracranial lesions in or near the hypothalamus.

This child had normal cranial imaging and no hormonal evidence of true precocious puberty and thus it was concluded that isolated adrenarche occurring with CT was a mere coincidence.
Developmental and educational progress was normal at age 10 years and the child was progressing well in all areas of the school curriculum, with academic performance well above average (STEN 8-10).

This case exemplified the difficulty that can be associated with serological diagnosis of CT in infants with low-level antibody production.
19.5 Case number 5

19.5.1 Screening and confirmation

The fifth confirmed case of CT was born by SVD at term following an uncomplicated pregnancy. The parents were Lithuanian migrants residing in county Tipperary for one year and this was their firstborn child. Heel blood was collected on day four of life; results were available on day nine and confirmatory mother-infant samples were taken on the same day for dispatch to the TRL. Confirmatory results were available on day 24 of life; the delay was accounted for by the Christmas period.

Infant serology confirmed CT. Comparative analysis of maternal postnatal and antenatal serology confirmed pregnancy seroconversion, likely during trimester 3. Mother and infant had strongly positive ELISA and ISAGA results on the first confirmatory sample (table 19.1 case number 5). The identified maternal risk factors for toxoplasma infection during pregnancy included ingestion of raw cured meat products and contact with cats with unawareness of the associated risks.

19.5.2 Infant evaluation and treatment

This infant was asymptomatic at birth. Blood and CSF were *T. gondii* PCR negative. Clinical evaluation in the newborn period revealed signs of CT. Ophthalmology examination demonstrated bilateral inactive chorioretinal scars in remote location to the macula. Brain imaging revealed multiple small punctate foci of intracranial calcification in both hemispheres and the right periventricular area (figure 19.2 a), thus by definition the infant had signs of severe CT at initial assessment. Audiology assessment was normal. Following in patient evaluation, antiprotozoal treatment commenced on day 32 of life and continued for one year.

Grades 1 to 4 neutropenia occurred during the first 10 months of treatment. More than five episodes of grade 1 neutropenia (ANC 1.03 x 10^9 - 1.240 x 10^9/L) occurred intermittently during months one to ten of treatment for which no treatment adjustments were made. More than five episodes of grade 2 neutropenia (ANC 0.76 x 10^9 - 0.96 x 10^9/L) occurred during months one to nine of treatment which responded to increased dosage of leucovorin. Two episodes of grade 3 neutropenia occurred. One occurred during the fifth month of treatment.
during an episode of viral gastroenteritis. ANC was 0.67 x 10^9/L. Treatment was temporarily discontinued and reinstated after one week at full dosage following recovery of the ANC. A second episode of grade 3 neutropenia occurred in the sixth month of treatment with ANC of 0.7 x 10^9/L. Antiprotozoal dose reduction by 25% was prescribed with increased leucovorin for one week following which antiprotozoal treatment was recommenced at full doses. One episode of grade 4 neutropenia occurred on one occasion during the third month of treatment (ANC 0.470 x 10^9/L), which required temporary antiprotozoal interruption for one week until recovery of the ANC. Therapy was re-introduced after 1 week at 75% of total dose for a further week and then returned to full dose. Hence the infant mainly experienced mild to moderate neutropenia during treatment. Three episodes of neutropenia required transient dose reduction or dose interruption; one in association with a viral infection.

The infant received treatment for 96% (50 weeks) of the intended duration of which 92% was at full dosage (48 weeks). Liver function and urinalysis remained normal throughout treatment.

19.5.3 Compliance with the management protocol and challenges encountered

This infant was a firstborn child to parents who were recent migrants to Ireland from Eastern Europe. The parents were able to communicate reasonably well in English but the mother was unable to read English. The father was employed by the construction industry. Parents accepted the diagnosis and adopted a positive attitude despite the infant’s clinical signs.

The mother was aware of toxoplasmosis as prenatal screening is routinely offered in Lithuania; however she was unaware of risk factors for acquisition. The parents were grateful that the diagnosis was made early and treatment initiated. They were fully co-operative with the management protocol.

Neutropenic episodes necessitated frequent blood testing which was done at the infant's local paediatric unit. In addition, the infant had regular hospital appointments at OLCH Dublin for clinical and ophthalmology review. The parents were compliant, despite the fact that the father's employment had to be interrupted for all hospital appointments as the mother was unable to drive.
Full blood count results and dose adjustments were communicated to the parents via telephone. Despite language and social barriers, the parents engaged well with medical services and coped well with the management protocol. They remained hopeful that the child would have no adverse long-term sequelae.

19.5.4 Clinical follow up and outcome to date
Chorioretinal scars have remained inactive and annual ophthalmology review continues. Repeat brain imaging following completion of treatment demonstrated almost complete resolution of calcification in the right lateral periventricular area (figure 19.2 b). Audiology assessments were normal up to the age of seven years at which point no further testing was scheduled. Progress reports through primary school demonstrated above average academic performance (STEN 8-10). The child at the age of 10 years is fluent in four languages, English, Russian, Lithuanian and Romanian.
19.6 Case number 6

19.6.1 Screening and confirmation
The sixth confirmed case of CT was born by SVD following an uncomplicated pregnancy. The parents were Polish migrants who had been residing in county Laois for 18 months and this was their firstborn child. Heel blood was collected on day four of life; results were available on day nine and confirmatory mother-infant samples were taken on the same day for dispatch to the TRL.

Infant serology confirmed CT. Comparative analysis of maternal postnatal and antenatal serology confirmed pregnancy seroconversion, likely in trimester 3 with a strongly positive maternal ELISA IgM detected postnatally (table 19.1 case number 6). The identified maternal risk factor for primary toxoplasma infection during pregnancy was ingestion of cured meat products and undercooked fresh meat with unawareness of the associated risk.

19.6.2 Infant evaluation and treatment
The infant was asymptomatic with no signs of CT on evaluation. Blood and CSF were *T. gondii* PCR negative. Following in-patient evaluation, antiprotozoal treatment commenced on day 26 of life and continued for one year. Grades 1 to 3 neutropenia occurred during the first six months of treatment. Grade 1 neutropenia was documented on three occasions during the first six months and required no intervention. Grade 2 neutropenia (ANC 0.8 x 10⁹ – 0.99 x 10⁹/L) was documented on more than five occasions during months one to four of treatment. All episodes responded to transient increase in the dose of leucovorin until normalisation of the ANC.

Two episodes of grade 3 neutropenia occurred. The first (ANC nadir 0.720 x 10⁹/L) occurred during the second month of treatment in association with an episode of viral gastroenteritis, hence it was necessary to reduce antiprotozoal doses by 50% rather than 25%. Medication was reinstated at full dose one-week later following resolution of viral symptoms and neutrophil recovery. The second episode of grade 3 neutropenia occurred in the fourth month of treatment with an ANC of 0.690 x 10⁹/L. Antiprotozoal dose reduction of 25% with increase in the leucovorin dose for one week allowed recovery following which antiprotozoal
treatment was recommenced at full doses. The infant received treatment for 100% of the intended time, albeit at a reduced dose during 4% of the treatment duration. Liver function and urinalysis remained normal throughout treatment.

19.6.3 Compliance with the management protocol and challenges encountered

The infant was a firstborn child of Polish parents. The mother was unable to communicate in the English language and the father had limited ability to communicate in English. The father was employed by the construction industry. The parents had no prior knowledge of toxoplasmosis or congenital infection. However they fully accepted the diagnosis and treatment protocol and were reassured by absence of signs on evaluation. The parents had no personal access to a motor-vehicle. It was thus necessary for both parents to attend all appointments as the infant’s mother was not confident to use public transport unaccompanied due to the language barrier. During the infant’s week-long hospital stay for clinical evaluation following confirmation of CT, the father’s employer presented himself to OLCH and verbally abused and threatened staff, as the infant’s management had interrupted his employee’s work.

A Polish interpreter was unavailable during the infant’s hospital admission and a Polish member of hospital staff acted as a translator on the day of infant discharge. Due to communication difficulties, the infant was administered incorrect doses of medication in the early weeks of treatment immediately post discharge from hospital. This was recognised during the planned early follow-up visits by a PHN and rectified with the use of a telephone translator service. An interpreter was necessary during out-patient clinic visits. Close liaison with the parents via the programme co-ordinator and local public health staff was necessary to ensure that therapy was correctly administered throughout the treatment course as the parents were reluctant to contact medical staff because of the language barrier.

Due to the lack of private transport and issues with the father’s employer, the infant was referred to local paediatric services after the age of two years for ophthalmology follow-up in an attempt to maximise compliance with necessary evaluation and minimise hospital appointments in Dublin.
19.6.4 Clinical follow up and outcome to date

Ophthalmology review was normal at age nine years, annual follow-up continues. Audiology testing was normal at the final scheduled assessment at age five years. Neurodevelopmental, behavioural and social function was normal at age nine. Academic progress at primary school was documented at high average (STEN 7-8).
19.7 Case number 7

19.7.1 Screening and confirmation

The seventh case of CT was the first-born infant of Irish parents, resident in Dublin and delivered at term following an uncomplicated pregnancy. Heel blood was collected on day five of life; results were available on day 11 and confirmatory mother-infant samples were taken on day 12 for dispatch to the TRL. Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from trimester 1 confirmed pregnancy seroconversion, likely during trimester 3. Mother and infant had a strongly positive ELISA IgM on the first confirmatory sample tested (table 19.1 case number 7). The only identified maternal risk factor for toxoplasma infection during pregnancy was ingestion of undercooked fresh meat with maternal unawareness of the associated risk.

19.7.2 Infant evaluation and treatment

The infant was asymptomatic at birth. Blood and CSF were *T. gondii* PCR negative. Ophthalmology examination revealed a right peripheral inactive chorioretinal scar (figure 19.3 a). Brain imaging and audiology assessment were normal. Following in-patient evaluation, antiprotozoal treatment was commenced on day 27 of life and continued for one year.

Grades 1 to 4 neutropenia occurred during the first eight months of treatment. Grade 1 neutropenia occurred intermittently reaching Grade 2 neutropenia on more than 5 occasions during months one to eight and responded to increased dosage of leucovorin. Two episodes of grade 3 neutropenia (ANC 0.7 x 10⁹/L) occurred, one during the third month of treatment and one in the seventh month. Both were managed with 25% reduction in antiprotozoal dosage and increased dosage of leucovorin for one week. Grade 4 neutropenia was documented on two occasions, each in association with an intercurrent respiratory viral illness, one in the fifth month of treatment with an ANC of 0.400 x 10⁹/L in and one in the sixth month of treatment with an ANC of 0.3 x 10⁹/L. Both necessitated antiprotozoal interruption for two weeks on each occasion until recovery of ANC following which therapy was re-introduced at 75% of total dose for a further week. Hence most neutropenic episodes which occurred during treatment were mild to moderate. A total of four neutropenic episodes required either transient treatment adjustment or
treatment interruption: two were grade 3; two grade 4 episodes occurred during a viral infection.

The infant received treatment for 92% of the intended duration, 84% of which was at the full antiprotozoal dosage. Antiprotozoal interruptions that were necessary for 8% of the intended treatment duration were due to episodes of grade 4 neutropenia that occurred during viral infection. Liver function and urinalysis remained normal throughout treatment.

19.7.3 Compliance with the management protocol and challenges encountered

The infant was a firstborn child; both parents were in full time employment. The mother was a speech therapist. Even though the parents had little prior knowledge of toxoplasmosis they fully accepted the diagnosis and treatment regimen, however tremendous maternal anxiety was generated from the beginning, particularly in relation to the child’s developmental outcome and long-term prognosis. The frequent hospital visits necessary during the first year of life because of neutropenic events contributed further to maternal stress, as the mother disliked witnessing infant upset during phlebotomy. In addition, the mother claimed that the treatment regimen did anything but normalise the experience of a firstborn child. Despite these issues, full adherence to recommendations was maintained throughout the treatment and follow up period.

19.7.4 Intraocular reactivation

Ophthalmology examination in the newborn period demonstrated an inactive chorioretinal scar at the periphery of the right retina (Figure 19.3 a). The left retina had no evidence of chorioretinitis. The child had ophthalmology examinations performed at regular intervals by a single consultant ophthalmologist. Intraocular reactivation occurred on three occasions from the age of 24 months. The first recurrence was noted 12 months after treatment completion shortly after age two years. Whilst undergoing routine ophthalmology examination, an active lesion in the periphery of the left retina was noted. Due to the location there was no visual threat, hence treatment was not prescribed and spontaneous regression was demonstrated at follow-up examination (lesion not shown).
The second recurrence was noted six months later at age 30 months, also in the left eye. On this occasion, the lesion was in the posterior pole of the retina and in close proximity to the optic disc (figure 19.3 b). The infant was prescribed three months of pyrimethamine, sulfadiazine and leucovorin with an eight-week course of tapering steroids. Therapy was well tolerated with no episodes of neutropenia recorded. During the three-month course of treatment, serial ophthalmology examinations revealed regression of the left chorioretinal lesion (figure 19.3 c). One week following completion of three months of treatment, follow-up retinal examination demonstrated that the left chorioretinal lesion was inactive; however a new focus of chorioretinitis was detected in the right eye at the temporal aspect of the right macula with adjacent vasculitis (figure 19.3 d).

A six-month course of treatment with antiprotozoals was prescribed. Treatment was well tolerated. Neutrophil counts remained above $1.1 \times 10^9$/L apart from two episodes of ANC of $0.9 \times 10^9$/L that responded to increased dosage of leucovorin.

Toxoplasma serology taken during each recurrence remained unchanged. There was no serological correlation with clinical episodes. Treatment response was demonstrated by a reduction in the DT. Type specific serology was sought from the UK-TRL to determine whether this child was infected with an atypical strain of *T. gondii*. However this service was not available at the UK reference laboratory. The TRL referred the request to a reference laboratory in the U.S.A, however serotyping was available only as a research tool to centres in the U.S.A. In addition, genotyping of isolates is usually only successful when performed on intraocular specimens, which were not possible to obtain from the child.

### 19.7.5 Seizure
At age three years, during the fourth month of the six-month course of retreatment with antiprotozoals, the child developed a febrile illness with lower respiratory symptoms. During the course of this illness, the child had a seizure which lasted approximately two minutes. The seizure activity was described by the mother as an episode of eye rolling, during which the child was unresponsive with associated stiffening and twitching of the whole body. This was followed by a period of drowsiness which lasted two hours. The child was admitted to OLCH and further evaluated. Blood counts were normal. Septic evaluation was normal with no abnormality revealed in blood, urine, CSF and chest x-ray. Repeat CCT brain scan
was performed one month after the seizure occurred and no abnormality was
detected. Neurological examination remained normal. At three years, the child was
beyond the common age for a first febrile seizure, albeit within the documented
age category for febrile seizures of six months to six years.

The final diagnosis following clinical evaluation for the seizure was: seizure with
fever, without a defined focus of infection. The child continued antiprotozoal
therapy during the febrile episode and for the remainder of the six-month course of
re-treatment. No further seizures have occurred after this episode.

19.7.6 Toe walking
The child walked at age one and all motor milestones in infancy were attained
within normal age limits. Sporadic toe walking was noted from the age of three and
a half years, approximately three months following the seizure episode, thus the
child was referred for neurological assessment, which was normal. There were no
hamstring or Achilles tendon contractures. Dermatomes, myotomes, lower limb
power and reflexes were normal.

Repeat CCT scan two months prior, i.e., one month following the seizure, was
normal. The neurologist concluded that there was no underlying neurological
disorder and toe walking was physiological or habitual with secondary tension
imposed on the gastrocnemius-soleus muscle complexes bilaterally. The child was
referred for physiotherapy assessment, which demonstrated that the child was
globally hypermobile with a bouncy gait and poor heel strike. There was mild
femoral anteversion and genu-valgum bilaterally with slightly cavovarus feet.
Physiotherapy sessions were commenced to target lower limb posture and heel
flexibility. Stretching exercises were also recommended and a single
physiotherapist reviewed the child regularly. Heel flexibility and gastrocnemius-
soleus muscle flexibility improved. Gait improved with better heel strike. The final
diagnosis was habitual toe walking.

19.7.7 Alopecia areata
At the age of six and a half years the child presented with scalp alopecia. A bald
patch was first noticed at the parietal area of the scalp. Further patches then
developed bilaterally at the temporal areas and the occipital region at the nape of
the neck. Dermatology consult was sought and a diagnosis of non-scarring
idiopathic alopecia areata was made. Autoimmune investigations were normal. The child was prescribed a prolonged course of topical Dermovate cream and Protopic ointment 0.1% to be applied once daily. Re-growth has occurred but new patches continue to appear sporadically at age nine.

19.7.8 Psychological issues
The child has been described by her parents as highly anxious, tense and emotionally stressed in general. Her mother attributed this to the CT diagnosis, reactivation and re-treatment, together with other medical issues which necessitated numerous hospital visits. The child admitted to fear and anxiety associated with hospital appointments that were initially generated by frequent blood monitoring during courses of antiprotozoal therapy. The fear of hospitals and medical staff persisted even after blood monitoring had ceased. The toe walking and alopecia areata were thought to represent manifestations of anxiety. Psychological support with counselling sessions have been attended to help the child cope and deal with her fears. In 2015 at the age of nine years, the child was formally diagnosed with anxiety disorder. Psychological support is ongoing.

19.7.9 Clinical follow up and outcome to date
Bilateral chorioretinal scars remained quiescent since the last reactivation at age 33 months. Visual acuity remained normal. Ophthalmology examinations were planned twice yearly but due to hospital associated anxiety, the frequency of ophthalmology evaluations was reduced to an annual review unless otherwise clinically indicated. Audiology testing last performed at the age of six years was normal at which point the child was discharged from audiology follow-up. Neurodevelopment and cognitive function were normal at age nine years. School teachers described the child as a tense and anxious child; however she continues to progress well academically and socially through primary school, with above average scores documented on all assessments to date (STEN 8-9).
19.8 Case number 8

19.8.1 Screening and confirmation
The eighth confirmed case of CT was born at term following an uncomplicated pregnancy. The parents were Irish residing in county Limerick and this was their firstborn child. Heel blood was collected on day four of life; results were available on day 10 and confirmatory mother/infant samples were taken on day 11 for dispatch to the TRL. Infant serology confirmed CT. Comparison of maternal postnatal serology with negative antenatal serology from trimester 1 confirmed pregnancy seroconversion, estimated to have occurred during trimester 3 (table 19.1 case number 8). Maternal risk factor for toxoplasma infection during pregnancy was ingestion of undercooked fresh meat with unawareness of the associated risk.

19.8.2 Infant evaluation and treatment
The infant was asymptomatic at birth. Blood and CSF were T. gondii PCR negative. Ophthalmology examination demonstrated that each eye had an inactive peripherally located chorioretinal scar. CCT brain scan revealed a small single focus of intracranial calcification, approximately one to two millimetres in size at the periventricular area adjacent to the frontal horn of the left lateral ventricle. Audiology testing was normal.

Antiprotozoal treatment commenced on day 20 of life and continued for one year. Episodes of grades 1 to 3 neutropenia were experienced during months one to five of treatment. Four episodes of grade 1 neutropenia occurred intermittently during the first five months of treatment for which no treatment adjustments were necessary.

Six episodes of grade 2 neutropenia occurred during the first three months of treatment which responded to increased dosage of leucovorin. Three episodes of grade 3 neutropenia with ANC of 0.7 x 10^9/L occurred during the first four months of treatment, antiprotozoal doses were reduced by 25% on each occasion for one week then reinstated at full doses following neutrophil recovery.

The infant received treatment for 100% of the recommended duration; 6% of the treatment time was on reduced dosage of antiprotozoals for grade 3 neutropenia.
Liver function and urinalysis remained normal throughout treatment.

19.8.3 Compliance with the management protocol and challenges encountered
This infant was a firstborn child to parents in the law profession. The parents accepted the diagnosis and were fully compliant with the management protocol. The child's mother experienced tremendous guilt regarding ingestion of undercooked meat during pregnancy and expressed anger at not being informed of the risk during pregnancy.

The parents, alarmed by the focus of intracranial calcification at birth, initially had many fears concerning possible sequelae associated with intracranial calcification. The presence of a chorioretinal scar in each eye was of even more concern to the parents, as they perceived the scars to be poor prognostic indicators for future visual function and acuity. This was augmented by the fact that they had an adult neighbour who became partially blind at age five years due to toxoplasmosis.

The parents educated themselves on congenital toxoplasmosis by review of reports in the literature and expressed concern regarding lack of studies on long-term follow-up and outcome of treated asymptomatic cases. Overall they had a positive opinion of the screening programme as they appreciated that it enabled diagnosis and treatment of a condition that could remain undetected in infancy. They were thankful that their child was born during the CT screening period which facilitated the diagnosis and management. As time progressed they were reassured by their child’s normal developmental progress.

19.8.4 Clinical follow up and outcome to date
The bilateral chorioretinal scars have remained unchanged and visual acuity was normal at age nine. Ophthalmology examinations continue annually. Repeat brain imaging at age one year was unchanged compared to imaging in the newborn period with a single focus of intracranial calcification detected. Audiology testing last performed at the age of six years was normal at which point the child was discharged from follow-up. Neurodevelopment and cognitive function was normal at age nine years. Excellent educational progress was reported in all areas of the school curriculum and academic records demonstrated scores well above average.
(STEN 8-10). The child at age nine years was the 500 metre athletics champion for his county in the under 10 age group.
19.9 Case number 9

19.9.1 Screening and confirmation
The ninth confirmed case of CT was born at term following an uncomplicated pregnancy. The infant’s mother was Russian and the father was Romanian. The parents had been residing in Dublin city for approximately one year and this was their firstborn. Heel blood was collected on day four of life; results were available on day seven and confirmatory mother-infant samples were taken on day 15 for dispatch to the TRL. Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from 29 weeks gestation confirmed pregnancy seroconversion most likely late in trimester 3. Mother and infant confirmatory serology demonstrated a strongly positive ELISA IgM and maternal IgG absorbance was too low to yield an avidity result, suggestive of very recent seroconversion (table 19.1 case number 9). The identified maternal risk factor for toxoplasma infection during pregnancy was ingestion of raw cured meat products with unawareness of the associated risk.

19.9.2 Infant evaluation and treatment
The infant was asymptomatic at birth with no signs of CT on clinical evaluation. Blood and CSF were T. gondii PCR negative. Antiprotozoal treatment commenced on day 28 of life and continued for one year.

There was one episode of grade 1 neutropenia during the second week of treatment. Antiprotozoal treatment was well tolerated. Neutrophil counts remained $\geq 1.100 \times 10^9/L$ for the duration of treatment. The infant completed 12 months of treatment at full dosage. Liver function and urinalysis remained normal throughout treatment.

19.9.3 Compliance with the management protocol and challenges encountered
The infant was a firstborn child to migrant parents from Eastern Europe. There were some language difficulties but the mother had reasonable command of English. The parents had migrated for employment opportunities one year previously and the father was employed by the construction industry. The infant’s mother was the sole contact with medical services and the father did not attend appointments. The infant’s mother was initially upset and shocked by the
diagnosis. She experienced tremendous guilt due to her unawareness of risk factors for toxoplasma infection. She fully accepted the diagnosis and treatment protocol and was reassured by absence of signs on the initial evaluation. The parents had no personal access to a motor vehicle and for this reason the infant’s follow-up continued at TCUH Dublin, which was geographically more convenient than OLCH Dublin.

Due to the minor language barrier the mother initially had some difficulty understanding drug dosage. The infant was administered incorrect doses on one occasion. This was rectified by the programme co-ordinator via a telephone translator service and no further problems were encountered. There was a degree of lack of maternal confidence regarding communication and as a result she liaised solely with the programme co-ordinator for all queries or issues and refused to liaise with other medical and public health staff. However full compliance with treatment was upheld throughout.

19.9.4 Clinical follow up and outcome to date
Clinical evaluation, ophthalmology, audiology and neurodevelopmental examinations were all normal when the child was last assessed at age two years, after which time the family were lost to follow up. The mother had communicated that following completion of infant treatment, it was likely the family would have to return to Romania due to unstable employment circumstances for the infant’s father in Ireland.

Appointments were not attended after the child was two years. Attempts to contact the mother by phone revealed the phone was disconnected. Social welfare records for the family confirmed that they had not collected welfare payments since the child was two years and two months and had left the country with no forwarding address. As the family left Ireland without forewarning or direct communication regarding the timing of departure, there was no opportunity to provide the mother with information on clinical follow-up that was necessary for the child in Romania. No further information on this child is currently available.
19.10 Case number 10

19.10.1 Screening and confirmation
The 10th confirmed case of CT was born at term following an uncomplicated pregnancy. The parents were Irish residing in a rural area of county Cork and this was their firstborn. Heel blood was collected on day four of life; results were available on day 10 and confirmatory mother-infant samples were taken on day 11 for dispatch to the TRL. Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from trimester 1 confirmed pregnancy seroconversion, likely during trimester 3 (table 19.1 case number 10). An identified maternal risk factor for toxoplasma infection during pregnancy was ingestion of undercooked cooked meat with unawareness of the associated risk.

19.10.2 Infant evaluation and treatment
This infant was asymptomatic at birth with no signs of CT on evaluation. Blood and CSF were *T. gondii* PCR negative. Antiprotozoal treatment commenced on day 26 of life and continued for one year. During the first six months of treatment, there were six episodes of self limiting grade 1 neutropenia. Additionally on six other occasions grade 2 neutropenia occurred and resolved following an increase in the dose of leucovorin. Grade 3 neutropenia occurred on two occasions; one in the third month of treatment (ANC 0.79 x 10^9/L) and the other in the fifth month (ANC 0.72 x 10^9/L). Both episodes responded to antiprotozoal dose reduction to 75% for one week. Grade 4 neutropenia occurred on one occasion in the fourth month of treatment (ANC 0.480 x 10^9/L), which necessitated temporary antiprotozoal discontinuation for one week with reinstatement thereafter at 75% of total dosage for a further week.

The infant received 51 weeks of treatment in total, i.e., for 98% of the recommended duration and 92% of treatment was at full dose. All treatment modifications were in response to neutropenia. Liver function and urinalysis remained normal throughout treatment.
19.10.3 Compliance with the management protocol and challenges encountered
The parents fully accepted the diagnosis and management protocol and were co-operative with treatment and follow up. They were reassured by absence of signs on evaluation.

19.10.4 Clinical follow up and outcome to date
Attendance of appointments in Dublin entailed a five-hour car journey, hence the infant's follow-up ophthalmology and audiology assessments were transferred to local services in Cork. The child attended the paediatric ID service at OLCH Dublin annually for developmental progress and review by the CT programme co-ordinator until the age of four years, thereafter progress was monitored by a paediatrician in Cork. Close liaison continued between the CT programme co-ordinator and the infant's local ophthalmology, audiology and paediatric service. Ophthalmology examination was normal at age nine years. Annual ophthalmology evaluation was scheduled to continue. Audiology testing last performed at the age of five years was normal at which point the child was discharged from audiology follow-up. Neurodevelopment was normal at age nine years with high and above average academic progress recorded at primary school (STEN 7-8).
19.11 Case number 11

19.11.1 Screening and confirmation
The 11th case of CT was born at term following an uncomplicated pregnancy. The infant’s mother was Irish and the infant’s father was of Middle Eastern ethnic origin. The parents were residing in county Cork and this was their third child. Heel blood was taken on day four of life and results were available on day 15. Confirmatory mother-infant samples were taken on day 16 of infant life for dispatch to the TRL and results were available on day 21. Infant serology confirmed CT. Comparative analysis of maternal postnatal serology and negative antenatal serology from trimester 1 confirmed pregnancy seroconversion, likely during trimester 3. Infant ELISA IgM was strongly positive (table 19.1 case number 11). No maternal risk factors for toxoplasma infection during pregnancy were identified. The mother was aware of toxoplasmosis and the risks for its acquisition.

19.11.2 Infant evaluation and treatment
Full evaluation and initiation of treatment were performed at the infant’s local paediatric unit under the care of a consultant paediatrician and in liaison with the CT programme co-ordinator. This was facilitated as the family lived a three-hour journey away from the tertiary children’s hospital in Dublin and the diagnosis was confirmed just prior to the Christmas period. The infant was asymptomatic at birth with no signs of CT on evaluation. Blood and CSF were *T. gondii* PCR negative. Antiprotozoal treatment commenced on day 28 of life for one year. During the 12 months of treatment neutropenia occurred on three occasions only. One episode of grade 1 neutropenia occurred in the fourth month of treatment. Two episodes of grade two neutropenia occurred; one in the second month of treatment and one in the fourth month of treatment with ANC of 0.980 x 10⁹/L and 0.860 x 10⁹/L respectively. Both responded to increased dosage of leucovorin. Treatment was completed at full dosage. Liver function and urinalysis remained normal throughout treatment.

19.11.3 Compliance with the management protocol and challenges encountered
The infant was a third born child. The parents fully accepted the diagnosis and management protocol and were co-operative with infant treatment and follow up.
They were reassured by absence of signs on initial evaluation, which remained unchanged at follow-up.

19.11.4 Clinical follow up and outcome

Infant clinical follow-up initially took place at OLCH Dublin. At age four, clinical follow up was transferred to local paediatric services in Cork for convenience. Ophthalmology examination was normal at age nine years. Annual ophthalmology evaluation continues. Audiologic testing last performed at the age of four years was normal at which point the child was discharged from audiology follow-up. Development and cognitive function were normal at age nine with academic progress documented as well above average thus far in most areas of the curriculum (STEN 8-9).
19.12 Case number 12

**19.12.1 Screening and confirmation**

The 12th case of CT was born by SVD following an uncomplicated pregnancy. The parents were Irish residing in county Donegal. Heel blood was collected on day four of life, results were available on day 17 and confirmatory mother-infant samples were taken on day 18 of life for dispatch to the TRL. Confirmatory results were available on day 33 of life as dispatch of samples to the UK coincided with the Christmas period.

Maternal postnatal serology was DT positive with a low avidity of 0.2 suggestive of infection within the previous three months. However ELISA IgM was borderline and likely represented the end of the IgM response and thus consistent with infection occurring six to nine months previously (table 19.1 case number 12). Stored maternal antenatal serology was not available for testing as the local hospital did not have the facility for storage of samples more than four months old. Hence timing of maternal seroconversion could not be definitively confirmed. Based on postnatal serology it was estimated that maternal infection and seroconversion likely occurred in trimester 1 or 2. Maternal risk factors for toxoplasmosis infection during pregnancy were not identified. The mother was aware of toxoplasmosis and the risks for acquisition.

**19.12.2 Infant confirmatory serology**

The first infant confirmatory sample taken on day 18 of life was inconclusive. The DT was positive at 2,000 IU/mL, ELISA IgM was negative, ISAGA IgM and IgA were positive. While the infant profile was consistent with CT, the ISAGA IgM was equivocal, lying between that level considered borderline and that definitively positive. Serial samples subsequently taken up to age 13 weeks remained inconclusive, at which point the samples were referred for WB analysis and the result was positive (table 19.1 case number 12).

Due to the delay incurred by the inability of serial serology to confirm or exclude infection, at the age of 13 weeks, clinical evaluation was initiated at the local paediatric unit whilst awaiting WB results. Ophthalmology examination revealed a right inactive chorioretinal scar superior to the macula, consistent with CT.
Shortly thereafter, WB results confirmed CT based on the finding of unique infant antibody bands compared with the maternal sample. Hence the diagnosis was confirmed clinically and serologically when the infant was three months of age.

19.12.3 Infant evaluation and treatment
A full evaluation was performed at OLCH Dublin when the infant was 17 weeks of age. The delay was due to unavailability of a hospital bed for an elective admission as the time period coincided with the busiest period of the year for hospital admissions.

Blood was *T. gondii* PCR negative. CSF sampling was traumatic and the CSF obtained was unsuitable for *T. gondii* PCR testing. Repeat ophthalmology examination confirmed the presence of a right inactive chorioretinal scar. Brain imaging and audiology assessment were normal. Antiprotozoal treatment commenced at age 17 weeks and continued for one year.

Episodes of grade 1 neutropenia occurred during months one to six of treatment. One episode of grade 3 neutropenia occurred in the fourth month of treatment during a viral infection and required interruption of antiprotozoal medication for one week, following which full dosage was reinstated and well tolerated. The infant received treatment for 98% of the intended time. Liver function and urinalysis remained normal throughout treatment.

19.12.4 Compliance with the management protocol and challenges encountered
The infant was the fourth born child to parents who were primary school teachers. The parents were compliant with serial testing in the first three months of life and they did not regard this as an imposition as they recognised the need to definitively confirm or rule out the diagnosis as soon as feasibly possible. The presence of a chorioretinal scar did not generate undue anxiety as they anticipated a good outcome and were aware that CT could present with more severe disease signs. The parents fully accepted the diagnosis and treatment protocol and were cooperative with infant monitoring during treatment and clinical follow up.
19.12.5 Clinical follow up and outcome to date

Attendance of appointments in Dublin entailed a four-hour car journey, hence the infant's follow-up ophthalmology and audiology assessments continued with local services in County Donegal. The child attended the paediatric ID service at OLCH Dublin annually for developmental progress and review by the CT programme co-ordinator until the age of five years. Thereafter, progress was monitored by a local paediatrician.

Ophthalmology examination was unchanged at age nine years with the right inactive chorioretinal lesion as detected in infancy. Audiology assessment was normal when last performed at age six years and the child was discharged. Developmental and educational progress were normal at age nine years with academic progress sustained well above average (STEN 8-9).
19.13 Case number 13

19.13.1 Screening and confirmation
The 13th case of CT was the second child, born by SVD following an uncomplicated pregnancy, to Irish parents residing in rural County Roscommon. Heel blood was collected on day three of life, results were available on day 17 and confirmatory mother-infant samples were taken on day 18 of life for dispatch to the reference laboratory. Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from 16 weeks gestation confirmed pregnancy seroconversion, likely in trimester 3 with a strongly positive maternal ELISA IgM detected postnatally (table 19.1 case number 13). Maternal risk factors for primary toxoplasma infection during pregnancy included ingestion of undercooked meat and use of untreated well water as their domestic water supply. Maternal lack of awareness of risk factors for infection was documented.

19.13.2 Infant evaluation and treatment
The infant was asymptomatic at birth. Blood and CSF were *T. gondii* PCR negative. There were no signs of CT on evaluation. Following in-patient evaluation at age four weeks, infant treatment was prescribed.

19.13.3 Compliance with the management protocol and challenges encountered
The parents reluctantly accepted the diagnosis and due to concerns regarding drug toxicity, they did not initially consent to infant antiprotozoal treatment. Based on their reading of some studies, they were unconvinced of the potential benefit of treatment of asymptomatic infants and were concerned that treatment risks might outweigh any potential benefits.

Following further discussions covering the current state of CT knowledge, its limitations and the need for further research, the parents then decided to commence the infant on antiprotozoal treatment, but for a shorter four-month duration; chosen based on their reading of reports in the literature of treatment durations as short as three months. Treatment was eventually initiated following parental consent when the infant was nine weeks old (day 62 of life).
During the first month of treatment the infant experienced gastrointestinal side effects. Failure to gain adequate weight was subjectively reported by the parents, hence they decided to withhold treatment for 3 of 4 weeks in the first month of therapy. Adequate weight gain during this time was documented by the local PHN. During the four months of treatment, it became apparent that antiprotozoals were being intermittently discontinued by the parents on a regular basis. The mother attributed these interruptions to a combination of reasons. Concerns regarding intermittent diarrhoea and inadequate weight gain persisted. Inability to acquire medication from their local pharmacy was another reason cited. Enquiries with the local pharmacy revealed that repeat prescriptions for medication, which the parents had in advance, were not delivered to the pharmacy in a timely manner. The local pharmacy was only able to dispense monthly supplies of antiprotozoals, sourced from a UK supplier. Hence the request for the repeat order was required by the local pharmacy from the parents one week before the month’s supply of medication was completed, but this did not occur. Following identification of this issue, the CT programme co-ordinator faxed prescriptions directly to the dispensing pharmacy, however medication was still not collected in a timely manner by the parents. As a result, the infant had many treatment interruptions during the four months of treatment that the parents had consented to.

A combination of factors contributed to the parents’ reluctance to administer antiprotozoals. The parents were unconvinced of the potential treatment benefit. They experienced technical difficulty with administration of medication to the infant. Diarrhoea following treatment initiation reinforced parental concerns regarding drug toxicity. In general, parental preference was expressed for alternative, natural, herbal and holistic treatments for all illnesses where possible and avoidance of prescribed medications. During the short period of treatment, the parents upheld compliance with infant blood monitoring.

One episode of grade 2 neutropenia occurred during the second month of treatment with an ANC of 0.8 x 10^9/L. This recovered with increased dosage of leucovorin. Two episodes of grade 1 neutropenia occurred during the fourth month of treatment. Liver function and urinalysis remained normal.

The infant received four months of intermittent treatment that commenced from week nine of life and ended after 16 weeks. In total, the infant possibly received a
maximum of 10 weeks of antiprotozoal medication, which was 19% of the recommended 12-month duration.

19.13.4 Clinical follow up and outcome to date

The parents were co-operative with clinical follow-up and continued to attend appointments for the child’s developmental assessments and ongoing clinical evaluation. They were reassured by absence of signs on evaluation. Ophthalmology and audiology evaluation continued with local services after the initial evaluation. Ophthalmology assessment was normal at age eight. Evaluation continues annually. At age three audiologic testing revealed mild bilateral conductive hearing loss secondary to otitis media effusions. Middle ear ventilation tubes were inserted at age four years. Audiology assessment was normal when last performed at age six years.

The child attended the paediatric ID service at OLCH Dublin annually for developmental progress and review by the CT programme co-ordinator until the age of five years, thereafter progress was monitored by their local paediatrician. Neurodevelopment was normal at age eight years. The child was progressing well in all areas of the school curriculum, i.e., behaviourally, socially and academically with scores ranging from high above to above average thus far (STEN 7-8).
19.14 Case number 14

19.14.1 Screening and confirmation
The 14th confirmed case of CT was born by instrumental delivery following an uncomplicated pregnancy. The parents were Irish residing in Dublin city and this was their first child. Heel blood was collected on day four of life, results were available on day 13 and confirmatory mother-infant samples were taken on day 18 of life for dispatch to the TRL.

Infant serology confirmed CT. Maternal postnatal serology compared with negative antenatal serology from 15 weeks gestation confirmed pregnancy seroconversion, likely in trimester 3 (table 19.1 case number 14). The identified maternal risk factor for primary toxoplasma infection during pregnancy was ingestion of undercooked meat with lack of awareness of the associated risk.

19.14.2 Infant evaluation and treatment
The infant was asymptomatic with no signs of CT on clinical evaluation. Blood and CSF were *T. gondii* PCR negative. Antiprotozoal treatment commenced on day 47 of life for one year. Hospital bed availability accounted for a delay in elective admission for evaluation and treatment initiation.

More than five episodes of grade 1 neutropenia occurred throughout months one to nine of treatment. Five episodes of grade 2 neutropenia occurred during months one to five of treatment that responded to increased dosage of leucovorin. During the fourth month of treatment, an episode of grade 3 neutropenia occurred with an ANC of $0.600 \times 10^9$/L that required temporary antiprotozoal dose reduction by 25% for one week. The infant received treatment for 100% of the intended treatment duration and 98% of treatment was at full dose. Liver function and urinalysis remained normal throughout treatment.

19.14.3 Compliance with the management protocol and challenges encountered
The infant was a first-born child. Whilst the parents were initially upset and overwhelmed by the diagnosis, they fully accepted the diagnosis and management regimen. They were co-operative throughout treatment with infant monitoring and
subsequent clinical follow-up. They were reassured by absence of signs on evaluation.

19.14.4 Clinical follow up and outcome to date
Ophthalmology evaluation was normal at age eight years and annual evaluation continues. Audiology assessment was normal when last performed at age six years and no further follow-up was scheduled. Developmental progress was normal. The child at the age of eight years had above average academic progress recorded at primary school (STEN 8-9).
19.15 Case number 15

19.15.1 Screening and confirmation

The 15th and final case of CT was born during the second to last week of the 24-month CT screening programme. The infant’s mother was from Estonia and the father from Poland. The parents had been residing in Ireland in county Westmeath for five years. This was the mother’s second pregnancy and the infant was delivered by EMCS following failure to progress after an uncomplicated pregnancy. Retrospectively the infant’s mother reported a flu-like illness during week 13 of gestation with symptoms of temperature, swollen neck glands and rash; medical attention was not sought. Heel blood was collected from the infant on day five of life; results were available on day 28 of life. The reason for the delay in the screening result was partially explained by a delay in transport of the filter paper card to the NNBSL in Dublin.

19.15.2 Symptoms in the newborn period prior to the screening result

At birth the infant was noted to have a head circumference of 40 cm, which was above the 99th centile. No further evaluation was performed and the infant was discharged home on day five of life from the local maternity unit. During the infant’s third week of life, a district health nurse performed a routine weight check and noted that the infant’s head appeared unusually large for his age. This prompted referral to the local paediatric service for further evaluation. A cranial ultrasound was performed and hydrocephalus was diagnosed. Following this, the infant was referred to the paediatric neurosurgical service at TCUH Dublin for further evaluation and VP shunt insertion.

The infant was admitted to TCUH on day 19 of life. Repeat cranial ultrasound performed on admission demonstrated severe ventriculomegaly of the lateral and third ventricles. Ventricular head quotient (VHQ) was 86%. The appearance on MRI scan confirmed aqueduct stenosis (figures 19.4 a and b). No developmental cortical abnormality was evident. A VP shunt was inserted on day 23 of life in the right transparietal area.

The positive newborn screening result was obtained on day 28, five days following VP shunt insertion and whilst the infant remained an in-patient at TCUH. Contact was made with the infant’s local screening liaison midwife who informed the CT
programme co-ordinator that the infant had been transferred to TCUH for management of hydrocephalus. The programme co-ordinator visited the mother and infant whilst the infant was an in-patient at TCUH. Given the infant’s clinical presentation and signs on evaluation, the diagnosis of congenital toxoplasmosis was highly likely and this was discussed with the mother with the help of an interpreter.

On day 30 of infant life, bloods were taken from mother and infant for confirmatory testing. Results were available on day 36 of life and confirmed the diagnosis of symptomatic congenital toxoplasmosis. Retrospective testing of maternal antenatal serology from eight weeks gestation was negative. Postnatal serology confirmed pregnancy seroconversion. Maternal ELISA IgM was borderline when tested one month post delivery, which suggested infection had occurred six to nine months prior, most likely in trimester 1 or early in trimester 2 (table 19.1 case number 15). Maternal risk factors for toxoplasma infection were ingestion of cured meat products and undercooked fresh meat.

The mother was informed of these results whilst the infant was an in-patient at TCUH Dublin. She had limited command of English and communication was difficult even with a translator present. The infant’s treatment was outlined to the mother by the programme co-ordinator. However she refused to engage fully and challenged the diagnosis of congenital toxoplasmosis despite clinical signs and serological confirmation. Up to the point of serology confirmation, the mother’s understanding was that the hydrocephalus was an isolated abnormality and she communicated that it was difficult for her to appreciate another diagnosis going forward. She was angry that the infant’s large head circumference was not investigated at birth and congenital hydrocephalus was not diagnosed until age four weeks. She requested time to accept the diagnosis and consider the treatment protocol.

Subsequently, the programme co-ordinator met with her a second time with an interpreter to again explain the condition, outline treatment, and possible prognosis. At this point the mother agreed to further infant evaluation and treatment for congenital toxoplasmosis. However she requested that all subsequent communication occur with the programme co-ordinator only and an interpreter. Her infant remained an in-patient during this time and she expressed
that she was overwhelmed by various discussions with medical staff regarding management of hydrocephalus and congenital toxoplasmosis.

19.15.3 Further infant evaluation and infant treatment

The infant had an uneventful post-operative period following VP shunt insertion. Blood was *T. gondii* PCR negative, CSF from a lumbar puncture was PCR negative and protein was elevated at 1.24g/L (range 0.1-0.4g/L). At VP shunt insertion on day 23 of life, a sample of CSF was sent by the neurosurgeon to the TRL Swansea for *T. gondii* PCR testing as this was part of an evaluation protocol for congenital hydrocephalus. A positive result was obtained on day 36 of life, which coincided with serological confirmation of CT. A CCT scan subsequently performed demonstrated multiple foci of calcification in the right periventricular area. Ophthalmology examination revealed a right inactive chorioretinal scar. Audiology examination was normal.

The infant commenced antiprotozoal treatment at almost eight weeks, on day 54 of life, with an eight week course of oral Prednisolone to be tapered depending on repeat CSF eight weeks after treatment commenced.

The mother appeared to comprehend the importance of treatment and liaised regularly with the programme co-ordinator via a telephone translator following infant discharge from hospital. Follow-up was arranged for infant admission to the day ward at TCUH during the eighth week of treatment for repeat CSF protein and PCR, the result of which would determine the duration of steroid treatment. However, it was discovered that the prednisolone had been abruptly discontinued during the eighth week of treatment. The mother had concerns regarding steroid toxicity, which she had read about in the literature but did not communicate with medical personnel. She did not wish for her infant to continue steroid treatment after eight weeks was completed and informed the programme co-ordinator of the abrupt discontinuation two days after its cessation. The importance of compliance with infant treatment was stressed. Repeat CSF-PCR and protein were negative. Adherence to recommendations did not pose a problem thereafter.

One episode of grade three neutropenia occurred during the fifth month of treatment with an ANC of $0.600 \times 10^9$/L which was managed with 25% reduction in antiprotozoal dosage. Less than a week later, while still on the dose reduction, the
infant developed symptoms of pyrexia and was assessed at the local hospital's accident and emergency department. The ANC was $0.540 \times 10^9/L$ prompting a full septic screen, which was unremarkable. The infant was diagnosed with viral illness. Antiprotozoal medication was temporarily withdrawn for one week and successfully reinstated a week later at full dosage. Liver function and urinalysis remained normal throughout treatment.

At nine months of age, the VP shunt failed due to blockage and the infant developed symptoms of persistent vomiting. Shunt replacement was required. During the period of persistent vomiting and shunt replacement, it was necessary to temporarily withdraw antiprotozoal treatment for approximately three weeks until the infant's condition was stable. Following discharge from hospital, the infant developed viral gastroenteritis and antiprotozoal medication was withheld for a further two weeks. The ANC remained normal throughout these episodes. The infant received 46 of 52 weeks of treatment, which was 88% of the intended duration.

VP shunt failure and associated symptoms necessitated three weeks of antiprotozoal interruption. Two occurrences of viral infection accounted for a total of three weeks of antiprotozoal interruption; neutropenia occurred on just one occasion of viral illness. Thus whilst it was necessary to discontinue treatment for six weeks in total, and reduce treatment dose for one week, antiprotozoal adverse effects were not a major contributor to those interventions, but rather viral illness and VP shunt complications.

19.15.4 Challenges encountered
An unfortunate sequence of events occurred with this infant, which resulted from failure of evaluation of a large head circumference at birth and the delay in the screening result. A reason was not obtained for why the documented head circumference was not deemed to be significant and acted upon by the local paediatric team. Even so, had the newborn screening result been obtained earlier, i.e., in the first 10 days of life, the diagnosis of CT could have been made earlier. Hydrocephalus was first detected when the infant was three weeks old. The newborn screening result was obtained five days after the infant had a VP shunt inserted for congenital aqueduct stenosis, presumed at the time to be due to a congenital anomaly rather than maternal toxoplasmosis in pregnancy. Hence
within the infant's first month of life, the parents perceived their infant to be healthy at birth, to be then informed at three weeks that the infant needed admission to a tertiary centre for evaluation and management of hydrocephalus, a diagnosis of isolated aqueduct stenosis was made, VP shunt sited, followed a week later by a positive newborn screen for CT that necessitated further interventions with serologic confirmation of CT at age five weeks. These events provided the foundation for maternal confusion and distrust of doctors, which persisted for some months.

The diagnosis of congenital toxoplasmosis was not initially accepted. Communication took place with the infant's mother on all occasions. The father was rarely met with at out-patient appointments. The language barrier posed difficulties initially, however as time went on the mother learned to communicate with reasonably good English. For minor infant ailments, attention was sought from the local general practitioner who experienced difficulty communicating with the mother. In addition, the infant required early intervention services. These were sought from local community teams. The infant's mother remained un-cooperative and indifferent towards local multidisciplinary services. On many occasions she would not present for appointments or was un-cooperative with home visits by members of the multidisciplinary team. Non-attendance was reported back to the programme co-ordinator by intervention services on many occasions with the threat of no further rescheduling of appointments if poor attendance continued.

The infant's mother perceived that the infant's condition represented irreversible brain damage that could not be helped by interventions other than a VP shunt. The programme co-ordinator held many discussions with the mother via telephone to convince her that her child's overall development could definitely benefit from early intervention aimed at maximising his developmental potential and outcome. The mother subsequently engaged with local early intervention services but full adherence to management recommendations was not reported, continuous persuasion was necessary for her to avail of services on behalf of her child. However the mother remained fully compliant with the CT treatment protocol after the initial issues. She adopted a policy of communicating only with the programme co-ordinator at all times and no other members of the team. This did pose some difficulty with the infant's management, however, she was facilitated with this request as the importance of ongoing communication was essential for the infant.
When the child was age three years, social workers and Gardai intervened into the family's domestic situation due to reports of domestic violence and concerns for the safety of the mother and her children. At that point the mother had another infant aged 12 months and an older child aged 10 years. Due to concerns for the mother’s and children’s welfare they were provided with alternative accommodation away from the children’s father. Challenging social circumstances further affected compliance with appointments for clinical evaluation and early intervention services. However additional social supports were put in place to maximise compliance.

19.15.5 VP shunt complications
VP shunt failure has occurred on four occasions to date. The first occurred whilst the infant was on antiprotozoal treatment at nine months of age as described above. At three years of age the patient presented with vomiting and was referred to TCUH for neurosurgical review. A blocked VP shunt was replaced. At age four years a routine CCT scan performed at TCUH for VP shunt review demonstrated increased ventricular dilatation compared with previous images. The child had no symptoms. The proximal shunt catheter was blocked and this was replaced. Annual shunt reviews performed by neurosurgical services at TCUH were reported as normal thereafter until the most recent episode of shunt failure which occurred in the summer of 2015 at the age of eight years when the child developed acute onset of severe headache and vomiting. The mother recognised the symptoms as those of VP shunt failure and promptly drove from county Westmeath to the accident and emergency department at TCUH. Cranial imaging demonstrated that the distal catheter of the VP shunt had disconnected. The child was transferred to neurosurgical services at Beaumont hospital and underwent emergency replacement of the VP shunt following which he made an excellent recovery. Annual neurosurgical review is scheduled to continue.

19.15.6 Clinical follow up and outcome to date
The child was reviewed annually at OLCH Dublin by the programme co-ordinator for developmental progress up to the age of five years. During this time the child was also participating in an early intervention programme. Thereafter developmental progress was monitored by local community paediatric services in county Westmeath. The child had numerous appointments in their local area, in
addition to ophthalmology assessments at OLCH and annual VP shunt reviews at TCUH. Following school entry, in order to maximise compliance, appointments in Dublin were maintained for only what was deemed necessary.

The child did not have repeat brain imaging at the age of one year as imaging had been performed at age nine months for VP shunt replacement. Subsequent CCT was performed at age three years at TCUH, which demonstrated normal ventricular size and resolution of periventricular calcification with one remnant focus detected (figure 19.5 a). Areas of PVL were demonstrated bilaterally but were not extensive (figure 19.5 b).

Ophthalmology follow up was performed at OLCH Dublin. The child developed a latent convergent squint in the left eye that required patch therapy, however the mother was not compliant with this recommendation. At age 16 months corrective surgery for strabismus was performed and corrective lenses were prescribed. Thus the strabismus likely represented a late ocular association of CT. At age eight years, ophthalmology evaluation remained unchanged from the newborn period with a right inactive chorioretinal scar; annual surveillance continues. Audiology follow up was performed by local services and was normal at the last scheduled visit when the child was age four years.

Access to local-community early intervention services were provided from early infancy. Assessments during the first year of life raised the possibility of early global developmental delay and an evolving left hemiparesis. Motor milestones were achieved slightly beyond the expected range for age. The child walked at 19 months of age, and by the age of two years had attained all gross motor milestones. At that point, speech and language delay, fine motor and co-ordination problems became evident.

The child received intensive multidisciplinary intervention services, which included speech and language therapy, physiotherapy and occupational therapy. At the age of four years the child had a full psychology assessment performed in preparation for primary school. His IQ score was 82, which was on the 13th centile and represented the low average range of ability on cognitive tasks. This suggested that with supplemental resource teaching he was capable of accessing a mainstream school curriculum along with age matched children. Speech and language
development was above average for age but expressive language was delayed and manifested with delay in vocabulary and grammar use for his age. The child continued to receive speech and language therapy for delayed articulation and demonstrated excellent progress.

The child was diagnosed with developmental co-ordination disorder in early childhood and continued to exhibit mild motor delay manifested primarily with balance and coordination problems with hopping, jumping and skipping. Gross motor skills were in range but fine motor object manipulation skills were below average. The child continued occupational therapy for development of fine motor and visual integration skills. Mild attention issues were displayed when asked to persevere with tasks however there were no specific psychological needs.

The child commenced main-stream primary school at the age of four years and three months. He was provided with resource teaching and a special-needs assistant. Excellent performance and progress was reported. School teachers noted no language deficits. The child’s ability to learn was reported as normal compared with peers and no specific intellectual deficit was identified. As a result, the child did not require continued input from resource teaching and this was withdrawn after the first year at school. Residual fine motor and visual motor integration delay manifested with writing problems and was the only area that required continued support with extra assistance at school; good progress was reported. The ability to learn in the areas of reading, spelling and maths was reported as excellent at age eight years.

The child continued to attend local paediatric services for annual evaluation; global neurodevelopmental progress was documented as excellent and within range at the age of eight years. The minor delay that persists with writing skills and fine coordination does not affect schooling, social, behavioural or academic function and overall quality of life. The child has been advised never to participate in contact sports.

The child at age eight years continues to display steady progress academically, with STEN scores documented as above average (7-8) in school tests thus far. The child has a special interest and talent in singing and drama and is a lead member of the school’s choir.
Figure 19.1: Cranial imaging for congenitally infected case number 3

**CCT scan of the brain at birth**

a) multiple foci of calcification (red arrows) in both frontal lobes with loss of cortical volume in the frontal lobe noted.
b) foci of calcification also noted bilaterally in the peri-ventricular regions adjacent to both frontal horns. Bilateral prominent ventricular system and diffuse hypo-density involving the cerebral white matter also noted.

**CCT scan following treatment completion**

c) Almost complete resolution of intracranial calcification. One focus of calcification is seen anterior to the anterior horn of the left lateral ventricle. Normal ventricular size.
Figure 19.2: CT case number 5: cranial imaging

a) Neonatal CCT scan
Multiple linear calcifications (red arrows) in the right lateral periventricular area. Calcification size and number were computed. Repeat cranial scans obtained at follow-up were reviewed by the same radiologist.

b) CCT scan following antiprotozoal treatment
Diminution in size and almost complete resolution of calcifications in the right lateral periventricular area.
Figure 19.3: CT case number 7: eye fundus photographs

**a) Right eye**
Sharply demarcated pigmented area represents a peripherally located inactive chorioretinal lesion (black arrow) present from birth. Infant treated for 1 year with antiprotozoals. Retinal examinations performed by a single ophthalmologist at specific intervals.

**b) Left eye**
Age 30 months, second recurrence. Active chorioretinal lesion in close proximity to optic disc (black arrow). Fluffy appearance and poor demarcation of the lesion indicates active inflammation. Patient treated with antiprotozoals and steroids.

**c) Left eye**
Healing focus of chorioretinitis. Cicatrisation occurring from the periphery towards the centre with pigmentary changes at the edges indicates regression.

**d) Right eye**
Age 33 months. One month following completion of three months of therapy for reactivation, new focus of chorioretinitis (black arrow) presented at temporal aspect of right macula with adjacent vasculitis. Patient retreated with six months antiprotozoals and tapering steroids. Therapy was successful and retinal lesion became dormant. All intraocular lesions subsequently remained inactive. Patient has normal visual acuity.
Figure 19.4: Intra-cranial imaging for CT case number 15 at age three weeks

MRI scan at age three weeks
a) Axial T2 image shows hugely dilated lateral ventricles and dilated third ventricle.
b) Sagittal T2 image shows that the aqueduct is dilated superiorly (black arrow) and occluded inferiorly consistent with aqueduct stenosis. Fourth ventricle has a normal size. The midbrain was slightly compressed. The cerebellum and brain stem were normal. The cortex was thin in the posterior parietal regions.
Figure 19.5: Case number 15; imaging at 3 years

a) CCT scan at 3 years old
Ventricles are normal size with shunt in place (green arrow); Focus of calcification is seen in the right periventricular area (white arrow); Shunt tubing outside skull (red arrow).

b) MRI scan at 3 years old
Some residual high signal is seen in the deep white matter posterior bilaterally (white arrows) and represents periventricular leucomalacia confined to the margins of the posterior ventricles. The brain parenchyma is otherwise normal. The ventricles have a normal size and configuration.
Table 19.1: Congenital toxoplasmosis; screening results and confirmatory serology for 15 mother-infant pairs

<table>
<thead>
<tr>
<th>Infant Case Number</th>
<th>AutoDELFIA IgM IU/mL</th>
<th>ISAGA IgM Screen</th>
<th>Infant Confirmatory Serology</th>
<th>Maternal Postnatal Serology</th>
<th>Maternal Antenatal Serology</th>
<th>Trimester of Maternal Seroconversion (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dye test IU/mL</td>
<td>Elisa IgM</td>
<td>ISAGA IgM</td>
<td>ISAGA IgA</td>
</tr>
<tr>
<td>1</td>
<td>188, 182, 191</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>206, 198, 218</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>166, 165, 179</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>210, 204, 218</td>
<td>Pos</td>
<td>125</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>595, 558, 568</td>
<td>Pos</td>
<td>4000</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
</tr>
<tr>
<td>6</td>
<td>1106, 926, 953</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>P0s</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>1070, 970, 948</td>
<td>Pos</td>
<td>2000</td>
<td>SP</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>8</td>
<td>450, 432, 424</td>
<td>Pos</td>
<td>1000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>1289, 1276, 1298</td>
<td>Pos</td>
<td>2000</td>
<td>SP</td>
<td>SP</td>
<td>SP</td>
</tr>
<tr>
<td>10</td>
<td>441, 282, 247</td>
<td>Pos</td>
<td>1000</td>
<td>BD</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>11</td>
<td>894, 897, 908</td>
<td>Pos</td>
<td>1000</td>
<td>SP</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>12</td>
<td>265, 285, 277</td>
<td>Pos</td>
<td>2000</td>
<td>1000</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>13</td>
<td>737, 713, 674</td>
<td>Pos</td>
<td>2000</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>14</td>
<td>161, 189, 171</td>
<td>Pos</td>
<td>4000</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>15</td>
<td>86, 91, 98</td>
<td>Pos</td>
<td>2000</td>
<td>Neg</td>
<td>BD</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Pos, positive; SP, strongly positive; VL, very low; Neg, negative; NT, not tested; BD, borderline, WB, Western Blot; NA, not available
Table 19.2: Serological profile for congenitally infected infant case number 4

<table>
<thead>
<tr>
<th>Age</th>
<th>Dye test IU/mL</th>
<th>ELISA IgM</th>
<th>ISAGA IgM</th>
<th>ISAGA IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>125</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>4 weeks</td>
<td>250</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>6 weeks‡</td>
<td>500</td>
<td>Negative</td>
<td>Borderline</td>
<td>Positive</td>
</tr>
<tr>
<td>12 weeks ‡ (WB)</td>
<td>1000</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>6 months</td>
<td>1000</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>9 months</td>
<td>500</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>12 months</td>
<td>500</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

‡ Infant blood PCR negative at 6 weeks

‡WB, (Western Blot) analysis negative
CHAPTER 20 - Final discussion summary and conclusions

20.1 Introduction

*T. gondii* can infect humans and all warm blooded animals. Despite a benign course in most immunocompetent humans, toxoplasmosis has been proven to impose a significant disease burden. At least one third of the world's human population is infected with *T. gondii* (Saadatnia et al., 2012). Congenital *T. gondii* infection of the fetus is associated with the potential for visual or neurodevelopmental sequelae. In Europe, CT has been reported to affect between 1 and 5 per 10,000 newborn infants of whom 4% to 30% develop retinochoroidal lesions which may lead to visual compromise and 1% to 2% develop significant neurological sequelae. (Villena et al., 2010).

CT prevention programmes in Europe primarily target pregnant women. Postnatal screening programmes for CT unlinked to antenatal screening have not been widely performed in Europe. However, the majority of European studies on infants with CT who were identified and treated from early infancy demonstrate good outcomes overall (Wallon et al., 2013).

Postnatal screening and treatment for *T. gondii* infection has not been studied using control groups. Therefore most conclusions are drawn from comparison with historic untreated groups or longitudinal observation of treated vs untreated groups that originated from similar or different populations. The on-going North American NCCCTS has provided direct comparison of treated and untreated groups of children with CT (McAuley et al., 1994; Phan et al., 2008b). However the population from the NCCCTS primarily consists of children referred with a clinically suspected or confirmed diagnosis of CT, rather than diagnosis from screening of the newborn. Thus selection bias is incurred and the majority of children have clinical symptoms or signs at referral.

In Europe, the topic of postnatal screening and treatment for CT, and of late, treatment of asymptomatic infants, continues to generate debate among the expert community. What has been unanimously agreed is that CT, symptomatic or asymptomatic, is a condition that needs to be diagnosed. However, the questions that yet require unanimous answers are what are the most effective means for early detection of CT and what is optimal treatment.
20.2 Postnatal screening for CT in the absence of randomised studies that prove benefit

The wide disease spectrum and potential sequelae of congenital *T. gondii* infection necessitates effective means for prevention, diagnosis and management of the condition. Postnatal screening identifies congenital infections and provides an opportunity for early diagnosis in areas where antenatal screening and interventions are not feasible or cost effective. Early treatment of CT may provide an opportunity to arrest disease progression in both symptomatic and asymptomatic infants and thus reduce the potential for long-term visual and neurodevelopmental sequelae. Even in infants with no apparent disease at birth, i.e., those with subclinical infection, early treatment may provide a more favourable outcome. Other early interventions may be necessary to minimise neurological sequelae in infants with CNS abnormalities that may be diagnosed only if specifically sought.

There is a lack of consensus and uniform approach to the early diagnosis and management of CT, not just in Europe but in resource rich settings globally. This is primarily due to ethical contraindication to a double blind randomised study that includes a group of untreated asymptomatic children, thus comparative evidence is unavailable to guide management protocols. Researchers from the EMSCOT study group proposed that due to the low risk of severe disease, overall good outcomes for CT in Europe and lack of proof that antiprotozoals benefit asymptomatic children or prevent recurrences, only the symptomatic children at risk of sequelae should be treated and rigorously followed up (Freeman et al., 2008). However, lack of RCTs and evidence based gain from treatment does not automatically exclude treatment benefit for infants with no signs of CT or for those with mild signs. In the absence of evidence, some countries in Europe have unified the approach to early diagnosis and management of CT through antenatal screening programmes. However policies and practices continue to vary, sometimes even within a country.

20.3 Proposal for newborn screening for CT in Ireland

The incidence and disease burden of CT in Ireland could only be determined by postnatal screening for the condition and follow up of an identified cohort for a defined time period to determine outcome.
Information on maternal toxoplasma seroprevalence in Ireland was first needed to evaluate the potential benefits of screening for CT. This led to a study in 1996 of randomly selected DBS to determine the prevalence of toxoplasma antibody in women of childbearing age (chapter 8). The low maternal seroprevalence of 25% and significant variation by county implied that the majority of pregnant women in Ireland remained susceptible to toxoplasma seroconversion in pregnancy, some significantly more so than others depending on geographical location. Thus the DoHC approved an application for screening of newborns and provided funding for a 2-year pilot programme of national newborn screening for CT in Ireland which commenced in July 2005.

A time delay of nine years occurred between the seroprevalence study in 1996 and the initiation of newborn screening in 2005. This was due to a number of contributing factors which are described as follows. Prior to submitting an application to the DoHC for the newborn screening proposal, the toxoplasma working group required evidence that screening of newborn DBS on filter paper was a feasible method for detection of CT in Ireland. Whilst the literature had reports of newborn screening for CT using DBS in other countries, feasibility of screening of a population similar to Ireland would strengthen the proposal for CT screening in Ireland. The toxoplasma working group was aware that Denmark, a country with a similar population to Ireland, had submitted a proposal for newborn screening for CT using DBS from infants' heel and a programme was commenced in 1999. The first published report on successful extraction of toxoplasma IgM from DBS following four years of newborn screening in Denmark was published in 2002 (Sorensen et al., 2002). Thus in 2003, following acceptance of the proposal by the DoHC, arrangements were commenced in Ireland for a programme of newborn screening for CT based on the methods in use in Denmark at that time. Two years of preparation were necessary for the programme methodology, in particular, for calibration of the screening assays and training of personnel at the NNBSL.

20.4 Programme implementation and execution
The national newborn screening programme for CT was successfully implemented on July 1st 2005 following minor obstacles. Newborn screening continued uninterrupted for the two-year period and the methods employed for all stages of the programme remained unaltered throughout.
20.5 Screening and confirmatory results and incidence of CT in Ireland

Uptake of newborn screening was approximately 99.75%. The low incidence of CT in Ireland (one per 10,000) derived from 24-months of screening was comparable with rates previously quoted for Western Europe and in particular Denmark (1.6 per 10,000).

Toxoplasma IgM was easily recovered from DBS on filter paper cards with a two-step protocol that utilised two IgM assays. Of the total screen positive population, 56% were confirmed negative by serology. Adaptations of the AutoDELFIA screening test were necessary to suit the Irish population. The AutoDELFIA threshold for a positive screen was set at a lower value than that recommended by the manufacturer. However, the median value of the AutoDELFIA was higher in serologically confirmed positive cases than confirmed negative cases which implied that perhaps the AutoDELFIA threshold for a screen positive result should have been higher. However such speculation can only be proven by screening of a control group at a higher threshold.

The low incidence of CT in the population, together with the low predictive value of the first IgM screening assay (AutoDELFIA) and the moderately predictive value of the second IgM screening assay (ISAGA) gave rise to a high rate of false positive screening results (1.3 per 10,000) that required confirmation. This demonstrated that ideally for mass screening of a population with a low incidence of CT, alternative screening assays would be desired, particularly if CT was to be considered for routine adoption into the existing newborn metabolic screening programme.

Despite the small number of infants in total who screened positive and required confirmation, a diversity of confirmatory serology was encountered. Six of 34 screen positive infants (18%) required more than one confirmatory serological; four of these were confirmed negative and two were infants with CT. Three of 34 (9%) screen positive infants required more than two serial tests for confirmation; one was confirmed negative and two were positive. One of the two infected was confirmed at three months with the assistance of WB testing and the presence of a clinical sign of CT. The other infant had no signs of CT and persistently inconclusive serial antibody profiles in addition to a negative result.
with WB analysis which resulted in late serological confirmation of CT at age nine months. Thus in the total cohort of 34 screen positive infants, there was just one infant (3%) who could not be serologically confirmed by the age of three months. This reinforced the importance of testing all confirmatory samples at a single Reference Laboratory with result interpretation assisted by an expert in the field. In addition, this demonstrated that the serological diagnosis of CT can be complex, and whilst this was represented by a minority in our cohort, it highlighted that studies on alternative and feasible methods for prompt serological diagnosis are necessary to confirm or rule out the diagnosis in infants with atypical or inconclusive serology. No screen negative cases born during the two-year programme were subsequently identified with CT.

### 20.6 Demographics of screen positive and confirmed positive cases

During the 24-month period, the majority of infants who screened positive were born to mothers resident outside county Dublin. Geographical distribution of the infants born to mothers with toxoplasma antibody as a result of seroconversion prior to or during pregnancy demonstrated that a minority of women resided in county Dublin; despite the fact that 40% of infants born in Ireland are born in county Dublin. These findings were consistent with the seroprevalence study performed in 1996 that demonstrated toxoplasma antibody seroprevalence was lowest in county Dublin. Hence despite the fact that there was a 10-year gap between the seroprevalence study in 1996 and the CT screening study, the geographical distribution of toxoplasma seroprevalence amongst women of childbearing age had not changed. Whilst the high rate of seronegativity in the pregnant population in county Dublin predicted a higher risk for pregnancy seroconversion, the rate of seroconversion in the county was low, likely due to urban-rural differences and less exposure to risk factors.

The migrant antenatal population, i.e.; women who were not of Irish ethnicity or Irish born, comprised 41% (14/34) and 33% (5/15) of the screen positive and confirmed positive cases respectively. This population was primarily represented by women from African and Eastern European countries and reflected migration trends during the study period 2005 to 2007 and in the five years preceding this time.
20.7 Clinical findings in the cohort of congenitally infected infants

Over the 24-month screening period, 15 cases of CT were identified giving an overall incidence of 1 per 10,000 live births. Fourteen of these were detected solely on the basis of the screening programme and the fifteenth, in whom an in-utero diagnosis had been made, was also identified through the screening programme.

Asymptomatic infants predominated and represented 87% of the infected cohort. This finding was expected and reflected results obtained from other screened European populations which demonstrate a low rate of symptomatic or severe disease (Roser et al., 2010). A contributing factor for the predominance of asymptomatic infants in our cohort was that the majority of women were estimated to have seroconverted in trimester 3, when the risk for infant signs and severe disease are lowest (Dunn et al., 1999). In addition, the avirulent type II strain of *T. gondii* predominates in Europe (Peyron et al., 2006). Of note in the group of asymptomatic infants in the study cohort, one had multiple intracranial calcification at initial assessment and thus by definition signs of severe CT which was detected only as a result of screening.

One symptomatic infant was diagnosed prenatally due to obvious signs during gestation, but would have also been detected by newborn screening if CT was not diagnosed antenatally. The infant had poor tone and head lag at birth. In the absence of newborn screening, such symptoms should have prompted intracranial imaging and further evaluation in the newborn period. Even if the diagnosis of CT was not made in early infancy, the child would have likely presented with developmental delay in the pre-school or early primary school years due to unilateral blindness, at which point treatment and early interventional benefits would have been missed. The areas of developmental delay which were promptly identified and targeted in this child was only as a result of services that were specifically sought because of the early diagnosis of CT and identification of signs in the newborn period that could affect long-term neurological outcome.

The second symptomatic infant had the classic triad at diagnosis. Despite obvious symptoms of an abnormally large head circumference at birth, this was only incidentally noted in the third week of life at which point the infant was referred for evaluation and further management. Even at the point of intervention for hydrocephalus, CT was not suspected until the positive screening result was
obtained, albeit on day 28 of life. A CSF sample was sent for *T. gondii* PCR at VP shunt insertion and a positive result was obtained on day 36 of life, which coincided with serological confirmation that was sought because of the positive screening result. This case reinforced that all infants with hydrocephalus at birth should have a prompt congenital infection screen and full neurological evaluation.

Denmark to date remains the only country in Europe that has performed national newborn screening for CT unlinked to antenatal screening. It seems reasonable to directly compare infant clinical findings obtained from screening in Denmark with Ireland. Both populations were demographically similar with a comparable seroprevalence in the pregnant population, methods used for CT screening were similar, screening years coincided and a similar incidence of CT was obtained in both countries.

Clinical signs at initial evaluation in congenitally infected infants in Ireland were higher compared with signs in the cohort of infants recruited from the first five years of screening in Denmark (chapter 12 table 12.4). Even though the total number of infected infants differed, it was clear that there was a higher incidence of signs demonstrated at birth in infants with CT in Ireland. The difference encountered can only possibly be explained by host genetic factors. Despite the higher incidence of signs in the Irish cohort overall, signs of inactive disease predominated. Severe signs were present 3 of 15 infants (20%).

The majority of CT in Europe is non-severe due to predominance of the typical type II strain of *T. gondii*. The type II strain exists in four main lineages that differ in their capacity to form cysts. Two lineages of the strain have the capacity to form fewer cysts and have been isolated from asymptomatic infants, whereas the other two lineages of the strain have an increased capacity to form cysts and have been isolated from symptomatic cases. It is proposed that in Europe, congenital infections are predominantly caused by the type II strains that have less capacity to form cysts (Gilbert et al., 2008; Brenier-Pinchart et al., 2010).

**20.8 Risk factors for seroconversion in women with infected infants**
The most common risk factor for pregnancy seroconversion in the small cohort of 15 women with infected infants was ingestion of meat that was either raw-cured or undercooked, which was similar to risk factors demonstrated in the large EMSCOT
study. The most striking finding on investigation of maternal risk factors was the overall lack of awareness of toxoplasmosis and risk factors for seroconversion amongst women who had congenitally infected infants. Thus this study identified epidemiological targets for future intervention which could potentially reduce the risks for pregnancy seroconversion.

Screening and interventions for CT are dictated by the disease burden in a country or region, prognosis of those affected and not least by availability of resources and funding. In Ireland, with a low incidence of CT and a predominance of asymptomatic infants, it seems justifiable to employ education as a formal CT prevention strategy. An active programme of education should ideally target all women of child bearing age and not just the antenatal population, at which point prenatal education may be too late in some cases. The recognition that during the study period 2005 to 2007, non-national women comprised approximately 20% of the population that gave birth in Ireland (available at http://www.cso.ie/en/media/csoie/releasespublications/documents/vitalstats/2007/chapter2_2007.pdf accessed November 2015) demonstrates the need for availability of comprehensive information on risk factors, with accessibility not just for the pregnant population but for the public domain in general.

20.9 Infant treatment and toxicity
Antiprotozoal adverse effects were common in this study, primarily transient gastrointestinal effects following treatment initiation, and neutropenic events during the 12-month course of treatment. All treated infants experienced neutropenia. Whilst neutropenic episodes in the cohort were predominantly mild or moderate (grades 1 and 2 events), some infants experienced more severe neutropenia, albeit infrequently. All episodes of neutropenia necessitated more frequent blood monitoring, even for less severe episodes when antiprotozoal adjustments were not indicated. One infant with severe CT required adjunctive neutrophil support with GCSF from three months of age, as interruption of antiprotozoal treatment was not feasible due to the possibility of significant visual compromise. Whilst neutropenic episodes were easily reversed with treatment adjustments and no significant clinical complications of neutropenia occurred, the frequency of events during a 12-month antiprotozoal regimen demonstrated the need for alternative, less toxic anti-toxoplasma regimens. Combination antiprotozoal therapy has been traditionally employed for CT for over 30 years. However there is
a recognised need for further studies in this area to address many questions that still remain unanswered, namely the optimal duration of treatment currently used and efficacy of other anti-toxoplasma agents that may have less adverse effects. The standard pyrimethamine/sulfadiazine combination only targets metabolism of parasite nucleotides by blocking tachyzoite folic acid synthesis, but cannot penetrate latent cysts. Alternative treatment regimens for CT should ideally demonstrate high ocular and cerebral penetration, efficacy against all parasite strains and not least be easily obtained.

A randomised study is ongoing in France to specifically address postnatal treatment options for non-severe CT with alternative and shorter regimens. However the recruitment phase of this study is due to end in 2016 and conclusions on treatment efficacy cannot be drawn unless ocular and neurological outcomes are studied both short and long-term to determine the effect of treatment on reactivation or disease progression. Hence it is possible that a minimum of five years following recruitment will be necessary before preliminary conclusions on this study are available. Thus, other treatment options for CT may not be provided in the very near future but are at least on the horizon.

20.10 Serological rebound
All treated children demonstrated a serological response to antiprotozoal therapy by suppression of the DT and other toxoplasma-specific immunoglobulin. Subsequently, serological rebounds were demonstrated by all children following treatment completion, most commonly during the second year of life and less frequently during the third year of life and beyond.

Serological rebound is also referred to as serological reactivation, its clinical significance has not been demonstrated in studies that have specifically addressed this phenomenon. The findings in our study also failed to demonstrate a clinical correlation with rebound episodes. Reappearance of IgM or IgA and high DT rebounds (4,000 IU/mL) did not correspond with disease progression or reactivation in our cohort at median serological follow-up of four years. Intraocular recurrences occurred in one child on three occasions between the second and third year of life, none of which were associated with a significant change in the serological profile.
Serological rebound is thought to result from re-exposure of the child's immune system to the parasite following discontinuation of the suppressive effect of antiprotozoals. As *T. gondii* exhibits latency, it has been proposed that either re-emergence of parasite from latent cysts occurs, or free tachyzoites are not totally eliminated following treatment for CT in the first year of life. However, an immunocompetent child is capable of controlling parasite replication to some extent after the age of one year and the renewed immune response to *T. gondii* activity is thought to be represented by serological rebounds. The rationale for treatment duration of one year for CT was initially based on the recognition that an infant under the age of one lacks immune competence and therefore antiprotozoals should be prescribed for the entire first year of life. Monitoring of the serological profile in our cohort, with demonstration of serological rebound in all children following treatment discontinuation, supports the recommendation for treatment until some degree of maturation of the child's immune system occurs at one year. In the studied cohort, treatment discontinuation after one year triggered a demonstrable immune response which suggests that under the age of one year, sufficient immunologic control may not be attainable without the support of antitoxoplasma therapy.

The inability of serological rebound to predict clinical consequences demonstrates that it is not necessary to monitor the antibody profile beyond the treatment period as it will not influence decisions on clinical monitoring or management. It is likely that serological rebound simply demonstrates an expected immune response.

**20.11 Outcome of the cohort**

**20.11.1 Ocular outcome**

Conclusions on treatment benefit in a population with CT are usually based on observation of one or more of the following 1) rate of disease progression, 2) demonstration of regression of signs and 3) neurodevelopmental outcome. The rate of disease progression is primarily based on the rate of ocular reactivation and visual impairment in the cohort at follow-up compared with initial findings at birth.

The incidence of chorioretinal lesions at baseline in the cohort was 40% (6/15), all lesions were inactive and only one child had macular involvement and visual
impairment at diagnosis. Despite reactivation in 7% (1/15) in the cohort, a favourable ocular outcome was demonstrated overall. The infant with recurrences in our cohort demonstrated that it is not always possible to accurately predict which children are at increased risk of ocular recurrence. Children with symptomatic CT or severe CNS and other extra-ocular signs are usually deemed to be at high risk for intraocular recurrences and disease progression overall. This was not demonstrated in our cohort. The child who experienced recurrences had no risk factors for prediction of intraocular reactivation apart from female gender. This case highlighted two issues that have been debated in CT.

Firstly, this case exemplified that all children with CT should have regular ophthalmology surveillance, and not just symptomatic infants or those at high risk for intraocular reactivation. Researchers of the EMSCOT study suggested that only children at high risk for ocular recurrence will benefit from regular ophthalmology examinations, low risk infants should have less frequent ocular surveillance in childhood, and more frequent surveillance should be guided by the appearance of ocular symptoms and signs (Freeman et al., 2008).

The infant in our cohort with recurrences would not have benefitted from prompt detection and treatment of recurrent chorioretinitis in the absence of the routine and frequent ophthalmology surveillance in early childhood that was included in our follow-up protocol. De-novo lesions were in proximity to the optic disc which posed a threat to vision. Without early detection, it is likely progression would have occurred and visual compromise may have resulted. Despite three intraocular recurrences within a time frame of less than 12 months, potential visual impairment was aborted by prompt retreatment.

Secondly, this case also exemplified that treatment for CT in the first year of life should not be restricted to children with symptoms or severe signs at birth, as has been proposed (Boubaker et al., 2008). Asymptomatic cases of CT with or without signs can potentially benefit from antiprotozoal treatment. Whilst it can be argued in this case that treatment during the first year of life did not prevent reactivation in early childhood, the counter argument is that treatment in infancy can prevent reactivation and potential visual sequelae during the first year of life when the
child’s vulnerable immune system is unable to prevent or effectively control recurrences.

20.11.2 Effect of treatment on intracranial lesions
Treatment effect was evident within the CNS of infants with multiple calcific foci, where dramatic resolution or regression of calcification was demonstrated after 12 months of antiprotozoal treatment. This proves that current antiprotozoal treatment for CT can effectively penetrate the CNS and directly target toxoplasma-specific lesions. Resolution or regression of calcific lesions implies that treatment of CT in infancy allows improved neurologic function. The finding also provides reassurance for treated infants with no obvious intracranial lesions, as it can be assumed that antiprotozoal effect within the brain will prevent or arrest parasite multiplication in the CNS, which is just as important in infants with no demonstrable intracranial signs. This further supports the argument for offering treatment to all infants with CT and not just those with symptoms evident at birth. Asymptomatic infants deserve the potential benefit of antiprotozoal treatment and protection conferred to the brain under the age of one year, during which time the CNS remains vulnerable to parasite activity that cannot be fully controlled by the immune system.

20.11.3 Neurodevelopmental outcome of the cohort in the first decade of life
A favourable neurodevelopmental outcome was demonstrated in the cohort at median 9-year follow up (range 2 to 10 years). Three asymptomatic children with no signs of CT were lost to follow up; two at age two years and one at age five years. Developmental abnormalities were identified under the age of one year in two symptomatic children who received intensive multidisciplinary early interventional services.

A surprising and difficult to explain outcome was demonstrated in one symptomatic child, for whom a poor prognosis was initially predicted, based on symptoms of ventriculomegaly apparent in gestation and severe signs of CT in the newborn period. Not only did this child progress to normal development by school entry, but throughout primary school demonstrated superior intelligence to such an extent that by the age of 10 years was identified as gifted with an intelligence score in the top 2% for age matched individuals nationwide. This led to selection for supplemental teaching at a centre for talented youth.
The second symptomatic child, one with significant hydrocephalus that was undetected until age three weeks, also demonstrated remarkable progress following treatment for CT with additional interventional services up to school entry. During infancy and the toddler years, the child displayed global developmental delay but by age four was categorised as functionally fit for mainstream primary school. Currently, the only deficits that remain for targeting at age eight are fine motor and some aspects of co-ordination, albeit minor. All other areas of development are normal, in particular cognitive function based on academic STEN scores and teacher assessments throughout school thus far. The child requires some assistance with writing skills in school, no interventions are required for other areas of the school curriculum. Hence this child has neurological sequelae of a very mild form, which was not as a result of disease progression but rather residual from brain injury in-utero. Given the degree of hydrocephalus in the newborn period, combined with residual periventricular leucomalacia and many VP shunt malfunctions, the outcome is no other than remarkable. Thus, as both symptomatic infants demonstrated the best possible outcome, they both exemplified that early detection, treatment and interventions for CT can maximise outcome, even in the severest of cases with poor prognoses expected.

At the time of this report, neurological deficits or sequelae were not identified in any of the asymptomatic infants with or without clinical signs at initial evaluation. Overall academic progress in primary school thus far for the asymptomatic children has remained at high average or above average. No deficits have been identified such as subtle neurological, cognitive or behavioural dysfunction. All children are progressing normally through school, both academically and socially. Some children have accomplished excellent performances academically and in extra-curricular activities also. No asymptomatic child has had to avail of learning support in school.

One child experienced intraocular reactivations, however there was no neurological sequelae. Whilst this child has anxiety disorder (probably related to the psychological traumas of medical events in early childhood), worse afflictions such as visual impairment and associated developmental and learning delay could have resulted if CT and recurrences were not detected and treated early.
Without control groups of untreated asymptomatic children for comparison, conclusions can only be made on longitudinal observation of the studied cohort. To summarise, it has been demonstrated that overall, during the first decade of life, prognosis to date has been excellent for the studied cohort. It is reasonable to state that it is unlikely that the three asymptomatic children lost to follow-up would have skewed the observational data and conclusions.

20.12 Comparison of outcome in the Irish cohort with the Danish cohort
Routine newborn screening for CT commenced in Denmark on January 1st 1999 and ended on July 31st 2007. Preliminary results and conclusions from the Danish screening programme were not available when newborn screening for CT commenced in Ireland on July 1st 2005.

The results of the Danish programme were subsequently the subject of two publications; the initial report detailed results based on the first four years of screening (Schmidt et al., 2006a) and the second and final report summarised the total nine year experience (Roser et al., 2010). Infants were treated for three months, and whilst follow up was to continue for 12 years, the published reports only displayed data on outcome after three years. The first report concluded that: 1) neonatal screening of DBS was feasible for diagnosing CT at birth in low endemic areas and 2) the rate of retinochoroiditis with macular lesions was higher at 3 years of age than at birth.

In the final paper that was published in 2010, based on a recruited cohort of 100 children in nine years of screening, the conclusions were that screening for CT: 1) did not prevent development of retinochoroidal lesions in children with and without previously detected lesions; 2) did not benefit children with few or no symptoms at birth; 3) had no impact on development and 4) generated high levels of anxiety in affected families. The overall conclusion was that CT was an unlikely candidate for screening in Denmark and different results might be obtained in regions with a higher prevalence or different strains of T. gondii.

Compared with the Danish cohort, a better ocular outcome was observed in the Irish cohort, where despite a higher incidence of ocular lesions at initial evaluation, the rate of reactivation was less with no increase in macular lesions or visual impairment at median 9-year follow up. In addition, despite the smaller number in
the Irish cohort due to the shorter duration of screening, it can be concluded that
the children in the Irish cohort had a better neurodevelopmental outcome than
those in the Danish cohort, given that there was a higher incidence of severe and
non-severe signs in the Irish cohort at initial evaluation (chapter 12 table 12.4).
The Danish researchers identified two areas that may have accounted for the
unfavourable outcomes in their cohort identified by screening. Firstly, the Danish
study group acknowledged that the 3-month pyrimethamine/sulfadiazine treatment
regimen may not have been sufficient to prevent ocular recurrences and
neurodevelopmental sequelae. A 3-month treatment regimen was employed, as
literature supporting and advocating a 12-month regimen were primarily based on
historic data at the time. Due to the controversial topic of treatment duration for
CT, the Danish toxoplasma study group decided on a 3-month regimen at the
point of programme initiation in 1999. Subsequently literature recommending 12
months of antiprotozoal treatment became available during the study period but
the treatment duration was not altered for the population in Denmark. Given all the
historic and recent observational data, a 3-month duration of treatment for CT is
possibly not adequate to prevent sequelae. The Danish study group
acknowledged this issue.

Secondly, the Danish study group retrospectively identified that there was lack of a
thorough and uniform follow-up programme for children with suspected and
confirmed CT and their parents. High levels of stress were found in parents and
families of infected children. This, together with treatment adverse effects greatly
affected compliance. During the first half of the screening programme, it became
evident that at the point of evaluation at three years, more than half the cohort
were lost to follow-up. The researchers acknowledged that it became apparent
during the programme that a thorough follow-up programme was necessary for
parents, with strategies to reassure and ensure compliance. This had not been
catered for at the outset.

Therein lies the two major differences in comparison with the CT screening
programme in Ireland. A standard 12-month antiprotozoal regimen was employed
in Ireland. Whilst the rate of adverse events was high, and 64% of treated children
required antiprotozoal treatment alterations for neutropenic events, parents were
provided with support to ensure maximum compliance during the treatment
period. A key member of the CT steering committee was the programme co-
ordinator. This person was the point of contact and liaison with not only all service providers involved, but also for parents of infected children for the duration of the programme and follow-up period. This provided and ensured uniform follow-up for all children identified by screening and proved to be essential for compliance in some cases, even at the early stages of the programme at the point of serological confirmation. It became evident from the outset that compliance issues were prominent for a minority of parents who required continued encouragement and support with acceptance of the diagnosis and management protocol. In hindsight, it is reasonable to conclude that overall compliance with the study in Ireland may have been worse than that encountered had there not been a designated person to remain in regular communication with parents throughout to reinforce the importance of adherence to recommendations and clinical follow-up. Thus the Irish study compared with the Danish study demonstrated the importance of aftercare to maximise outcome in screening for CT.

A major strength of the Irish study is the duration of follow-up, with particular reference to ophthalmology, which is on-going, co-ordinated by a single investigator thus ensuring robust and accurate data collection. A major limitation of this study is the small number of infected infants. However the cohort represented the clinical spectrum of CT, from complete absence of signs to an infant with the most severe clinical presentation manifested by the classic triad. All infants that remained for follow-up demonstrated good or better than expected outcomes.

**20.13 Report to DoHC**

The effect of screening should be evaluated at some point during the programme or following completion if screening is for a fixed time period. An estimate of the disease burden, magnitude of the health problem along with the effect of treatment, side effects, compliance and psychological aspects of screening should then be assessed. The economic costs should be assessed with availability of health resources. We did not employ a statistician to formally provide the financial cost or budget impact analysis of the programme. However we estimated that the cost would be the same as for Denmark, where the screening programme was estimated at €600,000 per year.

Towards the end of the 24-month pilot screening period in Ireland, in the last week of June 2007, a full report with the results thus far was dispatched to the Chief
Medical Officer (CMO) of the DoHC which included a request to fund an extension of the pilot period, from July 1st 2007 for a further three years, in order to acquire a recruitment period of five years and thus a larger cohort of infected infants from which conclusions could be drawn. The request was rejected; lack of funding was the primary reason stated. The CMO advised that a follow-up report should be submitted in 2009 to provide the department with data and conclusions on clinical outcome of the children at median three years. This was submitted to the DoHC in the latter half of 2009. The final feedback from the DoHC was that following consideration and analysis of the matter, it was not cost effective to employ screening for a further pilot period of a longer duration, as the disease burden was low and the magnitude of the health problem ranked below other diseases with higher morbidity and mortality that required urgent resources and funding at the time.

Thus, whilst the DoHC acknowledged that the study clearly demonstrated the importance of early detection of CT and the benefits to those recruited and treated, any available funding from the DoHC had to be allocated to other areas in the health sector deemed to be more of a priority at that time, given that the country was experiencing a recession.

### 20.14 Summary of the pilot programme of newborn screening for CT in Ireland

Twenty-four months of newborn screening for CT in Ireland that utilised a 2-step screening protocol detected an incidence of 1 per 10,000 in a population of low maternal seroprevalence (25%). A higher rate of false positivity (1.3 per 10,000) than true positivity (1 per 10,000) was detected. A diversity of confirmatory serology was encountered in a total of 34 screen positive cases, some required serial testing in addition to adjunctive methods for definitive serological diagnosis. For the 15 women with congenitally infected infants, the majority had seroconversion estimated in trimester 3 and lacked awareness of risk factors, with ingestion of undercooked or cured meat identified as the likely risk factor. In the cohort of infants with CT, 87% were asymptomatic; 13% were symptomatic; 20% had severe signs of CT; overall 40% of infants in the cohort had signs of CT in the newborn period.
Thirteen infants completed 12 months of combination antiprotozoal therapy. Treatment adverse events were common, all treated infants experienced reversible neutropenic events that were predominantly low grade, however compliance was upheld by the majority of those treated.

The rate of intra-ocular recurrence in the cohort was low (7%). A favourable ocular outcome was demonstrated for the cohort. One child had unilateral visual impairment at first assessment, bilateral impairment was not found in the cohort. At final assessment prior to this report, there was no increase in the number of children with retinochoroidal lesions, macular scars or visual impairment. A favourable neurodevelopmental outcome was demonstrated for all children at median 9-year follow-up. One symptomatic child demonstrated superior intelligence at age 10 years, one other symptomatic child had residual fine motor deficits at age 8 years that did not affect quality of life. All asymptomatic children had normal neurodevelopmental outcomes. Since cessation of the screening programme, only symptomatic cases of CT have been detected and reported to the HPSC, at a rate of approximately one per year.

20.15 Benefits vs harms of CT screening
In the studied cohort, the negative effects of screening were mainly generation of parental anxiety and adverse effects of antiprotozoal treatment in infancy. The positive effects of screening were the benefits gained from early detection and treatment, with subsequent follow up evaluations to promptly detect ocular recurrences. A favourable ocular and developmental outcome was demonstrated for the cohort.

Overall it can be concluded that screening did not have a negative psychological effect on parents and families. Parental anxiety was generated primarily during the diagnostic and treatment aspects of the programme, however this occurred in a minimum. High levels of anxiety where applicable were alleviated by provision of thorough follow-up and a named contact for communication.

For the 19 infants who had false positive screens, serologic confirmation of negativity was provided by the first sample in 79% of cases. For the 21% of false positives that required serial testing, the diagnosis was confirmed by one repeat sample at age six weeks in 75%. Thus in the group of false positives, parental
anxiety did not dominate. However, when screening for a condition, ideally confirmation with just one test following the screening test is desired to ensure rapid confirmation or exclusion of the condition.

A diagnosis of CT can potentially reduce the parental expectations of the new child even though the risk of severe disease with CT is low in Europe. Despite the initial trauma of accepting a diagnosis in the newborn period, the majority of parents welcomed the fact that their child had been screened and diagnosed, this was particularly the case for asymptomatic infants that were diagnosed with clinical signs. Evidence that the CT screening programme did not outweigh benefits with harms was demonstrated by parents of infected infants who volunteered to campaign for a continued period of screening in Ireland when the proposal for an extended period was rejected. These parents were happy that their child was born during the screening period and received treatment, despite antiprotozoal adverse effects. Most parents were thankful that their child had been detected and were willing to lend their voices for the benefit of future children. This, combined with the favourable clinical outcome of those affected, highlighted the positive effects of the screening programme.

In the absence of an untreated control group and larger numbers in a studied cohort, it is difficult to predict the frequency of prevented disease by screening, or how many children will suffer disability as a consequence of the infection. However, the medical and social costs of supporting a child with blindness or significant neurological disability for life are significantly high enough to warrant prevention of such handicap by implementation of feasible low-cost strategies where possible.

### 20.16 Future research

The CT screening programme highlighted two areas that require further study. Firstly, in areas with a low incidence and disease burden of CT, assays with a high predictive value would be preferable for mass screening of a population, in addition to alternative confirmatory serological methods for infants with atypical profiles. Such methods would benefit countries or regions that need to determine the true incidence of CT on a background of a low estimated incidence in the indigenous population.
Secondly, and of greater importance is the need for alternative less toxic treatment regimens of shorter duration with proven efficacy. In addition, whilst the researchers of this study do not advocate non-treatment of asymptomatic CT, we await future results on the outcome of asymptomatic children where others have taken the brave step of withholding treatment for asymptomatic CT.

Finally, as most human infection with *T. gondii* is sourced from animals, i.e., from ingestion of oocysts or bradyzoites, international researchers have recently reported on the proposal of cat vaccination against *T. gondii* as a strategy to reduce environmental oocyst contamination and thus disease burden in animals and humans (Schluter et al., 2014; Opsteegh et al., 2015). However it is anticipated that this measure may not be available for many years or widely applicable.

**20.17 Conclusions**

Newborn screening for CT in Ireland was feasible by testing of infant DBS on filter paper cards routinely collected for metabolic screening. Toxoplasma IgM was easily recovered from DBS using a 2-step protocol. The incidence of CT in Ireland was 1 per 10,000. The disease burden was low, 87% of infants were asymptomatic, some of who had signs of inactive disease at birth, one child in this group had recurrent chorioretinitis.

A limitation to this study was the small cohort represented by a total of 15 infected infants. However, a favourable ocular and neurodevelopmental outcome was observed in the cohort at median 9-year follow-up. Early diagnosis and treatment benefitted both symptomatic and asymptomatic children. CT is a clinical condition where, in the context of both historical and contemporary knowledge, postnatal treatment for infected infants is considered better than none. The potential benefit accrued relates not only to the initiation of specific anti-toxoplasma treatment, but importantly identifies this vulnerable group of infants who require detailed clinical evaluation and facilitates their enrolment in early intervention services where appropriate. Thus with current knowledge of the potential harms associated with CT; provision of early diagnosis, evaluation, treatment and follow up remains justifiable for infected infants who deserve every opportunity to become productive and valued members of society.
REFERENCES


62. Ferguson W., Mayne P.D., Cafferkey M., and Butler K., 2011. Lack of


71. Freeman K., Tan H.K., Prusa A., Petersen E., Buffolano W., Malm G.,


202. Pinto B., Castagna B., Mattei R., Bruzzi R., Chiumiento L., Cristofani R.,


212. Roizen N., Swisher C.N., Stein M.A., Hopkins J., Boyer K.M., Holfels E., Mets


251. Thalib L., Gras L., Romand S., Prusa A., Bessieres M.H., Petersen E., and


Appendix 1: The CT screening programme consent form and information leaflet for parents

Why is screening for congenital toxoplasmosis necessary?

Toxoplasma infection during pregnancy can affect the unborn baby. 90% of cases of congenital toxoplasmosis have no symptoms at birth and babies appear normal.

Congenital toxoplasmosis can cause varying degrees of visual and developmental handicap. In particular eye disease may not present until childhood or early adult life.

Early detection of cases and specific treatment of infected infants can prevent progressive visual damage and lead to overall health gain.

For further information about Congenital Toxoplasmosis visit:

Website: www.cdc.gov

This leaflet was designed for your information. It is approved by the National Newborn Screening Committee (Toxo), The Children’s University Hospital, Temple St. Dublin 1.
CONGENITAL TOXOPLASMOSIS SCREENING

All babies born in Ireland routinely have blood collected from the heel onto a filter paper card 72-120 hours after birth. This is commonly known as ‘the heel prick test.’ The blood spots on the card are currently tested for five disorders. The cards are analysed in the National Newborn Screening Laboratory, (The Children’s University Hospital Temple Street, Dublin.)

A 2-year programme is currently underway in Ireland to screen for a sixth condition called congenital toxoplasmosis on the filter paper card used for the heel prick test. (Not all infected infants will be detected by this method. Screening will detect 4 out of 5 infected infants.) All infants will be tested for congenital toxoplasmosis unless a parent specifically refuses.

In the event of a positive result parents will be informed immediately and a blood test will then need to be taken from the baby to confirm the screening result.

Further information on congenital toxoplasmosis and management of a confirmed positive case is available separately.

Screening will be carried out for 2 years to determine how much of a problem congenital toxoplasmosis is in Ireland, (e.g. 65,000 babies screened in 1 year, 20 positive cases) and to develop plans to improve outcome of infected babies.

WHAT IS TOXOPLASMOSIS?

Toxoplasmosis is an infection caused by a parasite called Toxoplasma gondii. This parasite can be transmitted to humans by ingestion of Toxoplasma cysts, which can be found in food that may have been contaminated with soil and water containing these cysts. In most healthy people infection produces no symptoms or presents with mild symptoms e.g. tiredness, enlarged glands and sometimes fever.

Toxoplasma infection is only dangerous to humans when the immune system is underdeveloped or not functioning properly e.g. the unborn child or people with immunodeficiency.

CONGENITAL TOXOPLASMOSIS NEWBORN SCREENING CONSENT FORM

All babies born in Ireland are routinely screened for five disorders as part of a national newborn screening programme. This test is usually performed 72-120 hours after birth whereby blood is collected from the baby’s heel onto a card, commonly known as ‘the heel prick test.’

Testing of the newborn for Congenital Toxoplasmosis is currently being included into this routine newborn screening programme for a 2-year period in Ireland.

Testing for Congenital Toxoplasmosis can be done using the same newborn screening card.

I consent [ ]

I do not consent [ ] (please tick as appropriate) for my baby to be tested for Congenital Toxoplasmosis as part of the routine newborn screening. I have received information about Congenital Toxoplasmosis and how it may possibly affect my child.

Baby’s Surname

Baby’s First Name

Date of Birth

Address

Hospital No (if applicable)

(Signature of parent/guardian)

(Date)

(Signature of witness)
Appendix 2: The CT Screening Programme information leaflet for health care professionals

INTRODUCTION

Newborn screening for Congenital Toxoplasmosis (CT) will take place as a pilot study in Ireland over a two year period from July 1st 2005. Cases of CT will be detected by linked testing of newborn screening cards for toxoplasma IgM antibody subject to parental consent. Parents can ‘opt out’ of having their baby tested for CT.

All first samples taken from July 1st 2005 will be tested for CT on the newborn screening card unless a parent specifically refuses.

A basic information leaflet will also be available to parents outlining the Toxoplasma screening. If confirmed positive a detailed information leaflet for parents is available.

Approximately 50% of cases detected by screening will be false positive. All positives detected by the screen are presumptive cases of CT. The initial screening test will then have to be confirmed.

Not all infected cases will be detected by this method. Screening should detect 4 out of 5 infected babies.

An infant found to be positive on the initial IgM screen will have a serum sample taken for immediate testing at the Toxoplasma Reference Laboratory in Swansea.

If a diagnosis of congenital toxoplasmosis is confirmed, the infant will be referred without delay for clinical examination, investigations and to commence specific antiprotozoal treatment. This referral will normally be to Dr. Karina Butler, Paediatric Infectious Diseases Consultant at The Children’s University Hospital (TCUH) Temple Street and Our Lady’s Hospital for Sick Children (OLHSC) Crumlin.

In some instances, referral may be to the Consultant Paediatrician in the area of residence of the infant’s family, who will liaise with Dr. Butler re: protocol for investigation and specific treatment.

Infants will also be referred to a neurologist if indicated. A standard protocol is attached.
DETAILED PROTOCOL

- When a positive case is detected on screening, the screening liaison midwife at the infant’s birth hospital will be informed by the programme co-ordinator, Dr. Wendy Ferguson, (Paediatric ID Registrar at The Rotunda Hospital Dublin, bleep #417) or Dr. Alida Talento (Paediatric ID Registrar at TCUH Temple St. Dublin, bleep #871) when Dr. Ferguson is unavailable.
- The local paediatric team will also be informed.
- The infant’s parents will then be contacted by the screening liaison midwife with this result and arrange for the infant to attend the local paediatric unit for a blood sample (1.3 ml of a clotted serum specimen) to be taken for confirmation as soon as possible. A maternal serum specimen will also need to be taken at the same time.
- These blood samples are to be sent immediately (courier or fast track service) to The Dispatch Laboratory at The Children’s University Hospital, Temple St. Dublin 1.
- Please inform Dr. Ferguson or Dr. Talento when these samples have been taken and sent to TCUH, Temple St.
- From there the samples will be separated and dispatched to The Toxoplasma Reference Laboratory in Swansea, Wales for confirmatory testing.
- If a diagnosis of congenital toxoplasmosis has been confirmed, Dr. Ferguson will liaise with the infant’s local paediatric team and arrangements made for admission to either TCUH Temple St. Dublin, or OLHSC Crumlin, under the care of Dr. Karina Butler.
- The infant will be admitted to hospital initially for up to 4 days to enable full investigation and initiation of treatment.
- The hospital admission will be arranged and co-ordinated by Dr. Wendy Ferguson and the infectious diseases team at the respective hospital.
- The investigations, which are the standard of care in congenital toxoplasmosis, will be put in place.
- Investigations will include CT brain, lumbar puncture, ophthalmologic examination, audiologic examination and neurologic evaluation if indicated.
- Specific antiprotozoal treatment with pyrimethamine and sulphadiazine will be prescribed for a period of one year.

FOLLOW-UP CARE

- Blood monitoring will be necessary during treatment and can be performed at the infant’s local hospital as follows.
- During the first month of treatment with pyrimethamine and sulphadiazine, a full blood count, differential, and platelet count will be performed weekly, with a serum creatinine and urinalysis at the end of the month.
- During subsequent months of treatment, FBC, differential and platelet counts will be repeated monthly.
- For so long as sulphadiazine is used, serum creatinine and urinalysis should be performed every 3 months at a local hospital.
- While on treatment, monthly outpatient reviews at a local hospital should be carried out for general physical examination and developmental assessment. At these times, drug supplies should be inspected to determine compliance, and dosages adjusted for weight.
- The following examinations will usually be performed at either of the 2 paediatric referral centres (OLHSC, TCUH). Ophthalmologic examinations will be necessary at 6-monthly intervals for 2 years or longer. Audiologic testing appropriate for age will be performed at 6-month intervals for the first 2 years in infants born with symptomatic or asymptomatic infection. Infectious diseases outpatient reviews with Dr. Karina Butler will be carried out annually. Referral to a neurologist for follow up will also be made if clinically indicated.
- Thereafter, annual or more frequent review will be arranged as required.

Programme sponsor: The Department of Health and Children
Programme supervisors: Dr. K. Butler (Paediatric Infectious Diseases Consultant) Professor M. Cafferkey (Consultant Microbiologist)
Programme co-ordinators: Dr. Wendy Ferguson (Paediatric Infectious Diseases Registrar, The Rotunda Hospital Dublin) in conjunction with The National Newborn Screening Laboratory
Appendix 3: AutoDELFIA® IgM Screen

B011-101

AutoDELFIA® Neonatal Toxoplasma-Screen

Time-resolved fluoroimmunoassay

Instructions for use. Reagents for 96 assays

Manufactured by:
PerkinElmer Life and Analytical Sciences, Wallac Oy,
Musionkatu 6, Turku, Finland

FOR IN VITRO DIAGNOSTIC USE

CE mark

387
SYMBOLS

**IVD**
For in vitro diagnostic use / Pour usage diagnostique in vitro / Zur in vitro-Diagnostik / Para diagnóstico in vitro / Per uso diagnostico in vitro / Para uso em diagnóstico in vitro / For anvendning vid in vitro-diagnostics / Til in vitro diagnostisk brug

**LOT**
Lot no. / Lot n° / Ch.-Nr. / N° de lote / No. lotto / No. lote / Lot nr. / Lot. nr.

**PN**
Packing no. / N° d’emballage / Pack-Nr. / N° de embalaje / No. confezione / No. de empacotamento / Pack nr. / Pakke nr.

**PROD**
Product no. / Produit n° / Produkt-Nr. / N° de referencia / Prodotto n. / No. do produto / Produkt nr. / Produkt nr.

**96**
Contains reagents for 96 tests / Contient des réactifs pour 96 dosages / Enthält Reagenzien für 96 Bestimmungen / Contiene reagenti per 96 dosaggi / Contém reagentes para 96 determinações / Innehåller reagens för 96 bestämningar / Indeholder reagenser til 96 bestemmelser

**i**
Note: Read the instructions for use / Remarque: Lire le mode d’emploi / Hinweis: Gebrauchsinformationen beachten / Nota: Leer las instrucciones de uso / Nota: Leggere le istruzioni d’uso / Nota: Leia as instruções para uso / OBS: Läs instruktioner för handhavande / Bemærk: Læs forskriften

**Manufacturer** / Fabricant / Hersteller / Fabricante / Produttore / Produtvido por / Tilverkare / Producent
AutoDELFIA® Neonatal Toxoplasma-Screen kit

INTENDED USE

This kit is intended for the determination of IgM antibodies to *Toxoplasma gondii* in blood specimens dried on filter paper as an aid in screening newborns for congenital (neonatal) toxoplasmosis using the 1235 AutoDELFIA® automatic immunoassay system.

SUMMARY AND EXPLANATION OF THE ASSAY

Toxoplasmosis is an infection caused by a single-celled, protozoan parasite called *Toxoplasma gondii*. The parasite is found throughout the world and can infect both humans and animals (especially cats and farm animals). Cats may develop toxoplasmosis after eating infected birds or rodents, and the infection can spread to persons through direct contact with the cat's feces or soils in contact with the feces. Undercooked infected meat is also a significant source of toxoplasma parasite. The infection is rather common, 23% of adolescents and adults in the United States show laboratory evidence of *Toxoplasma* infection (1), but very few have symptoms because the immune system usually keeps the parasite from causing illness. Once someone catches toxoplasmosis, the parasite remains inside one's cells as a latent (inactive) infection permanently. Latent infection with toxoplasmosis is most common in warm, mountainous and humid climates.

The infection may also be acquired congenitally. Congenital toxoplasmosis results from acute primary infection acquired by mother during pregnancy. In the United States today, an estimated 400 to 4000 cases of congenital toxoplasmosis occur each year (2). Unborn children infected in early pregnancy are most likely to suffer severe health effects including blindness, deafness, seizures, and mental retardation, whereas the infants who have acquired infection later in pregnancy quite often appear normal at birth, but develop symptoms months or years later. When symptoms do appear, they vary depending on the child's age and immune system maturity. Most common symptoms of congenital toxoplasmosis are impairment of visual or neurological functions.

If a child is born with congenital toxoplasmosis and remains untreated during infancy, there is almost always some sign of congenital toxoplasmosis by adolescence. Thus it is important to determine congenital infection status as soon as possible to prevent the emergence of eye lesions or to reduce the risk of neurological impairment in infected children. Postnatal treatment usually consists of an alternating regimen of spiramycin and pyrimethamine-sulphadiazine, and the duration of treatment varies widely from one country to another. In general, more clinical evidence of the efficacy of the treatments are warranted (3).

---

DELFIA and AutoDELFIA are registered trademarks of PerkinElmer, Inc.
PRINCIPLES OF THE ASSAY

The AutoDELFIA Neonatal Toxoplasma-Screen assay is a solid phase fluoroimmuno-metric assay designed for the determination of IgM antibodies to *Toxoplasma gondii* in blood specimens dried on filter paper. The assay can be divided into two separate phases, namely the collection of all IgM antibodies, and the detection of *Toxoplasma gondii* specific IgM. In the first phase, the casein buffer elutes IgM antibodies from the controls and test specimens (dried blood spots) and these react with immobilized polyclonal antibodies (rabbit) directed against μ-chain of human IgM. In the second phase, only the *Toxoplasma gondii* specific IgM antibodies are detected by incubating the immobilised IgM molecules with europium-labeled *Toxoplasma gondii* antigen prepare.

Enhancement Solution dissociates europium ions from the labeled antibody into solution where they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence in each well is then measured. The fluorescence of each sample is proportional to the concentration of *Toxoplasma gondii* specific IgM antibodies in the sample (4,5).
**KIT CONTENTS**

Each AutoDELFIA Neonatal Toxoplasma-Screen kit contains reagents for 96 assays.

The expiry date of the unopened kit is stated on the outer label. Store at +2 - +8°C.

Once opened, the kit components are stable for up to 2 weeks when used as described in the section "ASSAY PROCEDURE".

### Reagents

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Shelf life and storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma Calibrators (approx. values)</td>
<td>1 filter paper cassette (Schleicher &amp; Schuell no. 903) containing 3 sets of dried blood spots</td>
<td>Store refrigerated and protected from moisture and light in the original bag. Stable at +2 - +8°C until expiry date stated on the label.</td>
</tr>
<tr>
<td>Negative Calibrator 0 IU/mL</td>
<td></td>
<td>The exact Toxoplasma IgM concentration for the Positive Calibrator is given on the lot specific quality control certificate included in the kit.</td>
</tr>
<tr>
<td>Positive Calibrator 200 IU/mL</td>
<td></td>
<td>The target values for the controls are given on the lot specific quality control certificate included in the kit.</td>
</tr>
<tr>
<td>Toxoplasma Controls (approx. values)</td>
<td>1 filter paper cassette (Schleicher &amp; Schuell no. 903) containing 3 sets of dried blood spots</td>
<td>Store refrigerated and protected from moisture and light in the original bag. Stable at +2 - +8°C until expiry date stated on the label.</td>
</tr>
<tr>
<td>Q1 45 IU/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2 90 IU/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxoplasma Eu tracer (Toxoplasma gondii antigen ~5 μg/mL)</td>
<td>1 vial, lyophilized</td>
<td>+2 - +8°C until expiry date stated on the vial label.</td>
</tr>
</tbody>
</table>

The calibrators and controls have been prepared from human blood derivative with a hematocrit value of 50-55% and calibrated against The 3rd International Standard for Anti-Toxoplasma serum, human, code TOXM (NIBSC, Potters Bar, UK).

The tracer is in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, dextran, and < 0.1% sodium azide as preservative.

**NOTE:** The powder contains sodium azide (<1%) as preservative and it is harmful by inhalation, in contact with skin and if swallowed.
<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage and Expiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein Buffer</td>
<td>1 bottle, 50 mL</td>
<td>+2 - +8°C until expiry date stated on the bottle label.</td>
</tr>
<tr>
<td>Anti-human IgM Microtiteration Strips.</td>
<td>1 plate</td>
<td>+2 - +8°C until expiry date stated on the label.</td>
</tr>
<tr>
<td>Barcode labels for the reagent cassette</td>
<td>1 pcs</td>
<td>Note: barcodes are lot specific.</td>
</tr>
<tr>
<td>Extra barcode labels for the plate</td>
<td>1 pcs</td>
<td>Note: barcodes are lot specific.</td>
</tr>
<tr>
<td>Lot specific quality control certificate</td>
<td>1 pc</td>
<td></td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT**

This AutoDELFIA Neonatal Toxoplasma-Screen kit is for use with the 1235 AutoDELFIA automatic immunoassay system. The system requires the following items, which are available from PerkinElmer Life and Analytical Sciences or its distributors.

1. Wash Concentrate (prod. no. B117-100)
2. Enhancement Solution (prod. no. B118-100)
3. Dilution vessels for reagent dilution (prod. no. 1235-411)
4. Pipette tips (prod. no. 1235-402)
5. Automatic puncher - Wallac DBS Puncher (prod. no. 1296-071) or Wallac MultiPuncher™ 1 (prod. no. 1296-081), or a manual puncher to cut out filter paper disks with a diameter of approximately 3.2 mm (1/8 inch).

**SPECIMEN COLLECTION AND HANDLING**

Blood specimens should be taken directly from a heel prick onto filter paper. The assay has been validated for Schleicher & Schuell no. 903 filter paper. Lipemic (triglycerides ≤ 36 mmol/L) and icteric (bilirubin ≤ 250 mg/L) specimens do not interfere with the assay.

---

1 MultiPuncher is a trademark of PerkinElmer, Inc.
If the specimen is not applied directly onto the filter paper (not the method of choice), do not use EDTA or citrate tubes or capillaries to collect blood, as these anticoagulation reagents will affect the assay by chelating the europium label.

Neonatal screening programs differ from one another in the type of specimen required. In the United States, the recommendation is a blood spot, approximately 12.7 mm (½ inch) in diameter, collected by heel prick and spotted onto filter paper. The specimen collection device must comply with FDA regulations. A method based on dried blood specimens requires skillful collecting, handling and transport of specimens. The collection technique is described in detail in NCCLS document LA4-A4 (G), and the main points are listed below:

- Clean the skin with an alcohol swab and allow to air dry.
- Puncture the infant's heel with a sterile lancet or with a heel incision device to the depth of 1.0–2.0 mm. Puncturing deeper than 2.0 mm on small infants may cause bone damage.
- Wipe away the first drop of blood. Gently touch the filter paper against a large drop of blood and, in one step, allow a sufficient quantity of blood to soak through to completely fill a preprinted circle on the filter paper. Examine both sides of the filter paper to make sure that the blood penetrated and saturated the paper. Excessive milking or squeezing the puncture may cause hemolysis of the specimen or an admixture of tissue fluids with the specimen. Do not layer successive drops of blood in the collection circle (this causes caking).
- Allow the blood specimen to air-dry in a horizontal position for at least 3 hours at ambient temperature (+15–+22°C), not in direct light. Do not heat or stack the specimens during the drying process.
- Be sure that the required information on the specimen collection card has been completed. The minimum necessary information includes:
  - last name (and first, if available), sex, birth date (optional: time of birth), birth weight and age of the infant (indicate if < 24 h), and patient identification number
  - the first and last name of the mother
  - date of specimen collection (optional: time of collection)
  - the name and address of the submitter (optional: birth facility)
  - the name and phone number of the physician (health care provider)
  - the name of the newborn screening program and address
  - each should have a unique serial number and an expiration date
- Before placing the specimens in a container for transport, the dried blood spots on the collection cards should be separated by a physical barrier or rotated 180° from the blood spots on the cards in the stack immediately above and below. The blood spots can also be protected by a fold-over cover attachment or by placing glassine paper between the specimens.
- Follow local postal and transport regulations for specimen packing and transport. Specimens should not be placed in hermetically sealed containers (e.g. plastic or foil bags). If required, sufficient desiccant packages must be included. Humidity and moisture are detrimental to the dried blood spot specimen.
- Transport or mail the specimen to the laboratory within 24 hours after collection, unless otherwise directed by the screening laboratory.

Some screening laboratories may require additional information on the card, e.g. whether the infant was preterm or postdate, and if so, to what degree, whether he or she was or was not a twin, information about feeding and possible antibiotics and if blood transfusion has been performed. Consult local regulations and institutional policies for deviations from the minimum necessary information on the specimen collection device.

Toxoplasma IgM in dried blood spot specimens is known to be stable for at least 5 months at +4°C but for longer storage specimens should be stored in sealed plastic bags at -20°C (7).

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use.

This kit should only be used by adequately trained personnel.

This kit contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, anti-hepatitis C, anti-HIV antibodies, and syphilis and found to be negative/non-reactive. Nevertheless all recommended precautions for the handling of blood derivative should be observed. Please refer to the U.S. Department of Health and Human Services publication "Biosafety in Microbiological and Biomedical Laboratories" or any other local or national regulation.

This kit contains inactivated microbiological material, i.e. Toxoplasma gondii Level 3 antigen prepare. Although the infectious risk is very low, handle the material as if it were infectious.

Handle all patient specimens as potentially infectious.

Reagents contain sodium azide (NaNO₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Disposal of all waste should be in accordance with local regulations.
13905499-2 (en)

PREPARATION OF REAGENTS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reconstituted stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma-Eu tracer solution</td>
<td>2 weeks at -20°C</td>
</tr>
</tbody>
</table>

Add exactly 1.2 mL of distilled water to vial, and mix gently. Allow to stand for about 60 minutes before use.

NOTE: The powder contains sodium azide (<1%) as preservative and it is harmful by inhalation, in contact with skin and if swallowed. The dissolved tracer contains < 0.1% sodium azide and is not considered harmful.

Wash solution for plate processor    | 2 weeks at +2 - +25°C in a sealed container |

Pour the 250 mL of Wash Concentrate into a clean container and dilute by adding 6000 mL of distilled water (1:25) to give a buffered wash solution (pH 7.8).

ASSAY PROCEDURE

Please refer to the AutoDELFIA User manual for details. From the Settings/System/Neoscreening in the AutoDELFIA workstation software select Neoscreening. Samples and reagents must be brought to room temperature (+20 - +25°C) before use.

Calibration

The calibration is based on Toxoplasma Negative (NEG) and Positive (POS) Calibrators provided. Both calibrators should be run in triplicate on each plate. The results are calculated as in the formula:

\[
\text{IU/mL} = \frac{(\text{Sample}_{\text{POS}} - \text{NEG}_{\text{POS}})}{(\text{POS}_{\text{POS}} - \text{NEG}_{\text{POS}})} \times \text{IU/mL value of POS}
\]

Since the IU/mL value of Positive Calibrator may vary from lot to lot, the value should be checked from the lot specific quality control certificate before each run. If the lot has changed, the new value should be edited in the AutoDELFIA workstation software accordingly.

Check the MultiCalc protocol for "11 TOXO". If you change the replicate number for the unknowns please change the protocol accordingly (see the MultiCalc manual for editing the parameters).
ASSAY TYPE IS IFMA
01 DUAL ASSAY = NO
03 MEASURING PARAMETERS = Eu
20 X-AXIS (CONCENTRATION) = LIN
21 Y-AXIS (RESPONSE) = MEAS
22 FITTING ALGORITHM = LININT
26 STANDARD CURVE = NEW
26 STANDARDS ON 2 PLATES = NO

CODING
3 BLANK
3 STD = 200
1 UNKN

(CODING)
(Make sure that the Toxoplasma Positive Calibrator concentration corresponds to that given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)

CUTOFF = 10

(The proposed cut-off value is only preliminary. It is highly recommended that each laboratory establishes its own cut-off value.)

CODING 2
3 BLANK
3 STD = 200
1 UNKN

(See CODING)
(Make sure that the Toxoplasma Positive Calibrator concentration corresponds to that given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)

END

Procedure for a whole kit:

Note that the kit can be run in a maximum of 2 separate runs.

Note that blood spot samples must be punched into the plate separately, either with an automatic or a manual puncher.

The whole assay process can be programmed under the AutoMate button (see AutoDELFIA User manual). The same process can also be accessed through the individual workstation software commands, see below and AutoDELFIA User manual. Text in bold refers to instructions given in the AutoDELFIA workstation software.

1. Punch out calibrators, controls and samples into the wells (filter paper disks with a diameter of approximately 3.2 mm (1/8 inch)).

2. Enter information about samples and controls either from Load List or Create List in the AutoDELFIA workstation software.

3. Make Schedule.
4. Place the reagent cassette barcode sticker on the reagent cassette as shown in point 5 below. Note: the right end should extend beyond the reagent cassette to make it easier to peel the sticker off.

5. Remove the caps from the reagent vials and place the reagents into the reagent cassette as shown in the figure below. Place the black cap on the tracer vial.

1. Casein Buffer
2. Tracer
3. Empty

6. **Load reagents.** Place the reagent cassettes into the reagent rack in the order displayed on the screen.

7. **Load plates** (with the filter paper disks). The plate should be loaded with the barcode side to the plate processor and placed gently onto the plate holder. Check that all wells have a filter paper disk.

8. **Start run.** Make sure that there are enough pipette tips and dilution vessels in the reagent rack as displayed on the screen.

9. **Unload.** Unload plates, reagents and consumables at the end of the run.

10. Discard the used tracer and buffer. **DO NOT USE THEM AGAIN.** Remove the used dilution vessels from the reagent rack and the used pipette tips from the waste tray.

**Procedure for runs with partial plates:**

**Before run:**
- If less than a whole plate is used, remove the needed amount of strips and place them onto a frame, and label it with the enclosed plate barcode as shown in the figure below. If an odd number of strips is run, add one extra strip. This should be a dummy
strip. Note: Open the foil from three sides only and fold it aside leaving the plate-specific information on the package. Return the remaining strips into the package and press the foil cover back on as tightly as possible. Leave the desiccant in the package. Alternatively, store the remaining strips in a resealable plastic bag with the desiccant.

- do not throw away the buffer bottle and tracer vial caps

After run:
- recap the buffer bottle and put it back to +2 - +8°C
- recap the tracer vial and put it back to -20°C

PROCEDURAL NOTES

1. A thorough understanding of this package insert, the 1235 AutoDELFIA instrument manual and the AutoDELFIA workstation software is necessary for successful use of the AutoDELFIA kit. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed on the kit label.

2. Any deviation from the assay procedure may affect the results.

3. The avoidance of europium contamination and resulting high fluorescent background is important. Changing the Enhancement Solution bottle should be done with care, avoiding touching of tubings.

CALCULATION OF RESULTS

The AutoDELFIA system incorporates programs for data reduction, and the results are obtained as printouts of concentrations for samples etc.
Calibration

The Negative and Positive Calibrators should be run on each plate. The POS/NEG ratio generated by MultiCalc should be ≥ 3. If the ratio is < 3, there will be a warning "TEST NOT VALID".

Quality control

Control samples should always be used to assure the day-to-day validity of results. The controls should be run in the same way as the samples. Controls at two different levels are included in the kit. These controls should be run in each assay. Each laboratory should establish their own mean and acceptable range. The established mean should be within ±20 % of the values stated on the quality control certificate. It is recommended that the laboratories establish their own controls at different levels in addition to the controls included in the kit. Sample results should only be reported if control results for the assay meet the laboratory’s established criteria for acceptability (8).

We also recommend participation in external quality assessment schemes.

LIMITATIONS OF THE PROCEDURE

As with any other in vitro screening test, the data obtained using the AutoDELFIA Neonatal Toxoplasma-Screen blood spot immunoassay should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician. Any potentially positive or borderline result has to be confirmed by using another method with established sensitivity and specificity.

Conditions that are known to cause anomalous analytical assay results are:
- sample spot not uniformly saturated with blood
- sample spots punched too close to the edge of the blood spot
- poorly collected and improperly dried specimens
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity
- contamination of blood spot filter paper with maternal material
- the detection of IgM-class antibodies alone might miss subjects with very early intra-uterine infection due to the pre partum decline of Toxoplasma specific IgM antibodies
- very late Toxoplasma infections can be also missed as sufficient antibody response is yet to be evolved.

Please also refer to the section "PROCEDURAL NOTES".

EXPECTED VALUES AND INTERPRETATION OF RESULTS

The measurement of IgM-class antibodies for Toxoplasma from dried blood spots is used as a means of identifying a population of newborns who are at increased risk of having congenital toxoplasmosis. The identification is based on the use of a cut-off value or percentile, which distinguishes between the unaffected and affected populations to maximize both diagnostic sensitivity and specificity.
Proper interpretation of blood test results requires an understanding of the immune response in infants. High levels of immunoglobulin IgM present in the infant indicate congenital infection. The toxoplasma screening is useful in narrowing down the possibility of a congenital infection, but if the result is positive, a definite diagnosis will require additional testing. The mother will also need to be evaluated in order to interpret the newborn’s blood test (9).

Please note that the values mentioned in this section should only be used as a guideline, and each laboratory should establish its own reference range.

Toxoplasma IgM concentrations were measured from 969 neonatal samples with AutoDELFI A Neonatal Toxoplasma-Screen kit. The results ranged from 0 to 23.1 IU/mL in blood.

97.7% of the results were below the analytical sensitivity (6 IU/mL).

99% of the results were below 11.5 IU/mL.

Based on these results, the interpretation of results can be summarized as:

<table>
<thead>
<tr>
<th>Normal</th>
<th>&lt; 11.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline</td>
<td>11.5–23.1</td>
</tr>
<tr>
<td>Toxoplasma IgM positive</td>
<td>&gt; 23.1</td>
</tr>
</tbody>
</table>

Newborns with borderline or positive values should be recalled for confirmatory tests.

Screening sensitivity and specificity

The screening sensitivity and specificity of the AutoDELFI A Neonatal Toxoplasma-Screen kit for congenital toxoplasmosis are shown below:

Definition of congenital toxoplasmosis: physician confirmed
Definition of screening results: the cut-off values described above were used

<table>
<thead>
<tr>
<th>Congenital Toxoplasmosis</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Borderline</td>
<td>0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>964</td>
<td>964</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>989</td>
<td>992</td>
</tr>
</tbody>
</table>

**Screening sensitivity: 100%**

**Screening specificity: 98.5%**

---

2 Study performed at APAE, São Paulo, Brazil, in collaboration with CTN and Nobel RIE laboratories, Brazil.

3 as above
In addition to 3 clinically congenital toxoplasmosis positive specimens, the AutoDELFIA Neonatal Toxoplasma-Screen kit detected 35 specimens (clinical congenital toxoplasmosis negative) as positive/borderline. Of these specimens, 29 were previously screened as positive/borderline with methods routinely used in the study laboratories.

**ANALYTICAL PERFORMANCE CHARACTERISTICS**

**Precision**: The variation of the AutoDELFIA Neonatal Toxoplasma-Screen assay was determined with spiked blood spots in 18 runs with 8 replicates (4 replicates per plate; n = 144). 3 different kit lots and 3 AutoDELFIA systems were used, except for sample 1 which was determined in 11 runs with 1 kit lot and 1 AutoDELFIA system (n = 44). The analysis of variance approach was used to calculate the following variations (based on NCCLS document EP5-A) (10):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total mean value (IU/mL)</th>
<th>Intra-assay variation (% CV)</th>
<th>Inter-assay variation (% CV)</th>
<th>Total variation (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.1</td>
<td>15.2</td>
<td>16.0</td>
<td>22.1</td>
</tr>
<tr>
<td>2</td>
<td>28.5</td>
<td>8.3</td>
<td>15.4</td>
<td>17.5</td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>5.3</td>
<td>12.2</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>106</td>
<td>4.6</td>
<td>12.6</td>
<td>13.4</td>
</tr>
</tbody>
</table>

**Analytical sensitivity**: The analytical sensitivity of the AutoDELFIA Neonatal Toxoplasma-Screen assay is typically better than 6 IU/mL, if the analytical sensitivity is defined as the value which is 2 standard deviations above the mean of the zero standard measurement values (mean value + 2 SD). This was verified using 3 different kit lots, 96 replicates per lot.

**Functional sensitivity**: The functional sensitivity of the AutoDELFIA Neonatal Toxoplasma-Screen assay is typically better than 19 IU/mL, if the functional sensitivity is defined as the value at which total assay variation is <20%.

**Recovery**: Spiked blood spots were prepared by adding varying proportions of the Toxoplasma IgM positive sample (212 IU/mL) to whole blood containing known low Toxoplasma IgM values (n = 4, range 1–25 IU/mL). The prepared samples were tested with two kit lots. Recoveries were in the range of 93–145% with a mean recovery of 107%.

---

* Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.
Interfering substances: AutoDELFIA Neonatal Toxoplasma-Screen assay was evaluated for potential interference with 52 viral antibodies and disease state specimens. Each specimen was spiked with Toxoplasma IgM positive plasma (range 22–50 IU/mL). Sample spiking was carried out prior to preparation of dried blood spot specimens. The interference is presented as mean recovery % and 95% confidence interval in the following table.

<table>
<thead>
<tr>
<th>Disease state or interfering substance</th>
<th>Number of samples</th>
<th>Recovery %</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid 10 g/L</td>
<td>3</td>
<td>99</td>
<td>95–105</td>
</tr>
<tr>
<td>Bilirubin 250 mg/mL</td>
<td>3</td>
<td>106</td>
<td>104–108</td>
</tr>
<tr>
<td>Rheumatoid factor (RF)</td>
<td>5</td>
<td>99</td>
<td>93–104</td>
</tr>
<tr>
<td>Human anti-mouse antibodies (HAMA)</td>
<td>5</td>
<td>100</td>
<td>86–115</td>
</tr>
<tr>
<td>Antinuclear antibodies (ANA)</td>
<td>5</td>
<td>101</td>
<td>95–107</td>
</tr>
<tr>
<td>Syphilis</td>
<td>6</td>
<td>109</td>
<td>95–121</td>
</tr>
<tr>
<td>Varicella–zoster virus (VZV)</td>
<td>5</td>
<td>86</td>
<td>72–99</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>5</td>
<td>111</td>
<td>103–119</td>
</tr>
<tr>
<td>Herpes simplex virus (HSV)</td>
<td>5</td>
<td>89</td>
<td>79–103</td>
</tr>
<tr>
<td>Epstein–Barr virus (EBV)</td>
<td>5</td>
<td>89</td>
<td>84–84</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>5</td>
<td>92</td>
<td>88–97</td>
</tr>
</tbody>
</table>

Cross-reactivity: 13 specimens containing IgM antibodies to Varicella Zoster virus (n = 3), Cytomegalovirus (n = 4), Epstein-Barr virus (n = 2) and Herpes Simplex virus (n = 4) were tested. Each specimen was previously confirmed as Toxoplasma IgM negative / borderline, and none yielded Toxoplasma IgM positive result with AutoDELFIA Neonatal Toxoplasma-Screen.

Linearity: The linearity of the assay was tested by spiking 3 Toxoplasma IgM positive whole blood specimens (range 17–56 IU/mL) with varying proportions of 3 different Toxoplasma IgM low positive specimens (range 1–13 IU/mL). The observed vs. expected Toxoplasma IgM concentrations were determined in 5 different dilutions (6 replicates per dilution). The correlation coefficient (r) for the linearity was 0.98.

Dilution: Five different Toxoplasma IgM positive specimens in the range of 27–202 IU/mL were diluted serially with Toxoplasma IgM negative plasma, and dried blood specimens were prepared. The observed vs. expected Toxoplasma IgM concentrations were determined in 8 different dilutions (6 replicates per dilution). The correlation coefficient (r) for the dilution was 0.99.

8 Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.
9 as above
10 as above
11 as above
Method comparison:\textsuperscript{12} The 8011-101 AutoDELFIA Neonatal Toxoplasma-Screen kit was compared with an established neonatal solid-phase capture enzyme immunoassay with fluorometric detection using both newborn screening blood spot specimens (n = 45) and prepared blood spot specimens (n = 158) in the range of 0–1012 IU/mL blood. The latter blood spot specimens were prepared from 67 different serum specimens (some specimens were spiked with several concentrations). The agreement was found to be:

<table>
<thead>
<tr>
<th>Comparison method</th>
<th>Positive</th>
<th>Borderline</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AutoDELFIA Positive</td>
<td>75</td>
<td>3</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>Borderline</td>
<td>23</td>
<td>3</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>13</td>
<td>67</td>
<td>93</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>19</td>
<td>73</td>
<td>203</td>
</tr>
</tbody>
</table>

Relative specificity: 97% (67/69)
Relative sensitivity: 85% (75/88)
Relative agreement: 90% (142/157)

\textsuperscript{12} "Relative" refers to a direct comparison of AutoDELFIA Neonatal Toxoplasma-Screen kit results to that of a similar assay. No attempt has been made to correlate with disease presence or absence, and no judgement can be made regarding the comparison method's clinical accuracy.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event PerkinElmer Life and Analytical Sciences, Wallac Oy and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

PerkinElmer Life and Analytical Sciences, Wallac Oy, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

\textsuperscript{12} Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.
REFERENCES


Additional literature:


PATENTS

This test system is covered by the following patents:

Europe (Austria, Belgium, Italy, Switzerland, Holland, UK, France): 0064484, 0139675

Federal Republic of Germany: P32722605-08, P3462252.7

Sweden: 0102753-4

USA: 4,665,790, 4,808,541

Last revision January 2005
AutoDELFIA® Neonatal Toxoplasma-Screen kit
Summary of AutoDELFIA Assay Protocol

AutoDELFIA functions are carried out automatically according to a preprogrammed protocol.

**USER**
- Check Positive Calibrator concentration from QC certificate
- Check/fill consumables
- Punch out calibrators, controls and samples into the wells
- Load worklist
- Make schedule
- Load reagents
- Load plates (check that all wells have a disk)
- Start run

**AutoDELFIA**
- Dispense Casein Buffer (100 μL/well)
- Incubate (1 h)
- Measure (PreScan)
- Remove disks
- Wash (x 6)
- Dilute tracer (1 : 20)
- Dispense tracer dilution (100 μL/well)
- Incubate (2 h)
- Wash (x 6)
- Dispense Enhancement Solution (200 μL/well)
- Shake/incubate (11 min.)
- Measure

**REMINDERS:**
- Make sure reagents are in correct reagent cassette positions
- Reagent cassettes and plates should be labeled with appropriate barcodes
- Select Settings/System/Neoscreening
Appendix 4: Toxoplasma ISAGA IgM Assay: (Manufacturer’s leaflet)

### SUMMARY AND EXPLANATION (1-11)

Toxoplasma gondii, an obligate intracellular protozoan parasite, is a very common pathogen in humans. The parasite, whose definitive host is the cat, is disseminated in nature and infects numerous other mammals. Toxoplasmosis is usually benign or asymptomatic in immunocompetent subjects, but can have severe consequences if it occurs in immunodeficient subjects or fetuses. Women who are seropositive before they become pregnant are especially protected from transmitting the infection to their unborn child. Those who are seronegative are at risk of becoming infected during gestation. The possible ensuing fetal transmission is due to the transfusional migration of toxoplasmas during the acute phase of the infection. The frequency and severity of the fetal infection will depend on a number of factors including the virulence of the infecting strain of the parasite, size of the original inoculum, the immune response of the patient and when, during gestation, the mother became infected. The diagnosis of a T. gondii infection is most commonly made by biological examination, specific immunoglobulin detection (IgM and IgG).

The diagnosis of an acute acquired infection during pregnancy is established by demonstration of a seroconversion or by a significant rise in antibody titer in sequential sera assayed concomitantly.

### PRINCIPLE

It consists of a 2-step immunological reaction:
- the human IgM antibodies in the serum sample bind with the anti-human IgM monoclonal antibodies in the strip wells.
- specific toxoplasma IgM are then revealed by the addition of toxoplasma organisms.
- if the reaction is negative, sedimentation of toxoplasma occurs. If the reaction is positive, the toxoplasma are agglutinated in a mat.

This immunoprecipitation technique avoids interference due to rheumatoid factor and IgG/IgM competition known to occur when determining IgM levels.

### CONTENT OF THE KIT - RECONSTITUTION OF REAGENTS (384 wells, i.e., 192 screening tests):

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 x 8 strips with 2 x 8 round base wells presented as microtiter plates</td>
<td>R1</td>
</tr>
<tr>
<td>Toxoplasma antigen: 1 x 2.5 ml</td>
<td>R2</td>
</tr>
<tr>
<td>Diluent: 1 x 20 ml</td>
<td>R3</td>
</tr>
<tr>
<td>Positive serum: 1 x 0.5 ml</td>
<td>R4</td>
</tr>
<tr>
<td>PBS pH 7.2 2 x 1 liter (powder)</td>
<td>R5</td>
</tr>
<tr>
<td>Tween 20 1 x 1 ml</td>
<td>R6</td>
</tr>
<tr>
<td>* This product has been tested and shown to be negative for HBs antigen and antibodies to HIV1, HIV2, and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.</td>
<td></td>
</tr>
</tbody>
</table>

### MATERIAL REQUIRED BUT NOT PROVIDED

- Precision pipettes.
- Self-adhesive sheets.
- Wash bottle for PBS-Tween.
- Wash buffer for PBS.
- Incubator at 35-38°C.
- Reading system (optional): magnifying mirror for microtiter plates.
- Vortex-type mixer.

* SA English - 1
WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- For professional use only.
- This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use reagents after the expiry date.
- Do not use strips if the bag is visibly deteriorated.
- Do not use reconstituted R5 reagent if it is turbid, as this may be a sign of contamination.
- After removal from the refrigerator, allow reagents to come to room temperature before use.
- Do not mix reagents (or disposables) from different lots.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. Any liquid containing sodium azide is disposed of in the plumbing system, drain should be flushed with water to avoid build-up.
- Only remove the required number of strips from the aluminum bag. Store the remaining strips in the bag with the desiccant and seal hermetically using the dip seal.
- Do not use an antigen showing bumps after vigorous shaking and resuspension.
- Avoid vibrations during the demonstration step (see the instructions for use section).
- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- Store the kit at 2-8°C.
- Do not freeze reagents.
- If stored according to the recommended conditions, all components are stable until the expiry date indicated on the label.

SPECIMENS

Specimen type and collection

Serum. Serum samples can be stored for up to 5 days at 2-8°C, if longer storage is required, freeze at -25 ± 5°C. Avoid successive freezing and thawing. As the use of hemolized, lipemic or icteric samples has not been validated, it is recommended to collect a new sample.

INSTRUCTIONS FOR USE AND RESULTS

Remove the R3 reagent from the kit and place it in an upright position, leave to settle before taking a sample from the upper part.

Screening

Label the strips to be used appropriately on the frosted part provided (include 2 wells for each serum sample).

1. Titration of sera

At time of the test, dilute the sera in PBS (R5):
- serum samples 1/100;
- positive serum (R4) 1/10;
- serum samples from mean 1/20.

Dispense 100 μl of diluted R4 in 2 adjacent wells. Dispense each diluted serum sample in the same way. Replace sera by PBS in 2 wells for each batch of tests (antigen controls). Cover the strips with a self-adhesive sheet and incubate at 35-38°C for 2 hours.

2. Washing (wash bottle only)

Empty the wells by inverting the plate over a sink with a collection tray (containing bleach solution). Take care to squeeze the central part of the stand to hold the strips in place. Wash wells once in PBS-Tween avoiding overspilling. Empty immediately. Wash twice for 5 minutes in PBS-Tween, and then wash twice for 5 minutes in PBS only. After each washing step, drain the strips thoroughly on clean filter paper without allowing to dry. An automatic washer must not be used.

3. Demonstration

Dispense antigen (R2) diluted 1/20 in BABS buffer (R3) as follows for each serum sample and antigen control:

- 100 μl in the first well.
- 150 μl in the second well.

Cover the strips with a self-adhesive sheet and incubate overnight at 35-38°C. In a moist chamber.

4. Reading

- either with a reading system,
- or with the naked eye: read wells by placing the strip approximately 50 cm above a suitably illuminated white background. Each serum is tested with increasing concentrations of toxoplasma antigens. If the reaction is negative, sedimentation of the toxoplasma occurs. If the reaction is positive, the toxoplasma are agglutinated in a mat and any antigen in excess of the bound specific IgM forms an equivalent amount of sedimentation.

0 : total sedimentation in a button similar to the antigen control
1+ : large sedimentation button,
2+ : medium-sized sedimentation button,
3+ : very small sedimentation button,
4+ : mat covering the base of the well similar to the positive serum (R4).
Antigen control: total sedimentation of the toxoplasma (button).
Positive serum (R4); agglutination of the toxoplasma in a mat covering the base of the wall.
Serum sample: compare the size of the button with that of the corresponding antigen control and note the result obtained by referring to the scale given above.

5. Results
The ISAGA index of a serum corresponds to the sum of the values obtained for the 2 antigen volumes used.

Examples of ISAGA index calculation:

<table>
<thead>
<tr>
<th>Serum no.</th>
<th>100 μl of antigen</th>
<th>150 μl of antigen</th>
<th>ISAGA INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3+</td>
<td>2+</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>4+</td>
<td>4+</td>
<td>8</td>
</tr>
</tbody>
</table>

Interpretation:
- 0-5+: negative reaction.
- ≥ 6+: perform confirmatory test.

Confirmatory Test
Label the strips appropriately on the frosted part provided (include 2 wells for each serum sample).

1. Incubation of sera:
See point 1 of the Screening section.

2. Washing:
See point 2 of the Screening section.

3. Demonstration
Dispense R2 antigen diluted 1/20 in SAGS buffer (R3) as follows for each serum sample and antigen control:
- 150 μl in the first well,
- 200 μl in the second well.
Cover the strips with a self-adhesive sheet and incubate overnight at 35-39°C, in a moist chamber.

4. Reading
See point 4 of the Screening section.

5. Results
The ISAGA index of a serum corresponds to the sum of the values obtained for the 3 antigen volumes used (100 μl and 150 μl from the screening and 200 μl from the confirmatory test).

Examples of ISAGA index calculation:

<table>
<thead>
<tr>
<th>Serum no.</th>
<th>100 μl of antigen</th>
<th>150 μl of antigen</th>
<th>200 μl of antigen</th>
<th>ISAGA INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>11</td>
</tr>
</tbody>
</table>

RESULTS AND INTERPRETATION
Acquired toxoplasmosis (in adults and children):
ISAGA index:
- 0-5+: negative reaction.
- 6-9+: borderline reaction.
- 9-12+: positive reaction.

The IgM antibodies detected by the ISAGA technique are either residual or due to recent infection. Active infection can be demonstrated by a significant increase in specific IgG antibody titer shown on a subsequent sample (the two samples should be taken 3 weeks apart).

Congenital toxoplasmosis (in neonates):
Even a low ISAGA index (≥ 3) may indicate congenital toxoplasmosis. The test should be repeated and mother/infant antibody levels compared. Interpretation of Toxo-ISAGA assay results should be made taking into account the patient's history and any other tests performed.

As no international standard is available for the determination of anti-toxoplasma IgM, the Toxo-ISAGA reagent is calibrated against collection sera.

QUALITY CONTROL
A positive serum is included in each kit.
The positive serum and the antigen controls must be tested according to the protocol described in the INSTRUCTIONS FOR USE AND RESULTS section (Screening and Confirmation Test) each time a new kit is opened and then for each series of tests to check that the reagent performance has not been altered.
The user must check that the ISAGA index of the positive serum corresponds to the value indicated on the package insert (acceptable values: between 7 and 8 for the screening test and between 10 and 15 for the confirmatory test). If the index value deviates from the expected value, the results cannot be validated and the samples must be tested in a new run.

In the absence of complete sedimentation for the antigen controls, the results cannot be validated and the samples must be tested in a new run.

Note:
It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

PERFORMANCE
Within-run and between-run reproducibility
The ISAGA index of a serum must not vary by more than 2 crosses between the various liters.

Study 1
A study performed using 150 serum samples (including 29 cord blood samples) in comparison with the Remington method gave the following results:

<table>
<thead>
<tr>
<th>Remington test</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo ISAGA</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Specificity: 99.9% (95% confidence interval: 92.7 - 98.7%)
<table>
<thead>
<tr>
<th>Symbole</th>
<th>Signification</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF ou REF</td>
<td>Numéro de référence</td>
</tr>
<tr>
<td>IVD</td>
<td>Pour usage “in vitro”</td>
</tr>
<tr>
<td>Fabriquant</td>
<td></td>
</tr>
<tr>
<td>A conserver entre X - Y°C</td>
<td></td>
</tr>
<tr>
<td>Date de péremption</td>
<td></td>
</tr>
<tr>
<td>LOT</td>
<td>Numéro de lot</td>
</tr>
<tr>
<td>i</td>
<td>Se reporter aux instructions d'utilisation</td>
</tr>
<tr>
<td>÷</td>
<td>Nombre de tests</td>
</tr>
</tbody>
</table>
Appendix 5: Toxoplasma ISAGA IgA Assay: (Manufacturer’s leaflet)

Toxo-ISAGA IgA

The detection of anti-Toxoplasma IgA is also particularly suited to neonatal diagnosis and pediatric monitoring of congenital toxoplasmosis.

PRINCIPLE

It consists of a two-step immunological reaction:
- The human serum IgA in the sample bind with anti-human IgA monoclonal in the strip wells.
- Specific toxoplasma IgA are then revealed by addition of toxoplasma antigens.
- If the reaction is negative, sedimentation of toxoplasma occurs. If the reaction is positive, the toxoplasmas are agglutinated in a mat.

CONTENT OF THE KIT AND RECONSTITUTION OF REAGENTS (99 wells, including 48 screening tests):

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strips</td>
<td>Strips coated with anti-human IgA monoclonal antibodies (mouse)</td>
</tr>
<tr>
<td>Toxoplasma antigen</td>
<td>Suspension of formalin-treated toxoplasma, RH Sabin strain grown in mice (8). Store upright. Shake vigorously before use to ensure homogeneous suspension. At the time of test, dilute 1/10 in albumin saline buffer (BABS) (R3) in a glass bottle (do not store).</td>
</tr>
<tr>
<td>Diluent</td>
<td>Colored albumin saline buffer (BABS) pH 6.95 (1 g/l sodium azide). Ready-to-use. Avoid contamination.</td>
</tr>
<tr>
<td>Positive serum</td>
<td>Human serum * containing anti-Toxoplasma IgA + 1 g/l sodium azide. At time of test, dilute 1/10 in phosphate NaCl buffer (R5) (do not store).</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate NaCl buffer pH 7.2 Make up to 1 liter with distilled water. Stability after reconstitution: 2 months at 2-8°C. Avoid contamination.</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Dropper bottle.</td>
</tr>
</tbody>
</table>

* This product has been tested and shown to be negative for HIV1, HIV2 and HGV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

MATERIAL REQUIRED BUT NOT PROVIDED
- Precision pipettes.
- Self-adhesive sheets.
- Wash bottle for PBS-Tween.
- Wash bottle for PBS.
- Incubator at 35-39°C.
- Reading system (optional): magnifying mirror for microtiter plate.
- Vortex-type mixer.
WARNINGS AND PRECAUTIONS
• For in vitro diagnostic use only.
• For professional use only.
• This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - Latest edition).
• This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
• Do not use reagents after the expiry date.
• Do not use strips if the bag is visibly deteriorated.
• Do not use reconstituted R5 reagent if it is turbid, as this may be a sign of contamination.
• After removal from the refrigerator, allow reagents to come to room temperature before use.
• Do not mix reagents (or disposables) from different lots.
• Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
• Only remove the required number of strips from the aluminum bag. Store the remaining strips in the bag with the desiccant and seal hermetically using the clip seal.
• Do not use an antigen showing lumps after vigorous shaking and resuspension.
• Avoid vibrations during the demonstration step (see the Instructions for use section).
• The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS
• Store the kit at 2-8°C.
• Do not freeze the reagents.
• If stored according to the recommended conditions, all components are stable until the expiry date indicated on the label.

SPECIMENS

Specimen type and collection
Serum
Serum samples can be stored for up to 5 days at 2-8°C, if longer storage is required, freeze at -25 ± 6°C. Avoid successive freezing and thawing.

None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin: 0 to 300 μmol/L),
- lipemia (after spiking samples with lipids: 0 to 30 mg/dl equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 510 μmol/L).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

INSTRUCTIONS FOR USE AND RESULTS
Remove the R3 reagent from the kit and place it in an upright position; leave to settle before taking a sample from the upper part.

Screening
Label the test strips appropriately on the frosted part provided (include 2 wells for each serum sample).

1. Incubation of sera
At the time of the test, dilute the sera in PBS (R5):
• serum samples 1/100,
• positive serum (R4) 1/10.
Dispense 100 μl of diluted R4 in 2 adjacent wells.
Dispense each diluted serum sample in the same way. Replace sera by PBS in 2 wells for each batch of tests (negative controls).
Cover the strips with a self-adhesive sheet and incubate at 36-39°C for 2 hours.

2. Washing (wash bottle only)
Empty the wells by inverting the plate over a sink with a collection tray (containing bleach solution). Take care to squeeze the central part of the stand to hold the strips in place. Wash wells once in PBS-Tween avoiding over-spilling. Emty immediately. Wash twice for 5 minutes in PBS-Tween, and then wash twice for 5 minutes in PBS only. After each washing step, drain the strips thoroughly on clean filter paper without allowing to dry. An automatic washer must not be used.

3. Demonstration
Dispense antigen (R2) diluted 1/10 in BABs buffer (R3) as follows for each serum sample and antigen control:
• 100 μl in the first well,
• 150 μl in the second well.
Cover the strips with a self-adhesive sheet and incubate overnight at 35-39°C, in a moist chamber.
4. Reading
- either with a reading system,
- or with the naked eye: read the wells by placing the strip approximately 50 cm above a suitably lit white background.

Each serum is tested with increasing concentrations of toxoplasma antigens. If the reaction is negative, sedimentation of the toxoplasma occurs. If the reaction is positive, the toxoplasma are agglutinated in a mat and any antigen in excess of the bound specific IgA forms an equivalent amount of sedimentation.

- 0: total sedimentation in a button similar to the antigen control,
- 1+: large sedimentation button,
- 2+: medium-sized sedimentation button,
- 3+: very small sedimentation button,
- 4+: mat covering the base of the well similar to the positive serum (R4).

Antigen control: total sedimentation of the toxoplasma (button). Positive serum (R4): agglutination of the toxoplasma in a mat covering the base of the well.

Serum sample: compare the size of the button with that of the corresponding antigen control and note the result obtained by referring to the scale given above.

5. Results
The ISAGA IgA index of a serum corresponds to the sum of the values obtained for the 2 antigen volumes used.

Examples of ISAGA IgA index calculation:

<table>
<thead>
<tr>
<th>Serum no. 1</th>
<th>100 µl of antigen</th>
<th>150 µl of antigen</th>
<th>ISAGA IgA INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 +</td>
<td>2 +</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Serum no. 2</td>
<td>4 +</td>
<td>4 +</td>
<td>8</td>
</tr>
</tbody>
</table>

Interpretation:
- 0 to 2: negative reaction.
- ≥ 6: perform confirmatory test.

Confirmatory test
Label the test strips appropriately on the frosted part provided (include 2 wells for each serum sample).

1. Incubation of serum:
See point 1 of the Screening section.

2. Washing:
See point 2 of the Screening section.

3. Demonstration:
Dispense R2 antigen diluted 1:10 in RABS buffer (R3) as follows for each serum sample and antigen control:
- 150 µl in the first well,
- 200 µl in the second well.
Cover the strips with a self-adhesive sheet and incubate overnight at 35-38°C in a moist chamber.

4. Reading
See point 4 of the Screening section.

5. Results
The ISAGA IgA index of a serum corresponds to the sum of the values obtained for the 3 antigen volumes used (100 µl and 150 µl from the screening and 200 µl from the confirmatory test).

Examples of ISAGA IgA index calculation:

<table>
<thead>
<tr>
<th>Serum no. 1</th>
<th>100 µl of antigen</th>
<th>150 µl of antigen</th>
<th>200 µl of antigen</th>
<th>ISAGA IgA INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 +</td>
<td>2 +</td>
<td>2 +</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND INTERPRETATION

Congenital toxoplasmosis:
An ISAGA index < 3 is considered to be negative, an index ≥ 6 is considered to be positive and an intermediate index requires confirmation using a serum collected at least 10 days later.

Toxoplasmosis in adults
An ISAGA index < 6 is considered to be negative, an index > 7 is considered to be positive and an intermediate index requires confirmation using a serum collected at least 10 days later.

Interpretation of Toxo-ISAGA IgA assay results should be made taking into account the patient’s history and the results of any other tests performed.

Toxo-ISAGA IgA is metrologically connected with Standard E1l (range 5).

QUALITY CONTROL
A positive serum is included in each kit.
The positive serum and the antigen controls must be tested according to the protocol described in the INSTRUCTIONS FOR USE AND RESULTS section (screening and confirmatory test) each time a new kit is opened and then for each series of tests to check that reagent performance has not been altered.

The user must check that the ISAGA IgA index of the positive serum corresponds to the following values:
- Screening test: 7-8.
- Confirmatory test: 10-12.

If the index value deviates from the expected value, the results cannot be validated and the samples must be tested in a new run.

In the absence of complete sedimentation for the antigen controls, the results cannot be validated and the samples must be tested in a new run.

Note
It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.
PERFORMANCE
Within-run and between-run reproducibility
The ISAGA IgA index of a serum must not vary by more than 2 crosses between the various tiers.

Sensitivity and specificity

Study 1
A study was performed using 218 sera corresponding to 116 infants:
- 35 infants with congenital toxoplasmosis (n = 60 sera),
- 80 infants without congenital toxoplasmosis (n = 158 sera).
Sensitivity was established using sera obtained between birth and the 10th day of life. Thirty-eight of the 35 infants with congenital toxoplasmosis were positive for IgA, i.e. 86.86% sensitivity (95% confidence interval: 71.91 – 95.96%).
Specificity was established using 158 sera from 80 infants without congenital toxoplasmosis, obtained between birth and the 14th month of life. One hundred and forty-one of the 158 samples were negative for IgA, i.e. 89.24% specificity (95% confidence interval: 83.31 – 93.04%).

Study 2
A study was performed retrospectively on 807 patients (pregnant women, immunocompetent and immunocompromised adults, infants) with or without toxoplasmosis. This study revealed the following points:
- In non-immunized patients, detection of high titers of IgA using ISAGA was not observed.
- If low IgM titers are detected using ISAGA, a negative ISAGA IgA result will not enable to distinguish the start of seroconversion from a non-specific response.
- In the case of seroconversion, detection using ISAGA occurs earlier than for IgM. The best detection levels for IgA with ISAGA occur during the second and third month of seroconversion. After the 60th day of seroconversion, detection levels for IgA with ISAGA decrease more significantly than those for IgM with ISAGA.
- In the case of reactivation or reinfection, detection using ISAGA is less frequent for IgM than for IgA.
- In the case of congenital toxoplasmosis, detection using ISAGA proves to be less effective for IgM than for IgA.

LIMITATIONS OF THE METHOD
The sole detection of anti-Toxoplasma IgA can in no case enable the diagnosis of T. gondii infection.

EPIDEMIOLOGY
Toxoplasma gondii is a strict pathogen whose prevalence differs from one country to another or even one region to another. Contamination by T. gondii can vary according to cultural customs and eating habits, resulting in a prevalence ranging from less than 10% in certain regions of Northern Europe to more than 90% in Africa.

WASTE DISPOSAL
Dispose of unused R2 and R4 reagents, and used R1, R2 and R4 reagents following procedures for contaminated products. All other reagents can be disposed of without taking any special precautions.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES
### INDEX OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>US</td>
<td>Catalog number</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>M</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>T</td>
<td>Temperature limitation</td>
</tr>
<tr>
<td>U</td>
<td>Use by</td>
</tr>
<tr>
<td>L</td>
<td>Batch code</td>
</tr>
<tr>
<td>C</td>
<td>Consult Instructions for Use</td>
</tr>
<tr>
<td>D</td>
<td>Contains sufficient for n tests</td>
</tr>
</tbody>
</table>

### WARRANTY

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMÉRIEUX’S LIABILITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMÉRIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.
Appendix 6: Publications and presentations associated with the thesis

Published papers
   **W Ferguson, PD Mayne, B Lennon, K Butler, M Cafferkey**

2. Lack of Awareness of Risk Factors for Primary Toxoplasmosis in Pregnancy.
   **W Ferguson, PD Mayne, M Cafferkey, K Butler**

Published abstracts
1. Pilot Study of Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland.
   *INFECTIO* 2007;11,S1:1-2

2. 2-Year Pilot Programme of National Newborn Screening for Congenital Toxoplasmosis: preliminary results.
   *IMJ* 2007 Oct;100:9

Invited speaker presentations
1. **European Society for Paediatric Infectious Diseases (ESPID)**
   May 2014, Dublin
   Meet the professor session: congenital toxoplasmosis and CMV
   Dr W Ferguson: Congenital Toxoplasmosis
   Professor D Kimberlin (U.S.A): Congenital CMV

2. **Paediatric Infectious Diseases Master class**
   May 2010, The Royal College of Physicians Ireland
   Title: Congenital Toxoplasmosis
3. Annual National Neonatal Nurse Study Day
   January 2008, Croke Park conference centre, Dublin
   Title: Screening Results from 2 years of National Newborn Screening for Congenital Toxoplasmosis in Ireland

4. 3rd International Conference on Infection in the Immunocompromised Child
   December 2007, Cambridge UK
   Title: Controversies in Neonatal Congenital Toxoplasmosis Screening

5. 1st National Symposium on Infection in Pregnancy and the Neonate, Setting Up the Network for Diagnosis and Treatment
   November 2007, The Rotunda Hospital Dublin
   Title: Screening of the Neonate for Congenital Toxoplasmosis

6. Irish Society of Clinical Microbiologists, Autumn Scientific Meeting
   November 2006, Dublin
   Title: Serological Diagnosis of Congenital Toxoplasmosis

Oral presentations

1. Infectious Diseases Society of Ireland – Inaugural meeting
   June 2008, The Royal College of Physicians Ireland
   Title: Screening of the Newborn for Congenital toxoplasmosis in Ireland: Results from a 2-year Programme

2. 3rd International Congress on Congenital Toxoplasmosis
   May 2007, Colombia
   Title: Preliminary Results of Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland

3. Irish Perinatal Society Meeting (IPS)
   March 2007, Belfast
   Title: Implementation of a 2-year pilot programme of National Newborn screening for Congenital Toxoplasmosis in Ireland
**Poster presentations**

1. **3rd International Congress on Congenital Toxoplasmosis (ICOCT)**
   - November 2010, Marseilles France
   - Title: Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland and clinical outcome of infected infants to date

2. **European Academy of Paediatric Societies (EAPS)**
   - October 2010, Copenhagen
   - Poster walk
   - Title: Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland: serological and clinical variety

3. **The American Academy of Paediatrics (AAP), National Conference and Exhibition**
   - October 2010, San Francisco
   - Title: Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland and clinical outcome of infected infants to date

4. **European society for Paediatric Infectious Diseases (ESPID): Prize awarded for best poster**
   - May 2007, Portugal
   - Title: Newborn Screening for Congenital Toxoplasmosis in the Republic of Ireland

**Courses attended**

1. **Toxoplasma and Food**
   - May 2006, Palermo Italy (2 day meeting)

2. **Toxoplasma Serology Study Day**
   - December 2005, Royal College of Pathologists, London (1 day course)