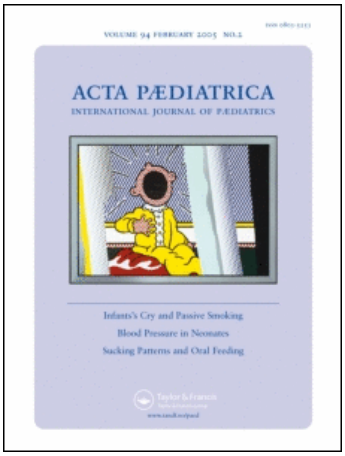

Acta Paediatrica
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618451

Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: A cohort study in 13 European centres

Luuk Gras [a]; Martine Wallon [b]; Arnold Pollak [c]; Mario Cortina-borja [a]; Birgitta Evengard [d]; Michael Hayde [c]; Eskild Petersen [e]; Ruth Gilbert [a]
[a] Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, London, UK [b] Laboratoire de Parasitologie, Hôpital de la Croix Rousse, Lyon, France [c] Department of Paediatrics, Division of Neonatology and Intensive Care, University of Vienna, Vienna, Austria [d] Clinic of Clinical Bacteriology, Karolinska University Hospital Huddinge, Stockholm, Sweden [e] Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

Online Publication Date: 01 December 2005

To cite this Article Gras, Luuk, Wallon, Martine, Pollak, Arnold, Cortina-borja, Mario, Evengard, Birgitta, Hayde, Michael, Petersen, Eskild and Gilbert, Ruth(2005)'Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: A cohort study in 13 European centres',Acta Paediatrica,94:12,1721 — 1731
To link to this Article: DOI: 10.1080/08035250500251999
URL: http://dx.doi.org/10.1080/08035250500251999

Acta Pædiatrica, 2005; 94: 1721–1731

Taylor & Francis
Taylor & Francis Group

Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: A cohort study in 13 European centres

LUUK GRAS[1], MARTINE WALLON[2], ARNOLD POLLAK[3], MARIO CORTINA-BORJA[1], BIRGITTA EVENGARD[4], MICHAEL HAYDE[3], ESKILD PETERSEN[5] & RUTH GILBERT[1], FOR THE EUROPEAN MULTICENTER STUDY ON CONGENITAL TOXOPLASMOSIS*

[1]*Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK,* [2]*Laboratoire de Parasitologie, Hôpital de la Croix Rousse, Lyon, France,* [3]*University of Vienna, Department of Paediatrics, Division of Neonatology and Intensive Care, Vienna, A-1090, Austria,* [4]*Clinic of Clinical Bacteriology, Karolinska University Hospital Huddinge, Stockholm, Sweden, and* [5]*Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark*

Abstract

Aim: To determine the effectiveness of prenatal treatment for clinical manifestations of congenital toxoplasmosis. *Methods:* We prospectively identified 255 live-born infants with congenital toxoplasmosis using prenatal or neonatal screening. We determined the effect of prenatal treatment on the risks of intracranial or ocular lesions in infancy, accounting for gestational age at maternal seroconversion. *Results:* Prenatal treatment within 4 wk of seroconversion reduced the risk of intracranial lesions compared with no treatment (odds ratio, OR 0.28; 95% CI: 0.08–0.75), but there was no significant effect when initiated after 4 wk (OR 0.76; 95% CI: 0.35–1.59; overall *p*-value 0.19). Compared to spiramycin alone, no treatment doubled the risk of intracranial lesions (OR 2.33; 95% CI: 1.04–5.50), but the risk did not differ with pyrimethamine-sulphonamide treatment (overall *p*-value 0.52). There was no consistent relationship between the type or timing of treatment and the risk of ocular lesions. Gestational age at maternal seroconversion was inversely associated with the risk of intracranial but not ocular lesions.

Conclusion: Only early versus no prenatal treatment for intracranial lesions showed a statistically significant benefit. A large randomized controlled trial and/or meta-analysis of individual patient data from cohort studies is required to confirm these findings.

Key Words: *Congenital toxoplasmosis, intracranial lesions, retinochoroiditis, treatment*

Introduction

The aim of prenatal treatment for toxoplasma infection is to prevent neurological or visual impairment by reduction of mother-to-child transmission of infection, or, once fetal infection has occurred, by limiting cell damage caused by the parasite. *Toxoplasma gondii* infects all fetal tissues but is found in proportionately larger numbers in the central nervous system [1,2]. As a result, the most common clinical manifestations, affecting 14 to 17% of infected infants, are retinochoroiditis and intracranial calcification or ventricular dilatation [3,4]. Although information is lacking on the significance of these manifestations for long-term developmental and visual function, most affected children appear to be developmentally normal in early childhood [5–8].

Due to the lack of randomized controlled trials [9], information on the effectiveness of prenatal treatment is limited to retrospective cohort studies that took account of gestational age at maternal infection [4,6,10,11]. The findings are inconsistent and may reflect different analytical approaches or selection bias. Biological studies suggest that the effectiveness of

Correspondence: R. Gilbert, Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK. E-mail: r.gilbert@ich.ucl.ac.uk

*Members listed at end of report.

(Received 18 April 2005; revised 14 June 2005; accepted 11 July 2005)

ISSN 0803-5253 print/ISSN 1651-2227 online © 2005 Taylor & Francis
DOI: 10.1080/08035250500251999

treatment for toxoplasma infection depends partly on the timing of treatment after infection [2]. Although the tachyzoite form of the parasite, which causes inflammation and necrosis, is highly sensitive to antibiotics [12], it rapidly transforms into the latent encysted bradyzoite form, which is impenetrable to antibiotics [1,2,13]. Information is lacking on how fetal immune responses initiate and sustain cyst formation, but if free tachyzoites persist in the fetus there may be a prolonged period when prenatal treatment could reduce parasite damage. The type of drug used may also be important. Initial treatment is usually with spiramycin after diagnosis of maternal infection. However, pyrimethamine-sulphonamide combination treatment is universally prescribed after a positive diagnosis of fetal infection, as maternal-to-fetal drug transfer and penetration of the blood – brain barrier is higher than for spiramycin [12,14].

We report findings from a multicentre prospective cohort study of live-born infants with congenital toxoplasmosis identified by prenatal or neonatal screening. The study examined the association between prenatal treatment and the timing and type of treatment on the risk of intracranial or ocular manifestations of congenital toxoplasmosis detected in infancy.

Methods

Study population

We prospectively studied a cohort of live-born infants with congenital toxoplasmosis identified between 1996 and 2000. Infected infants in 10 centres (Lyon, Paris, Vienna, Reims, Grenoble, Marseille, Nice, Toulouse, Naples and Milan) were identified by prenatal screening for maternal toxoplasma infection. These patients have been included in previous reports [15,16]. In three centres (Stockholm, Poznan and Copenhagen), identification was by universal neonatal screening for congenital toxoplasmosis. Neonatal screening was based on the detection of toxoplasma-specific IgM in Copenhagen [8], specific IgM or IgA in Poznan (both used an in-house ELISA test) [17], and specific IgM and IgG with retrospective testing of stored prenatal blood samples to identify seroconverting women in Stockholm [18]. Prenatal screening methods have been described in detail elsewhere [16].

Women suspected of having acquired toxoplasma infection during pregnancy and infected infants identified by neonatal screening were enrolled prospectively, prior to the collection of follow-up data. To minimize selection bias due to referral of mother – child pairs at higher risk of an affected infant, we excluded cases with non-sequential dates for the detection of maternal infection, and fetal infection or abnormality. In the neonatal centres, we excluded children with non-sequential dates for screening and confirmatory tests. Fetal deaths were excluded, as detection of manifestations by autopsy is not comparable with ophthalmoscopy or cranial ultrasound in infancy.

Congenital toxoplasmosis was diagnosed if specific IgG antibodies were still detectable after 11.5 mo of age. To avoid bias due to exclusion of infants with insufficient data to meet this criterion, we included infants with a probability of congenital toxoplasmosis of at least 90%, based on PCR analysis of amniotic fluid, specific IgM or IgA in the infant, age at last positive IgG result, and/or the weeks of gestation at maternal seroconversion. Further details are reported elsewhere [16].

Treatment

The prenatal testing and treatment schedules have been described in detail elsewhere and are summarized in Table I [16]. In brief, susceptible women were re-tested to detect maternal seroconversion each month in France, three monthly in Austria, and one to three monthly in Italy. None of the women in the neonatal screening centres were treated.

All centres recommended immediate postnatal treatment with pyrimethamine-sulphonamide, with or without alternating cycles of spiramycin, for infants with biological and/or clinical evidence of infection. Postnatal treatment lasted between 3 mo (in Denmark) and 2 y (in some French centres) (Table I).

Clinical manifestations

We used a standard questionnaire for all infected infants to record clinical findings at paediatric examinations in the neonatal period, and at 6 and 12 mo, and at ophthalmoscopy before 4 mo and at 12 mo of age. Cranial ultrasound was performed within the first 4 mo of life. Results for additional examinations, including those based on CT scan or ultrasound scan performed according to local clinical policy or in response to clinical findings, were also recorded to determine whether the initial ultrasound findings were confirmed. No centre routinely performed CT scans on all infected children.

Intracranial lesions were present if intracranial calcification and/or ventricular dilatation were detected at least once by cranial ultrasound after birth, as the number of lesions were too few to analyse these findings separately. Ocular lesions were present if there was one or more retinochoroidal lesion. Due to variable attendance for the 12-mo examination, we accepted manifestations recorded before 16 mo in children who were not examined at 12 mo as representative of clinical findings during infancy.

Analyses

Gestational age at maternal seroconversion. As previous studies [4,6,19,20] have reported a significant inverse

Table I. Treatment protocols and number of infants with congenital toxoplasmosis and clinical manifestations by centre.

	Prenatal treatment					Infants with clinical manifestations	
	Trimester						
	1st and 2nd	3rd	After positive prenatal diagnosis	Postnatal treatment	Infected infants	Retino-choroiditis	Intracranial lesions
France							
Lyon	Spira	P-S	P-S	P-S for 3 wk[a], Spira till >5 kg[b], Fansidar for 12 mo[c]	44	5	4
Paris	Spira	Spira	P-S	P-S for 12 mo[d,e]	64	7	4
Marseille	Spira	Fansidar	Fansidar	*No manifestations*: Fansidar for 12 mo[c] *Manifestations*: Fansidar for 24 mo[c]	20	2	2
Grenoble	Spira	Fansidar	Fansidar	*No manifestations*: Fansidar for 12 mo[c] *Manifestations*: Fansidar for 24 mo[c]	6	1	1
Nice	Spira	P-S	P-S	P-S for 3 wk[d], Fansidar for 24 mo[c]	8	2	2
Toulouse	Spira	Spira	Fansidar	Fansidar/spira for 12 mo[c]	22	1	1
Reims	Spira	Spira	Fansidar	Fansidar for 24 mo[f]	8	1	0
Austria							
Austria	P-S[j]	P-S	P-S	*No manifestations*: P-S/spira for 12 mo[d] *Manifestations*: P-S for 6 mo, P-S/spira for 6 mo[d]	24	2	3
Italy							
Naples	Spira	Spira	P-S	*No manifestations*: P-S/spira for 12 mo[d,g,h] *Manifestations*: P-S for 6 mo, P-S/spira for 6 mo[d,g,h]	11	3	3
Milan	Spira	Spira	P-S	P-S for 12 mo Spiramycin[d]	2	0	0
Sweden							
Stockholm	Nil	Nil	Nil	*No manifestations*: S/spira for 12 mo[d,g,h] *Manifestations*: P-S for 6 mo, P-S/spira for 6 mo[d,g,h]	3	1	1
Poland							
Poznan	Nil	Nil	Nil	*No manifestations*: S/spira for 12 mo[b,d] *Manifestations*: P-S for 6 mo, P-S/spira for 6 mo[b,d]	29	2	6
Denmark							
Copenhagen	Nil	Nil	Nil	P-S for 3 mo[d,h,i]	14	3	3
TOTAL				Total = 51	255	30	30

P-S: pyrimethamine-sulphonamide; Spira: spiramycin; P-S/spiramycin indicates 4–6 cycles alternating with Spiramycin; all prenatal treatment was continued until delivery. Folinic acid was prescribed to all infants receiving P-S or Fansidar.
[a] Pyrimethamine (3 mg/kg every 3 d), sulfadiazine (75 mg/kg/d).
[b] Spiramycin (125 mg/kg/d).
[c] Fansidar consists of pyrimethamine (1.25 mg/kg every 10 d) and sulphadoxine (25 mg/kg every 10 d).
[d] Pyrimethamine (1 mg/kg/d), sulfadiazine (75–100 mg/kg/d).
[e] Pyrimethamine dose reduced to 1 mg/kg/3 d after 3 wk treatment.
[f] Fansidar consists of pyrimethamine (1.25 mg/kg every 15 d) and sulphadoxine (25 mg/kg every 15 d).
[g] Spiramycin (100 mg/kg/d).
[h] Pyrimethamine 2 mg/kg/d for 1–3 d, then reduce to 1 mg/kg/d.
[i] Prednisolone given if intracranial manifestations or active retinochoroiditis.
[j] P-S given after 15 wk of gestation, otherwise spiramycin used.

association between gestational age at seroconversion and the risk of clinical manifestations, we adjusted for gestational age at seroconversion when examining treatment effects. We used a statistical method, previously described, to take into account the fact that maternal IgM seroconversion occurred on an unknown date between the last negative and first positive antibody test (interval censored) [3], or between conception and the first positive test in women who were IgM and IgG positive at the first prenatal test. To take account of the fact that women who were IgG negative at their first positive IgM test (85/207 seroconverters, 41%) would have seroconverted shortly beforehand, we used the function reported in a previous analysis [16] which is described in part 1 of the Appendix.

Infants identified by neonatal screening had no prenatal tests to date maternal seroconversion. However, seroconversion is more likely to have occurred later in pregnancy, as all of them had specific IgM and/or IgA antibodies detected soon after birth, and these antibodies are produced less frequently in infected babies born to mothers infected early in pregnancy [21,22]. To take account of the weeks of gestation at maternal seroconversion in these infants, we derived a function (Appendix, part 2) to reflect the probability of detecting specific IgM in any peripheral blood sample before 2 mo postnatal age according to the type of maternal treatment (spiramycin alone/untreated versus pyrimethamine-sulphonamide) and the week of maternal seroconversion [16]. The function was based

on infants born to women identified by prenatal screening who were tested for IgM status and had congenital toxoplasmosis confirmed at 12 mo. We used this function for women treated with spiramycin or not treated to estimate the distribution of the gestational age at maternal seroconversion for infants identified by neonatal screening.

Effect of prenatal treatment. We determined the effect of the timing and type of prenatal treatment on intracranial and ocular lesions. We considered intracranial and ocular lesions separately in view of biological evidence suggesting that pathogenesis may differ [23], the fact that retinochoroiditis is associated with visual impairment whereas intracranial manifestations may be markers of neurological impairment, and an earlier report that gestation at maternal seroconversion was inversely associated with the risk of intracranial but not ocular lesions [6]. Postnatal treatment was not considered in any of the analyses as lesions detected during infancy were assumed to be congenital and the timing of postnatal treatment may have been related to the presence of lesions.

Timing of treatment. The effect of the timing of treatment, measured as the interval between seroconversion and treatment (subsequently termed treatment delay) [3,6], tested the hypothesis that the earlier treatment is given, the lower the risk of intracranial or ocular manifestations. In the main analysis, treatment delay was categorized as less than 4 wk or more than 4 wk [24], and untreated women were considered as a separate category.

Type of treatment. To determine the effect of the type of prenatal treatment, we compared women treated with spiramycin alone with those first treated with spiramycin and later changed to pyrimethamine-sulphonamide, those given pyrimethamine-sulphonamide as the first treatment, and untreated women. This analysis tested the hypothesis that drugs with better maternal – fetal transfer and penetration of the blood – brain barrier would be more effective.

To test the effect of timing and type of prenatal treatment, we restricted analyses to treated women, and, using treatment delay as a continuous variable, we determined the additional effect of treatment type. We also tested whether the effect of the timing of treatment differed according to gestation at seroconversion by including an interaction term.

Statistical methods. We used a logistic regression model to estimate the effect of treatment variables on the risk of clinical manifestations (part 3 in the Appendix). All analyses took account of the interval censoring of gestational age at maternal seroconversion, and we assumed that the effects of treatment and gestational age at seroconversion were additive on a logistic scale. The effect of other potential confounders (maternal age, parity, lymphadenopathy during pregnancy) was investigated, and none were significant at a level of $p < 0.20$. Fractional polynomials were fitted for continuous variables [25]. Maximum likelihood estimates were obtained numerically using optimization routines from the NAg Fortran Library (NAg Group, Oxford, Mark 19). Further details are given in the Appendix. Approximate 95% confidence limits were derived using profile likelihoods [26].

Results

Study population

A total of 258 live-born infants were identified with congenital toxoplasmosis. Three infants were excluded because it was not clear that they were initially identified by screening. Of the 255 infants included, 46 (18%) were identified by neonatal screening in Poznan ($n = 29$), Copenhagen ($n = 14$), and Stockholm ($n = 3$), and 209/255 by prenatal screening. All of the 209 women identified by prenatal screening seroconverted during pregnancy. Eighty-five of 209 (41%) had a negative IgG test at their first positive IgM test before becoming positive on subsequent IgG tests. They were therefore likely to have seroconverted shortly before the IgM-positive date. Of the remaining seroconverters, 114/209 (55%) had a negative IgM or IgG test after conception, and 10 (5%) had no negative IgG or IgM test during pregnancy and conception was therefore imputed as the last negative test date (six had their first positive test date after 20 wk).

Congenital toxoplasmosis was confirmed by the persistence of specific IgG for more than 11.5 mo of age in 207 infants. All 48 infants with missing data for IgG persistence were classified as infected based on a positive prenatal diagnosis and/or positive specific IgM or IgA, and all except one case were corroborated by their clinician. One hundred and thirty-three of 255 (52%) were boys.

Treatment

There were 78/255 (31%) untreated women, including 15% (32/209) of the women identified by prenatal screening. Spiramycin was the first treatment prescribed to 138/209 (66%) women identified by prenatal screening, of whom 58/138 (42%) subsequently changed to pyrimethamine-sulphonamide. Pyrimethamine-sulphonamide was the first treatment in 39/209 (19%) women (two women were given pyrimethamine-sulphadoxine and the remainder pyrimethamine-sulphadiazine). Ninety-nine of 209

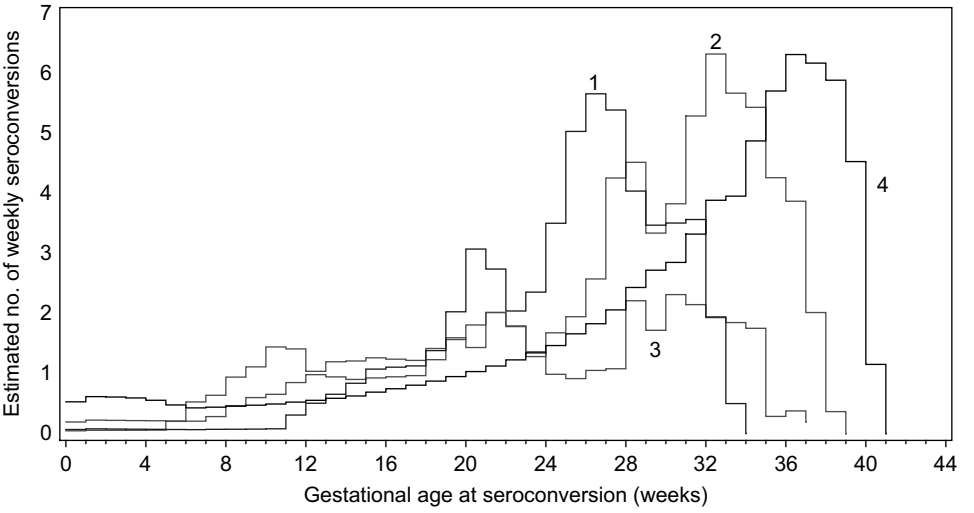

Figure 1. The estimated number of women ($n = 255$) treated with spiramycin alone (solid line, 1), spiramycin changed to pyrimethamine-sulphonamide (dotted line, 2), pyrimethamine alone (dotted line, 3) or not treated (solid line, 4) is plotted against the gestational age at seroconversion (weeks).

(47%) women identified by prenatal screening underwent prenatal diagnosis, and of these, 66 (66%) had a positive PCR result. Pyrimethamine-sulphonamide treatment was prescribed prenatally to all but 6/66 women: five of these six women had amniocentesis performed within 9 d of delivery.

The estimated number of women who seroconverted at each week of gestation according to type of treatment is shown in Figure 1. The gestational age at seroconversion for untreated women is skewed towards the end of pregnancy as 29/32 untreated women identified by prenatal screening had their first positive test within 1 mo of delivery. Treatment was stopped in 6/177 (3%) women due to adverse events (five were receiving pyrimethamine-sulphonamide, and one spiramycin).

Postnatal treatment was prescribed to all but two of the 255 infants. One died aged 1 wk due to severe congenital toxoplasmosis and parents refused treatment for the other. Postnatal treatment was started 3 d after birth (median, IQR 0–24) in infants without clinical manifestations, and 11 d after birth (IQR 1–25) in the remainder. This partly reflects the older age at initiation of postnatal treatment in neonatal screening centres: median age at start of treatment in prenatal centres was 2 d (IQR 0–14, range 0–287), and in neonatal screening centres 26 d (IQR 22–33, range 0–123).

Effects on clinical manifestations

Table I shows the number of infected infants with clinical manifestations in each centre. A total of 51/255 (20%) infants had one or more clinical manifestations, and nine had both intracranial and ocular lesions. Four babies died after birth, three were identified by neonatal screening: one died at 13 mo from respiratory insufficiency due to pulmonary dysplasia (birthweight 1230 g, gestational age 29 wk, and intracranial calcification and neurological impairment were detected); one died at 11 mo due to pneumonia (birthweight 1800 g, gestational age 32 wk; intracranial calcification, ventricular dilatation, and neurological impairment were detected); and one died of staphylococcal sepsis at 3 mo in very poor social circumstances (birthweight 3260 g, gestational age 40 wk; no clinical manifestations of congenital toxoplasmosis detected). Finally, one baby died at 7 d of age with disseminated toxoplasmosis (birthweight 2400 g, gestational age 34 wk; hydrocephalus, cortical atrophy, microphthalmia, retinochoroiditis, convulsions and hepatosplenomegaly were present). In the latter case, seroconversion occurred between weeks 5 and 31 of gestation and spiramycin treatment was given from 32 wk until delivery.

(1) Intracranial lesions. Of 244/255 infants with at least one cranial ultrasound examination after birth, 19 had intracranial calcification, five had ventricular dilatation and six had both detected at least once (total with intracranial lesions 30/244, 12%). All but two were diagnosed within 40 d (the other two cases were diagnosed at 2 and 6 mo).

Intracranial imaging (ultrasound or CT scan) was subsequently performed in 24/30 children, and abnormalities were confirmed in 19/24. The risk of intracranial lesions was similar in boys (16/133, 12%) and girls (14/122, 11.5%).

Effect of prenatal treatment. Table II shows that, compared with no treatment, prenatal treatment before 4 wk of seroconversion significantly reduced the risk of intracranial lesions (odds ratio 0.28;

Table II. Effect of prenatal treatment on intracranial and ocular lesions in infants with congenital toxoplasmosis.

Treatment comparison	Number of women ($n=224$)	Adjusted odds ratios for intracranical lesions (95%CI)	Number of women ($n=225$)	Adjusted odds ratios for intracranical lesions (95%CI)
(1) Timing of treatment	$n=30$ with intracranial lesions		$n=30$ with retinochoroiditis	
No treatment	75	1.0	78	1.0
Any treatment <4 wk[a]	91.4	0.28 (0.08–0.75)	96.2	1.067 (0.54–2.10)
Any treatment after 4 wk[a]	77.6	0.76 (0.36–1.59)	80.8	0.518 (0.21–1.20)
		Overall $p=0.19$		Overall $p=0.502$
(2) Type of treatment		$n=30$		$n=30$
Spiramycin alone	77	1.0	80	1.0
Spiramycin changed to pyrimethamine-sulphonamide	55	1.19 (0.50–2.97)	58	2.314 (1.13–4.86)
Pyrimethamine-sulphonamide throughout pregnancy[b]	37	1.37 (0.55–3.37)	39	0.492 (0.134–1.45)
No treatment	75	2.33 (1.038–5.50)	78	1.675 (0.811–3.561)
		Overall $p=0.52$		Overall $p=0.44$

Note: all analyses are adjusted for gestational age at maternal seroconversion.
[a]Numbers are estimated by summing the probability of seroconversion for all women according to treatment delay.

95%CI: 0.08–0.75), but there was no significant effect for treatment 4 or more weeks after seroconversion (OR 0.76; 95% CI: 0.36–1.59; overall p-value 0.19). The effect of pyrimethamine-sulphonamide, alone or following spiramycin, was similar to spiramycin alone. The risk of intracranial lesions was increased in infants of untreated mothers compared to those treated with spiramycin alone, but the lower confidence limit was close to 1.0 (OR 2.33; 95% CI: 1.04–5.50). Among treated women, there was no significant increase in the risk of intracranial manifestations per additional week of treatment delay (OR 1.06; 95% CI: 0.98–1.16). The inclusion of treatment type did not improve the model ($p=0.94$), and there was no evidence of an interaction between the timing of treatment (measured as a continuous variable) and gestation at maternal seroconversion ($p=0.99$).

Gestational age at maternal seroconversion. Figure 2a shows that later gestational age at seroconversion was associated with a significantly reduced risk of intracranial lesions (odds ratio per week of gestation 0.89; 95% CI: 0.86–0.93). However, the results for early pregnancy reflect an extrapolation of the risk of lesions as few women seroconverted before 10 wk of gestation (shown in Figure 1).

(2) Ocular lesions. All 255 infants had at least one ophthalmoscopy examination. In 169/255 (65%) infants, at least one examination was by indirect ophthalmoscopy. Retinochoroiditis was detected in 30/255 (12%) infants. The median age at diagnosis was 2 mo (minimum 3 d, maximum 16 mo). Of these, 27/30 had at least one further examination and lesions were confirmed in 26/27. The risk of retinochoroiditis was similar in boys and girls (16/133, 12% vs 14/122 11.5%).

Effect of prenatal treatment. Table II shows that there was no evidence that the risk of ocular lesions differed significantly in infants of women treated before or 4 wk after seroconversion compared with those not treated. There was a significant increase in the risk of ocular lesions when treatment was changed from spiramycin to pyrimethamine-sulphonamide, but not for pyrimethamine-sulphonamide alone. Among treated women, there was no significant increase in the risk of ocular lesions per additional week of treatment delay: OR 0.93 (95% CI: 0.83–1.02). Inclusion of treatment type did not improve the model ($p=0.21$).

Gestational age at maternal seroconversion. There was no evidence of an effect of gestational age at seroconversion on the risk of retinochoroiditis (OR 0.97; 95%CI: 0.94–1.01; Figure 2b).

Discussion

We found evidence for a 72% reduction in the odds of intracranial lesions in infants of mothers who were treated within 4 wk of seroconversion compared with infants born to untreated women. As the overall p-value was not significant, this result should be treated with caution and requires confirmation. We could not detect a significant effect of the type of prenatal treatment on the risk of intracranial lesions. There was an increased risk of intracranial lesions in babies of untreated women compared to those treated with spiramycin alone, which was of borderline significance. We found no evidence of a consistent or significant effect of either timing or type of prenatal treatment on the risk of ocular lesions.

This is the largest cohort study of children with congenital toxoplasmosis. Strengths include minimization of selection biases by prospective enrolment

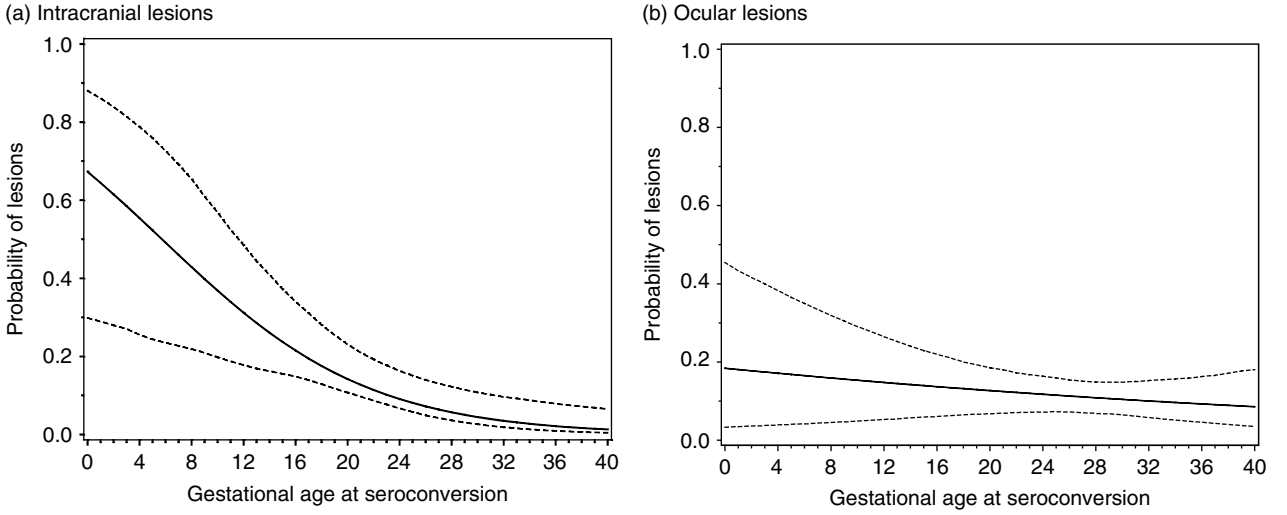

Figure 2. The probability of (a) intracranial manifestations and (b) ocular lesions is plotted according to gestational age at maternal seroconversion (dotted line = 95% confidence intervals).

and data collection, and a high rate of follow-up for cranial ultrasound and ophthalmoscopy. Systematic variations in treatment between centres and individuals were unrelated to outcomes and therefore made it possible to examine associations between prenatal treatment and clinical manifestations. All analyses took into account the effect of gestational age at maternal seroconversion due to its strong association with the risk of intracranial lesions. As the date of maternal seroconversion was not known precisely, we used a statistical method to take into account the possibility of seroconversion at all points between the last negative and first positive IgM test dates. A further strength was that the gestational age at seroconversion for women given alternative treatments or not treated was distributed throughout most of pregnancy. Although untreated women identified by prenatal screening seroconverted in late pregnancy, inclusion of neonatal screening centres allowed comparison with untreated women who seroconverted earlier in pregnancy. As mothers of infants identified by neonatal screening were not tested during pregnancy and had no information on the gestational age at seroconversion, we used a distribution function to estimate the gestational age at maternal seroconversion in IgM-positive infants identified by neonatal screening (Appendix, part 2). This method compares favourably to alternative approaches [27].

A weakness of the study was the variability between clinicians in their ability to detect and interpret abnormalities on intracranial ultrasound and ophthalmoscopy. Provided the quality or number of examinations was not dependent on whether the mother had been treated or not, poor sensitivity (failure to identify infants with clinical manifestations) would not alter the estimate of treatment effect [28]. On the other hand, poor specificity (finding manifestations in unaffected infants) would underestimate the treatment effect. We could not confirm intracranial lesions in 5/24 (21%) children who were re-examined. This may reflect poor specificity of the initial ultrasound examination or spontaneous resolution of lesions [29]. Finally, we cannot exclude the possibility that centre- or region-specific factors influenced the findings. For example, neonatal centres are grouped in northern Europe, whereas prenatal centres are in central and southern Europe. Consequently, the contribution of regional variation possibly due to organism characteristics such as the parasite form (tissue cysts or oocysts), or centre differences in outcome measurement, could not be investigated.

Our findings agree closely with those of a retrospective study by Foulon et al. who reported a 70% reduction in the relative risk of any clinical manifestations in infants born to treated versus untreated women (19/64 infected infants had lesions: ocular (6), intracranial (6), ocular and intracranial (2), intrauterine death (3), and neurological abnormality (2)) [4]. Another cohort study, using similar statistical methods to the present study, reported a significant protective effect of delayed treatment or no treatment compared to treatment within 4 wk of seroconversion on the risk of intracranial lesions found in 18/181 (10%) infected children by 3 y old, but the authors attributed the finding to chance [6]. All previous studies that accounted for gestational age at seroconversion agree with our results that there is no evidence that pyrimethamine-sulphonamide is more effective than spiramycin for clinical manifestations [4,6,10,11]. This finding contrasts with the widely held view that pyrimethamine-sulphonamide is more effective that spiramycin. We explored whether the lack of an association could be explained by pyrimethamine-sulphonamide being given after a longer treatment

delay than spiramycin, but found no evidence of this in analyses restricted to treated women. It is possible that our study and others have failed to detect an association between pyrimethamine-sulphonamide and risk of clinical manifestations as few women ($n=37$) were initially treated with this combination.

One possible explanation for the similarity of effect of spiramycin and pyrimethamine-sulphonamide on intracranial lesions could be that they both reduce the infective parasite burden. This burden, along with individual host resistance, route of infection and parasite strain, has been related to brain and ocular lesions in experimental animal studies [30,31]. If reduction of parasite burden is the mechanism, the timing of treatment might be critical, as conversion from tachyzoite to bradyzoite cyst, which is impenetrable to antibiotics, has been found to take place between 4 and 14 d after infection in immune-competent experimental animals [1]. However, why treatment should have an effect on brain but not ocular lesions remains puzzling as experimental and human fetal studies of ocular toxoplasmosis have found that severe lesions are associated with a locally increased burden of free tachyzoite [31,32]. We were unable to explore the effect of prenatal treatment on long-term development of retinochoroiditis. However, a recent cohort study with follow-up until adolescence found no evidence of an effect of timing or type of treatment on the occurrence of ocular lesions in childhood. These results should be viewed with caution as the study was not designed to answer this question [20]. Nevertheless, these long-term results highlight the fact that, of infected children with ocular lesions by 6 y of age, only one-third had lesions detected in infancy.

The effect of prenatal treatment on clinical manifestations in infancy is just one of the factors that policy-makers need to consider when deciding about prenatal screening. We have previously reported no evidence of an effect of the timing or type of prenatal treatment on mother-to-child transmission of toxoplasma infection [16], but were unable to examine the effect of treatment on fetal loss (abortion or stillbirth) [15]. However, we have found no evidence that the rate of fetal loss in toxoplasma-infected women is higher than expected [33].

Although several authors have reported an inverse association between clinical manifestations and gestational age at maternal seroconversion [4,19], only one study has examined this effect separately for ocular lesions, and, like us, found no association. This finding, together with the lack of evidence of an effect of treatment on ocular lesions [10], supports the hypothesis that the pathogeneses of intracranial and ocular lesions differ [23]. These results, and possible reasons for varying findings between studies of the effects of prenatal treatment, will be further examined in an individual patient data meta-analysis which is currently in progress [34]. Studies on pharmacokinetics of treatment in pregnancy are also required to determine optimal dosing schedules.

Implications

The benefit of early versus no treatment on the risk of intracranial lesions has been estimated to be maximal for women who seroconvert at 28 wk of gestation, when the combined risk of mother-to-child transmission and clinical manifestations in an infected child are greatest [3]. At this gestational age, the risk of mother-to-child transmission is 48% [16], the risk of intracranial lesions identified by ultrasound examination in untreated women is 12.43%, and for women treated within 4 wk, 3.55% (risk difference 8.88%; 95% CI: 0.08–19.79). The number of women who seroconvert at 28 wk of pregnancy that need to be treated within 4 wk of seroconversion compared to those not treated to prevent one live-born child with intracranial lesions is 24 (95% CI: 11–2604) ($8.88\% \times 48\% = 4.3\%$). The number needed to treat would be higher for seroconverters at all other weeks of gestation. Only an estimated 47% of women in the French centres received treatment within 4 wk of seroconversion. More women would be treated earlier, but at greater cost, if testing for seroconversion were performed more frequently than monthly.

A further consideration is the uncertain prognostic significance of intracranial lesions. Most children appear to be neurologically normal in early childhood [3,5,7,35], but information is lacking on the long-term risk of impairment. Investigation of the association between intracranial lesions and functional deficit at 3 and 4 y of age is reported for the EMSCOT cohort elsewhere [36].

Finally, the consistent lack of evidence for a beneficial effect of pyrimethamine-sulphonamide treatment compared with spiramycin [4,6] highlights the need for controlled trials to compare different strategies such as pyrimethamine-sulphonamide versus spiramycin (or other macrolides), and no treatment.

Acknowledgements

We are grateful to David Dunn for help with the study design and implementation, and the development of the statistical approach. We are grateful to Rodolphe Thiebaut, Genevieve Chene and Gwendoline Poizat who carried out an independent statistical analysis of the EMSCOT. The project was funded by the European Commission (BIOMED II No. BMH4-CT98-3927 and QLG5-CT-2000-00846). Contributions to this work were made by the "Verein unser kind, Verein zur Durchfuehrung der wissenschaftlichen Forschung auf dem Gebiet der Neonatologie und Kinderintensivmedizin" in Vienna. The project complied with the research ethics requirements in each participating country. As the study was based on routinely collected data which was anonymized prior to central collation, no patient consent was required by the respective ethics bodies nor was it sought.

References

[1] Denkers EY, Gazzinelli RT. Regulation and function of T-cell-mediated immunity during Toxoplasma gondii infection. Clin Microbiol Rev 1998;11:569–88.

[2] Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington JS, Klein J, editors. Infectious diseases of the fetus and newborn infant. Philadelphia: WB Saunders; 2001. p. 205–346.

[3] Dunn D, Wallon M, Peyron F, Petersen E, Peckham CS, Gilbert RE. Mother to child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet 1999;353:1829–33.

[4] Foulon W, Villena I, Stray-Pedersen B, Decoster A, Lappalainen M, Pinon J-M, et al. Treatment of toxoplasmosis during pregnancy: a multicentre study of impact on fetal transmission and children's sequelae at age 1 year. Am J Obstet Gynecol 1999;180:410–5.

[5] Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, Stechenberg B, et al. Neonatal serologic screening and early treatment for congenital Toxoplasma gondii infection. The New England Regional Toxoplasma Working Group [see comments]. N Engl J Med 1994;330:1858–63.

[6] Gras L, Gilbert RE, Ades AE, Dunn DT. Effect of prenatal treatment on the risk of intracranial and ocular lesions in children with congenital toxoplasmosis. Int J Epidemiol 2001;30:1309–30.

[7] Berrebi A, Kobuch WE, Bessieres MH, Bloom MC, Rolland M, Sarramon MF, et al. Termination of pregnancy for maternal toxoplasmosis. Lancet 1994;344:36–9.

[8] McCabe RE. Antitoxoplasma chemotherapy. In: Joynson DHM, Wreghitt TG, editors. Toxoplasmosis: a comprehensive clinical guide. Cambridge: Cambridge University Press; 2001. p. 319–59.

[9] Peyron F, Wallon M, Liou C, Garner P. Treatments for toxoplasmosis in pregnancy (Cochrane Review). Chichester, UK: John Wiley & Sons, Ltd.; 2003.

[10] Gilbert R, Dunn D, Wallon M, Hayde M, Prusa A, Lebech M, et al. Ecological comparison of the risks of mother-to-child transmission and clinical manifestations of congenital toxoplasmosis according to prenatal treatment protocol. Epidemiol Infect 2001;127:113–20.

[11] Couvreur J, Thulliez P, Daffos F, Aufrant C, Bompard Y, Gesquiere A, et al. In utero treatment of toxoplasmic fetopathy with the combination pyrimethamine-sulfadiazine. Fetal Diagn Ther 1993;8:45–50.

[12] Derouin F. Drugs effective against Toxoplasma gondii. Present status and future perspective. In: Amboise-Thomas P, Petersen E, editors. Congenital toxoplasmosis. Scientific background, clinical management and control. Paris: Springer Verlag; 2000. p. 95–110.

[13] Luder CG, Giraldo-Velasquez M, Sendtner M, Gross U. Toxoplasma gondii in primary rat CNS cells: differential contribution of neurons, astrocytes, and microglial cells for the intracerebral development and stage differentiation. Exp Parasitol 1999;93:23–32.

[14] Trenque T, Marx C, Quereux C, Leroux B, Dupouy D, Dorangeon PH, et al. Human maternofoetal distribution of pyrimethamine-sulphadoxine. Br J Clin Pharmacol 1998; 45:179–80.

[15] Freeman K, Oakley L, Pollak A, Buffolano W, Petersen E, Semprini AE, et al. Congenital toxoplasmosis and preterm birth, low birth weight, and small for gestational age birth. BJOG 2004;112:31–7.

[16] EMSCOT. Effect of timing and type of treatment on the risk of mother to child transmission of Toxoplasma gondii. Br J Obstet Gynaecol 2003;110:112–20.

[17] Paul M, Petersen E, Pawlowski ZS, Szczapa J. Neonatal screening for congenital toxoplasmosis in the Poznan region of Poland by analysis of Toxoplasma gondii-specific IgM antibodies eluted from filter paper blood spots. Pediatr Infect Dis J 2000;19:30–6.

[18] Evengard B, Petterson K, Engman M-L, Wiklund S, Ivarsson SA, Tear-Fahnehjelm K, et al. Low incidence of toxoplasma infection during pregnancy and in newborns in Sweden. Epidemiol Infect 2001;127:121–7.

[19] Desmonts G, Couvreur J. [Congenital toxoplasmosis. Prospective study of the outcome of pregnancy in 542 women with toxoplasmosis acquired during pregnancy.] Ann Pediatr Paris 1984;31:805–9.

[20] Binquet C, Wallon M, Quantin C, Kodjikian L, Garweg J, Fleury J, et al. Prognostic factors for the long-term development of ocular lesions in 327 children with congenital toxoplasmosis. Epidemiol Infect 2003;131:1157–68.

[21] Naessens A, Jenum PA, Pollak A, Decoster A, Lappalainen M, Villena I, et al. Diagnosis of congenital toxoplasmosis in the neonatal period: a multicenter evaluation. J Pediatr 1999; 135:714–9.

[22] Ecochard R, Landrivon G, Colin C, Creac'h P, Wallon M, Gandilhon F, et al. [Decision analysis of congenital toxoplasmosis in the absence of exact knowledge of the treatment benefits and secondary effects.] Rev Epidemiol Sante Publique 1996;44:145–54.

[23] Roberts F, McLeod R. Pathogenesis of toxoplasmic retinochoroiditis. Parasitol Today 1999;15:51–7.

[24] Gilbert RE, Gras L, Wallon M, Peyron F, Ades AE, Dunn D. Effect of prenatal treatment on mother to child transmission of Toxoplasma gondii: a cohort study of 554 mother-child pairs in Lyon, France. Int J Epidemiol 2001; 30:1303–8.

[25] Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964–74.

[26] Sprott DA. Maximum likelihood estimation. In: Sprott DA, editor. Statistical inference in science. New York: Springer; 2000. p. 163–96.

[27] Gomez G, Espinal A, Lagakos W. Inference for a linear regression model with an interval-censored covariate. Stat Med 2003;22:409–25.

[28] Rothman K, Greenland S. Precision and validity of studies. In: Rothman K, Greenland S, editors. Modern epidemiology. Philadelphia: Lippincott-Raven; 1998. p. 115–34.

[29] Patel DV, Holfels EM, Vogel NP, Boyer KM, Mets MB, Swisher CN, et al. Resolution of intracranial calcifications in infants with treated congenital toxoplasmosis. Radiology 1996;199:433–40.

[30] Gormley PD, Pavesio CE, Luthert P, Lightman S. Retinochoroiditis is induced by oral administration of Toxoplasma gondii cysts in the hamster model. Exp Eye Res 1999;68: 657–61.

[31] Freyre A, Falcon J, Correa O, Mendez J, Gonzalez M, Venzal JM, et al. Cyst burden in the brains of Wistar rats fed Toxoplasma oocysts. Parasitol Res 2003;89:342–4.

[32] Roberts F, Mets MB, Ferguson DJ, O'Grady R, O'Grady C, Thulliez P, et al. Histopathological features of ocular toxoplasmosis in the fetus and infant. Arch Ophthalmol 2001;119:51–8.

[33] Thalib L, Gras L, Romand S, Prusa A, Bessieres MH, Petersen E, et al. Prediction of congenital toxoplasmosis by polymerase chain reaction analysis of amniotic fluid. BJOG 2005;112:567–74.

[34] Thiebaut R, Gilbert RE, Gras L, Chene G. Timing and type of prenatal treatment for congenital toxoplasmosis (Protocol for a Cochrane Review). The Cochrane Library, Chichester, UK: John Wiley & Son, Ltd.; 2003.

[35] Lebech M, Andersen O, Christensen NC, Hertel J, Nielsen HE, Peitersen B, et al. Feasibility of neonatal screening for

toxoplasma infection in the absence of prenatal treatment. Lancet 1999;353:1834–7.

[36] Freeman K, Salt A, Prusa A, Malm G, Ferret N, Buffolano W, et al. Association between congenital toxoplasmosis and parent-reported developmental outcomes, concerns, and impairments in 3 year old children. Biomed Central Pediatrics 2005; in press.

Appendix

The likelihood function consists of three parts corresponding to the estimation of gestational age at seroconversion (GASC) based on: 1) maternal IgM and IgG test results; and 2) the child's IgM status. The third part models the effect of GASC and treatment variables on clinical manifestations in infancy. The equations are as follows:

(1) To estimate the probability distribution of gestational age at IgM seroconversion for women who were IgG negative and IgM positive at their first prenatal test, we defined a function F, which can be written as:

$$F_j(u) = 1 - \frac{1}{1 + (0.824(RR_j - u))^{2.549}}$$

where u is GASC, and RR_j is the gestational age at the first IgM-positive test (measured in weeks from the last menstrual period).

The function was based on 415 women in a cohort from Lyon, who were observed to seroconvert for both IgM and IgG [3]. Analyses used methods for doubly censored data. The same methods were applied to the EMSCOT and the results were similar.

(2) To determine the probability distribution of GASC for mothers of babies identified by neonatal screening for toxoplasma-specific IgM, we derived a logistic function with a linear predictor consisting of three parameters corresponding to an intercept, a quadratic effect for GASC in weeks, and a two-level factor for class of treatment: pyrimethamine-sulphonomide vs spiramycin or no treatment. The function was derived from women who seroconverted during pregnancy and were identified by prenatal screening, provided their baby had at least one test for toxoplasma-specific IgM in the first 2 mo of life. The interval censoring of GASC was taken into account in the same way as reported in this paper. For the j-th subject it can be written as:

$$O_j(u) = P[\text{IgM at birth}] = 1 - \frac{1}{1 + \exp(-2.12 + 0.0038\,u^2 - 1.09PS_j)}$$

where u is GASC in weeks, and PS_j is 0 for women treated with pyrimethamine-sulphonomide and 1 otherwise.

(3) To determine the effect of GASC and treatment variables on clinical manifestations in infancy we derived a logistic function $L_j\,(g(u), X;\beta)$ based on a linear predictor for covariates $g(u)$, $X = X_1, \ldots, X_p$ with parameters $\beta = \beta_0, \beta_1, \ldots, \beta_p)$ to model the presence of brain or eye lesions based on an intercept, GASC and the treatment variables. The function $g(u)$ represents the effect of GASC on the probability of clinical manifestations and was a linear function for all the analyses presented.The likelihood function is:

$$\begin{aligned} Lik(\beta) = \sum_{j=1}^{N} \sum_{u=LL_j+1}^{RR_j} \{ & ((F_j(u))^{IgG_j} \\ & \times (1 - F_j(u))^{1-IgG_j})^{Prenatal_j} \\ & \times ((O_j(u))^{IgMB_j} \\ & \times (1 - O_j(u))^{1-IgMB_j})^{IgMBP_j} \\ & \times (L_j(g(u), X;\beta))^{CM_j} \\ & \times (1 - L_j(g(u), X;\beta))^{1-CM_j} \} \end{aligned}$$

where β is a vector of parameters for the linear predictor modelling probability of lesions; N is the number of subjects in the study; u is GASC; g is a function of GASC; X is a vector of covariates; LL_j is GA at the last negative IgM test for subject j; RR_j is GA at the first positive IgM test for subject j; IgG_j is 1 for women who were IgM+ and IgG− at the first prenatal test, and 0 otherwise; $Prenatal_j$ is 1 if the mother was screened prenatally and 0 otherwise; $IgMB_j$ is 1 if the child was IgM positive after birth and 0 otherwise; $IgMBP_j$ is 1 if the child was tested for IgM within 2 mo of birth and 0 otherwise; CM_j is 1 if there were any clinical manifestations detected during infancy for child j and 0 otherwise.

Members of the European Multicenter Study on Congenital Toxoplasmosis (EMSCOT)

Coordinating Committee: M.-H. Bessieres, W. Buffolano, H. Dumon, R. Gilbert, E. Petersen (chairperson), A. Pollak, P. Thulliez, M. Wallon.

Writing Committee: Luuk Gras, Martine Wallon, Arnold Pollak, Mario Cortina-Borja, Birgitte Evengard, Michael Hayde, Eskild Petersen, Ruth E. Gilbert (principal investigator).

Clinical centres contributing data (number of patients contributed to this report): P. Thulliez, S. Romand (64; Institut de Puericulture, Paris), M. Wallon, F. Peyron (44; Hôpital de la Croix Rousse, Lyon), M. Paul (29; University Medical Sciences, Poznan), A. Prusa, M. Hayde, A. Pollak (24; University

Children's Hospital, Vienna), M.-H. Bessieres (22; Hôpital de Rangueil, Toulouse), J. Franck, H. Dumon, P. Bastien, E. Issert (20; Hôpital de la Timone, Marseille; CHU de Montpellier), E. Petersen, D. Remmer Schmidt (14; Statenseruminstitut, Copenhagen), W. Buffolano (11; Universita di Napoli, Naples), C. Chemla (8; Hôpital Maison Blanche, Reims), N. Ferret, P. Marty (8; Hôpital de l'Archet, Nice), H. Pelloux, H. Fricker-Hidalgo, C. Bost-Bru (6; Centre Hospitalier Universitaire de Grenoble), B. Evengard, G. Malm (3; Huddinge Hospital, Stockholm), E. Semprini, V. Savasi (2; Milan).

Study design and coordination: R. Gilbert (principal investigator), L. Gras, J. Rickett, A. Salt, L. Valenti, Hooi Kuan Tan (Institute of Child Health, London).

Statistical analysis: L. Gras, M. Cortina-Borja, K. Freeman (Institute of Child Health, London).