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# **Individual patient data meta-analysis of prenatal treatment effect for congenital toxoplasmosis.**

The SYROCOT (Systematic Review on Congenital Toxoplasmosis) Study Group\*

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## **Abstract**

**Background.** Despite three decades of prenatal screening for congenital toxoplasmosis in some European countries, uncertainty remains about the effectiveness of prenatal anti-toxoplasma treatment.

**Methods.** We conducted a systematic review of cohort studies based on universal screening for congenital toxoplasmosis. We did a meta-analysis using individual patient data to examine the effect of the timing and type of prenatal treatment on mother to child transmission of infection and clinical manifestations before one year of age. Analyses were adjusted for gestational age at maternal seroconversion and other covariates.

**Findings.** We included 25 cohorts in the review. In 1438 treated mothers identified by prenatal screening, we found weak evidence that treatment started within 3 weeks of seroconversion reduced mother to child transmission compared with treatment started after 8 or more weeks (Odds ratio [OR] 0.48,  $p=0.05$ ). Among 550 infected infants identified by prenatal or neonatal screening, we found no evidence that prenatal treatment significantly reduced the risk of clinical manifestations in infected live born infants (OR for treated vs not treated 1.11, 95% Confidence interval [CI]: 0.61, 2.02). Increasing gestational age at seroconversion was strongly associated with an increased risk of mother-to-child transmission (OR 1.15, 95%CI: 1.12, 1.17) and a decreased risk of intracranial lesions (OR 0.91, 95%CI: 0.87, 0.95), but not with eye lesions (OR 0.97, 95%CI: 0.93, 1.00).

**Interpretation.** Further evidence from observational studies is unlikely to change these results and would not reduce biases due to confounding. Only a large randomized controlled clinical trial would provide clinicians and their patients with valid evidence of the potential benefit of prenatal treatment.

## Introduction

*Toxoplasma gondii* is a common parasitic infection acquired by ingesting oocysts excreted by cats and contaminating soil or water, or by eating tissue cysts which remain viable in undercooked meat of infected animals <sup>1,2</sup>. Mother to child transmission of the parasite occurs only when infection is acquired for the first time during pregnancy. The risk of transmission rises steeply with gestational age at maternal infection <sup>3</sup>. Overall, about one third of infected mothers give birth to an infant with congenital toxoplasmosis <sup>3,4</sup>. Most children with congenital toxoplasmosis are developmentally normal <sup>5</sup> but up to four per cent die or have evidence of permanent neurological damage or bilateral visual impairment during the first years of age <sup>6,7</sup>.

Toxoplasma infection in pregnancy is usually asymptomatic. Consequently, infected mothers can only be detected by serological testing. Prenatal testing for toxoplasmosis is routinely offered in many European countries in order to treat infected mothers with antibiotics to reduce the risk of mother to child transmission and, if fetal infection has occurred, to reduce impairment in the child <sup>8</sup>. There is no consensus about the most effective screening strategy or the best type of treatment. Uncertainty about the benefits of prenatal treatment <sup>9</sup> and concerns about adverse treatment effects and the infrastructure and costs required to implement prenatal screening have led to diverse policies including no screening, neonatal screening <sup>6,10,11</sup> and prenatal screening with monthly or 3-monthly re-testing schedules <sup>4,8,12,13</sup>. In those countries where prenatal screening applies, recommendations for treatment may differ. In most centers, including those in France, spiramycin is prescribed immediately after diagnosis of maternal infection and changed to a pyrimethamine-sulphonamide combination if fetal infection is diagnosed or if infection is acquired in late

pregnancy<sup>4</sup>. In contrast, in Austria, mothers are initially treated with pyrimethamine-sulphonamide (after 15 weeks of gestation), and changed to spiramycin if fetal diagnosis is negative<sup>4</sup>.

So far, two systematic reviews have evaluated the effect of prenatal treatment on mother to child transmission<sup>9,14</sup>. No randomized controlled trials were found and we know of no subsequent trial. Meta-analysis of the effect of prenatal treatment was not possible in these reviews due to differences in the analytic methods and the way aggregate data were presented. However, new observational data have been published since then: three analyses of retrospective cohort studies<sup>12,13,15,16</sup> and the results of a large prospective, multicentre cohort study<sup>4,7</sup>. None of these studies reported a significant effect of treatment on mother to child transmission but none could exclude clinically important effects. The findings for the effect of prenatal treatment on the risk of clinical manifestations of congenital toxoplasmosis (intracranial and ocular lesions) have been inconsistent<sup>7,12,13,16</sup>.

Our aim was to estimate the effects of timing and different type of prenatal treatment on the risk of congenital toxoplasmosis and its clinical manifestations during infancy, based on a systematic review using individual patient data to undertake a meta-analysis.

## **Methods**

### ***Study selection***

Any cohort study of women identified during pregnancy by universal screening for *T.gondii* infection was eligible for inclusion provided the following data were recorded. For the analysis of mother to child transmission: sample dates for the last negative and first positive specific antibody tests; date prenatal treatment was started; date of birth or last menstrual period; and congenital infection status based on specific antibody tests beyond 11 months postnatal age. Exact dates for testing and treatment were required to reduce inaccuracy in the measurement of the timing of treatment and the gestational age at maternal seroconversion. For analysis of clinical manifestations in infancy, we included studies meeting the above criteria and those based on neonatal screening for congenital toxoplasmosis provided at least one ophthalmoscopy or intracranial imaging examination was recorded during the first year of life. We excluded studies of mothers enrolled before 1985 as diagnosis using IgM was widely used only after this time.

### ***Searches and study selection***

We searched MEDLINE®, EMBASE® and PASCAL® from 1980 to 2002 (figure 1) and updated searches using Current Contents® (based on Medline®) in November 2005 (full details of searches are reported elsewhere <sup>17</sup>). We also searched reference lists of identified papers and contacted researchers in the field with the results of the initial searches to ask for any additional studies. There was no language restriction. Two reviewers (RT and RG) independently scanned abstracts for potentially eligible studies, and selected studies using a checklist of inclusion criteria. Datasets were requested and individual patient data were examined by four reviewers (RT, SL, SDC, RG) before inclusion was confirmed.

## ***Analyses***

We performed separate meta-analyses of the effect of prenatal treatment on mother-to-child transmission and on clinical manifestations in infancy. This was because there were differences in the cohorts and pregnancies that could be included in each analysis. To avoid selection bias due to referred cases, we excluded mothers that had prenatal diagnosis or initiation of treatment before documented seroconversion.

### ***Mother-to-child transmission***

#### *Study population*

Analyses of the effect of prenatal treatment on mother-to-child transmission were confined to mothers who seroconverted during pregnancy and who were identified by prenatal screening. Neonatal screening cohorts with retrospective testing were excluded because the diagnosis of maternal infection is less specific than in prenatal cohorts<sup>18</sup>. Primary analyses were further restricted to treated mothers because delayed detection of untreated mothers, who were clustered in late pregnancy, introduced potential selection bias.

#### *Treatment exposure*

We compared the risk of transmission according to the time interval (categorized by quartiles) between seroconversion and the initiation of any type of prenatal treatment. We also examined the effect of the type of this first treatment (spiramycin alone or pyrimethamine-sulphonamide combination [P-S]). Patients who changed from spiramycin to pyrimethamine-sulfadiazine after prenatal diagnosis of fetal infection were considered as treated with spiramycin. We did not analyse specific dosages or types of pyrimethamine and sulphonamide treatment due to lack of power.

### *Outcome*

Congenital infection status was based on serologic tests for IgG and IgM antibodies in live-born infants. The presence of congenital infection was defined by the persistence of specific IgG antibodies beyond 11 months of age. The absence of congenital infection was defined by undetectable IgG after 2 months of age in the absence of anti-toxoplasma treatment<sup>18</sup>. In the case of stillbirth or termination of pregnancy, congenital infection status was positive given a PCR test of amniotic fluid result or any detection of the parasite in fetal tissues, and negative if all tests were negative.

### ***Clinical manifestations in children***

#### *Study population*

We confined the analyses to European cohorts of live born children with congenital toxoplasmosis identified by prenatal or neonatal screening. Studies from South America were excluded because ocular disease is more frequent and more severe than in Europe<sup>19</sup>. In addition, North and South American studies (mainly based on neonatal screening) were excluded because they used computed tomography (CT) scan to screen for intracranial lesions. This method is more sensitive than cranial ultrasound (US) scan<sup>20,21</sup>.

When analysing the risk of intracranial lesions, patients who presented with ocular lesions alone were excluded (and vice versa when ocular lesions was the outcome) to avoid bias in favour of no effect.

#### *Treatment exposure*

We compared the effect of timing and type of prenatal treatment strategies, defined as: no treatment, spiramycin alone started within 5 weeks, or 5 or more weeks after



seroconversion, pyrimethamine-Sulphonamides and spiramycin followed by pyrimethamine-Sulphonamides. The 5-week threshold was based on the median treatment delay.

### *Outcome*

Clinical manifestations were defined as ocular (retinochoroiditis or microphthalmia) and/or intracranial lesions (intracranial calcification or ventricular dilation) detected by cranial ultrasound screening during the first year of life. Infants that had no ophthalmic examination or cranial ultrasound scan were excluded from analyses for that specific outcome. Other (rare) clinical manifestations were not systematically assessed or reported and were not taken into account in analyses.

### ***Statistical analyses***

The gestational age at seroconversion was considered in all the analyses. This variable is defined by the dates of last negative and first positive tests and is therefore interval-censored. Other factors considered in the analysis were the latitude of the centre (representing the geographical variation of the epidemiology of *Toxoplasma gondii*) and the period of the study distinguishing studies predating PCR for prenatal diagnosis (<1991), studies based on the same standard prospective data collection form as used in the EMSCOT studies<sup>4,7</sup> (>1994), and studies in the interim period (1991-1994). We did not examine the effect of maternal age or gender of the child as these data were absent from most databases. We examined whether the treatment effect was modified according to explanatory variables using the Likelihood ratio test for interaction between variables.

We used logistic models. The addition of a random effect, to allow a differential baseline risk (of transmission or clinical manifestation) according to centre, did not improve

the model likelihood. Results are therefore presented using fixed effect logistic regression. Model parameters were estimated using an integrated maximum likelihood method to take into account the interval censoring of the gestational age at seroconversion and the timing of treatment initiation (further details given in the appendix)<sup>7,22</sup>. Consequently, the uncertainty relating to these variables was included in all estimations of model parameters. We used information from mothers who were IgG negative at the first positive IgM test, and from the child's postnatal serology (presence of IgM, IgG titre) to modify the probability of seroconversion at each possible date between the last negative and first positive date<sup>7</sup>. In sensitivity analyses, the results were robust to each of the functions used to estimate the gestational age at seroconversion (data not shown). Women with no IgG negative test date during pregnancy were excluded from the analyses of mother to child transmission but included in the analyses of clinical manifestations by assuming that the last IgG negative test occurred at conception.

## **Results**

### ***Systematic literature search and included studies***

We found no randomized controlled trials. 26 observational cohorts were included in the review comprising a total of 1745 infected mothers and 691 infected live born infants. Three cohorts from the same study<sup>12</sup> had relevant data but declined to participate (96 mothers, 43 infected infants). Investigators for 4 further studies<sup>23-26</sup> accounting for 288 mothers and 49 infected infants did not respond (Figure 1). Studies of prenatal screening varied from a monthly to three-monthly re-testing schedule for susceptible mothers (Table 1). Details of the prenatal treatment regimens are published elsewhere<sup>4,7,12,13,15</sup>. The crude risk of mother to child transmission shown in Table 1 varies between cohorts due mainly to differences in gestational age at maternal seroconversion. Among the 1745 infected mothers, the risk of fetal death, whether due to therapeutic termination (n=22) or stillbirth (n=13), was very low (2%).

Four cohorts from outside Europe (2 in Brazil, 1 in Colombia, and 1 in Massachusetts; N=141 infected infants), mainly based on neonatal screening, were excluded. The crude risk of ocular lesions diagnosed the first year of life was much higher in the South American cohorts (47%; 18/38) than in Europe (14%; 79/550), and intermediate in the Massachusetts cohort (27%; 28/103). The crude risk of intracranial lesions detected by CT scan was much higher in the cohorts from North (19%, 19/103) and South America (53%, 20/38) than those from Europe (9%, 49/550), where cranial ultrasound was used.

### ***Mother-to-child transmission***

Overall, there were 1721 infected mothers with 506 infected fetus/children from 20 cohorts. 24 women (and one infected child) were excluded because they started prenatal treatment before the date of positive serology (potential referred cases). The estimation of

maternal-fetal transmission rate according to gestational age at seroconversion was 15% (95% CI 13; 17) at 13 weeks, 44% (95% CI 40; 47) at 26 weeks and 71% (95% CI 66; 76) at 36 weeks. The odds of transmission increased by 12% (95% CI 10; 14) per week of maternal gestation at seroconversion (Figure 2). This estimation was similar when excluding mothers from neonatal screening with retrospective testing.

The primary analysis was based on 1438 infected mothers who were treated during pregnancy (from 18 prenatal screening cohorts): 398 of their fetuses/children were infected. The sooner prenatal treatment was started after seroconversion, the lower the adjusted odds of mother-to-child transmission (Odds ratio [OR] 0.94 per week, 95% CI 0.90, 0.98). Compared with mothers treated after 8 weeks of seroconversion (upper quartile of delay from seroconversion), mothers treated earlier tended to have a lower odds of mother to child transmission, particularly if prenatal treatment was initiated within 3 weeks after seroconversion (OR=0.48, CI=0.28; 0.80, Table 2). There was no significant effect of the type of prenatal treatment (Table 2). The effect of the timing and type of treatment was not modified according to gestational age at seroconversion (test for interaction:  $p=0.54$  and  $p=0.61$ , respectively). The effects of the period when the study started was not significant ( $p=0.14$ ). The odds of transmission decreased significantly with higher latitude (OR=0.71 for 5° higher, 95% CI [0.53; 0.96],  $p=0.03$ ).

### ***Clinical manifestations in infected infants***

This study was initially based on 26 cohorts based on prenatal or neonatal screening. Overall, in 691 infected infants, 13% developed intracranial lesions during the first year of life, 18% ocular lesions, and 24% had at least one of both types of lesions (Table 1). When restricting the analysis to the sample of 550 infected infants from Europe, 105 (19%) infants presented with at least one type of clinical manifestation, 79 (14%) had ocular lesions, and 49

(9%) had intracranial lesions. The odds of clinical manifestations during infancy decreased with older gestational age at seroconversion (OR=0.96, p=0.01; Table 3). However, when the type of lesion was considered, we found a marked reduction in the odds of intracranial lesions with gestational age at seroconversion (p<0.0001), whereas the decline in odds with ocular lesions was less significant (p=0.04) (Figures 3 a and b).

The adjusted odds of any clinical manifestations in infants of treated mothers compared with untreated mothers was not significantly different (OR=1.11, p=0.74). We further analysed the treatment effect by distinguishing the type and the timing of treatment initiation (Table 3). We found no evidence of reduced odds of clinical manifestations in infants born to mothers treated with spiramycin throughout pregnancy or with P-S alone, compared with untreated mothers (all confidence intervals including 1). However, infants born to mothers treated with spiramycin followed by P-S had a higher odds of any clinical manifestations compared with those treated with P-S alone (OR=1.29, CI=1.42; 9.34). The effect of prenatal treatment was not modified by gestational age at seroconversion (test for interaction, p=0.56). An intent-to-treat analysis of prenatal treatment (patients switching their treatment being considered in the spiramycin group) did not show any significant effect of treatment (p=0.39). Compared to the untreated group, there was no significant difference in the odds of clinical manifestations in infants of mothers treated with spiramycin within 5 weeks of seroconversion (OR=1.18, 95%CI=0.58; 2.40), after 5 or more weeks after seroconversion (OR=1.22, 0.63; 2.38), or those treated by P-S (OR=0.63, 0.25; 1.61). The odds of clinical manifestations was not significantly different according to the period of the study (p=0.08) or the latitude of the centre (OR=1.31 for 5° higher, p=0.13).

## **Discussion**

### *Summary of the results*

We found weak evidence for an increased risk of mother-to-child transmission the later prenatal treatment was started after maternal seroconversion. This result may reflect a true protective effect of early treatment or confounding due to selective treatment of mothers at high risk of fetal infection whose infection was diagnosed late (ie. outside the standard monthly or 3 monthly re-testing schedule). We found no evidence that prenatal treatment significantly reduced the risk of clinical manifestations in infected live-born infants. Gestational age at seroconversion was strongly associated with mother-to-child transmission and with the risk of intracranial lesions but marginally with eye lesions.

### *Strengths and limitations*

To our knowledge, this is the first meta-analysis of the effect of prenatal treatment for congenital toxoplasmosis. Almost all eligible cohorts were included but three cohorts with appropriate data, representing 96 infected mothers (43 infected children), declined to participate. Four further cohorts were unlikely to have been eligible because of selection bias and enrolment before 1985<sup>23-26</sup>. Analysis of individual patient data made it possible to examine the effect of systematic differences in treatment schedules within and between cohorts and we used a statistical method to minimize bias and reflect uncertainty due to the interval censored variables (gestational age at seroconversion and timing of prenatal treatment).

The main limitation is that our results for prenatal treatment may be partly explained by biases in the way the cohort studies were designed and conducted. Although we adjusted for the strong confounding effects of gestational age at seroconversion, we can not exclude

effects due to unmeasured confounders.<sup>27</sup> In the analysis of mother to child transmission, we included only prenatal screening cohorts as all used multiple tests on repeated samples to confirm maternal infection. We excluded neonatal screening cohorts as retrospective testing of a single stored prenatal sample could result in mislabelling of uninfected women as infected, thereby reducing the risk of transmission in untreated women. We further restricted the primary analysis of prenatal screened cohorts to treated women because of potential biases causing untreated women to have a lower risk of mother to child transmission than treated women who seroconverted at the same gestational age. One possible explanation for this finding is that women were less likely to be treated after a long delay from seroconversion and shortly before delivery, unless there were signs of fetal infection or complications. Such indication bias could have increased the risk of transmission in women treated after a long delay and could partly explain the weak association between early treatment and the risk of mother to child transmission.

There were several sources of potential bias in the analyses of clinical manifestations, which included neonatal and prenatal screening cohorts. Firstly, although the criteria for congenital infection were similar across all cohorts, neonatal screening is less sensitive than prenatal screening. Insensitivity is associated with the gestational age at maternal seroconversion and, as it is most marked in the first half of pregnancy when intracranial lesions are more likely, could reduce the observed effect of prenatal treatment<sup>28</sup>. We minimised this problem by adjusting all analyses for gestational age at seroconversion. A further source of bias is the large uncertainty in the estimated gestational age at seroconversion in neonatal screened untreated cohorts compared with prenatal screened cohorts. Whether such error would bias in favour of under- or overestimating the treatment effect is difficult to predict. Thirdly, bias could have been introduced if the accuracy of cranial

ultrasound or ophthalmic examinations differed between neonatal and prenatal screened cohorts. This seems unlikely as it is standard practice in European centres to perform a single cranial ultrasound in early infancy (repeated if abnormalities are detected) and to perform at least two ophthalmic assessments, one in early infancy and the other at one year. Prenatal centres stated that their protocol was to examine children 3 to 6 monthly, while neonatal centres reported 3 monthly examinations. We could not verify these practices as most datasets did not record each examination. Fourthly, indication bias is likely to explain the apparently harmful effect of changing treatment from spiramycin to pyrimethamine-sulphonamide compared with no treatment. This finding may be because clinicians performed prenatal diagnosis or changed treatment more readily if they detected fetal or maternal complications. Such a response would also overestimate the benefits of treatment for mothers who remained on spiramycin. We minimised this problem by conducting an ‘intention to treat’ analysis. Fifthly, inclusion of ocular lesions detected at older ages could diminish the treatment effect if, as seems likely, the impact of prenatal treatment is greatest on lesions detected soon after birth. Sixthly, treatment effects in both analyses could be diminished by poor compliance with treatment. Unfortunately, data on compliance were not recorded in any cohort. A major limitation of ours and all published cohort studies to-date is the lack of information on the clinical consequences of intracranial lesions for subsequent development.

We could not investigate the potential impact of missing data on these results. As is commonly the case in studies based on routine practice, investigators recorded their caseload of patients undergoing follow up not all seroconverting women who were eligible for follow up. Hence, we could only identify cases with missing outcome data in the prospective EMSCOT study: 15% of infants born to infected women, and 19% of all infants classified as infected had insufficient follow up to meet the reference criteria for congenital infection status<sup>4,7</sup>. Infection status was imputed for these cases based on prenatal PCR results, postnatal IgM



tests, and the postnatal age when last IgG positive <sup>4,7</sup>. In the remaining cohorts, we excluded only 21 mother-child pairs due to missing infection status and relied on the investigators' classification of infection status. Similarly, for most cohorts, we did not have data on dates and results of all postnatal ophthalmic and cranial ultrasound examinations, and relied on the investigators' classification of findings. Information on type or timing of treatment were rarely missing (less than 10 women) and were imputed according to the protocol performed in the given centre.

We excluded cohorts from America in the meta-analysis because of differences in the burden of the disease, the risk of clinical manifestations <sup>19</sup>, the parasite strain <sup>29,30</sup>, and the way in which intracranial lesions were measured (CT versus ultrasound scan) <sup>20,21</sup>. Further studies are required to compare outcomes in treated and untreated mothers within South America and other endemic tropical areas.

### *Policy implications*

From our results, it is unclear whether prenatal treatment has any effect on transmission or the presence of clinical manifestations. However, confidence intervals were wide and consistent with a beneficial effect and with no effect. Further evidence from observational studies is unlikely to change these results. Valid evidence of any benefit of prenatal treatment should be obtained through a large randomized controlled clinical trial.

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Figure 1. Flow-chart to show results of searches and study selection.

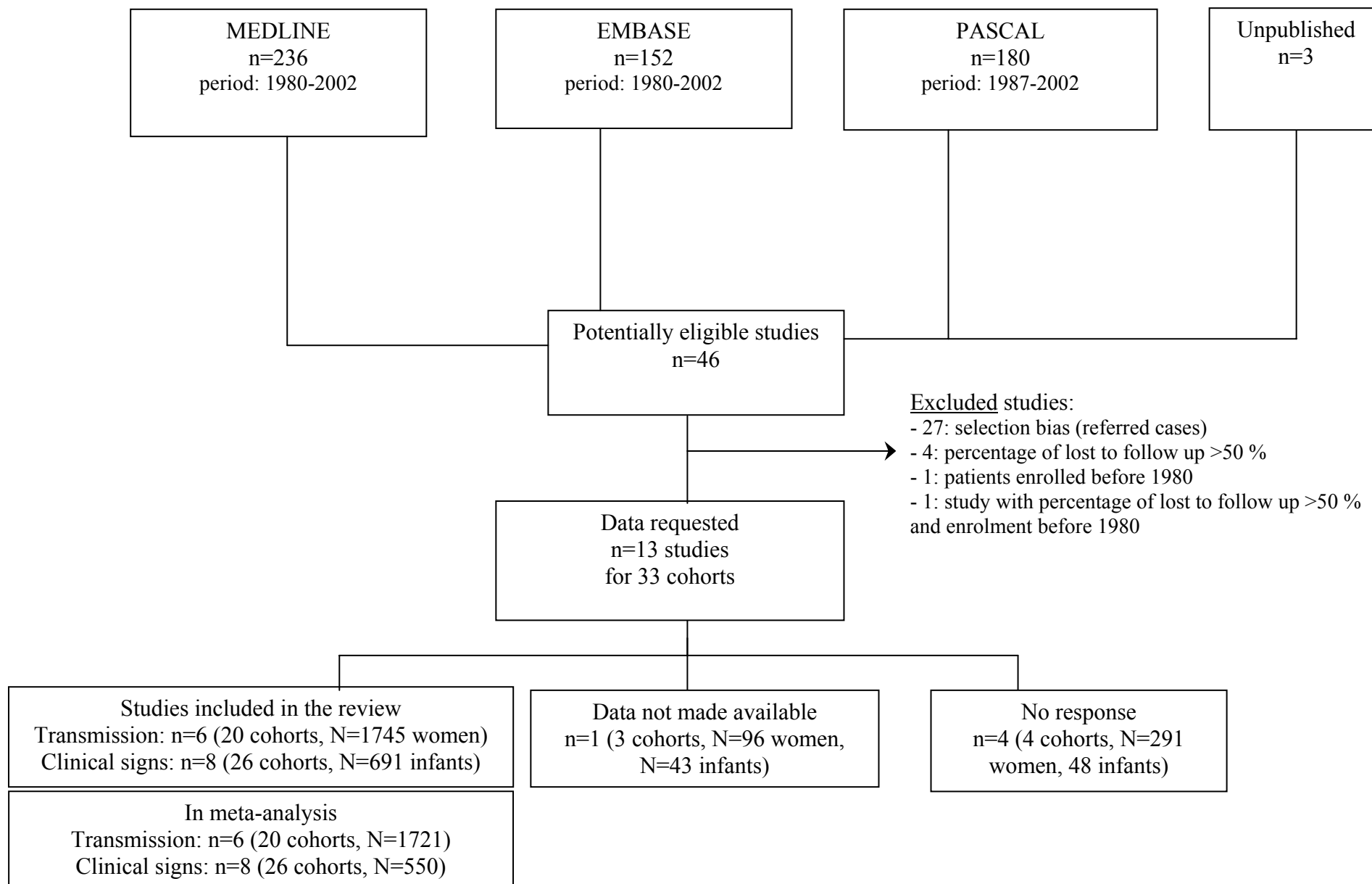


Figure 2. Risk of mother to child transmission of *T. gondii* according to gestational age at maternal seroconversion. Dotted lines are bounds of 95% confidence interval. SYROCOT Study, N=1721.

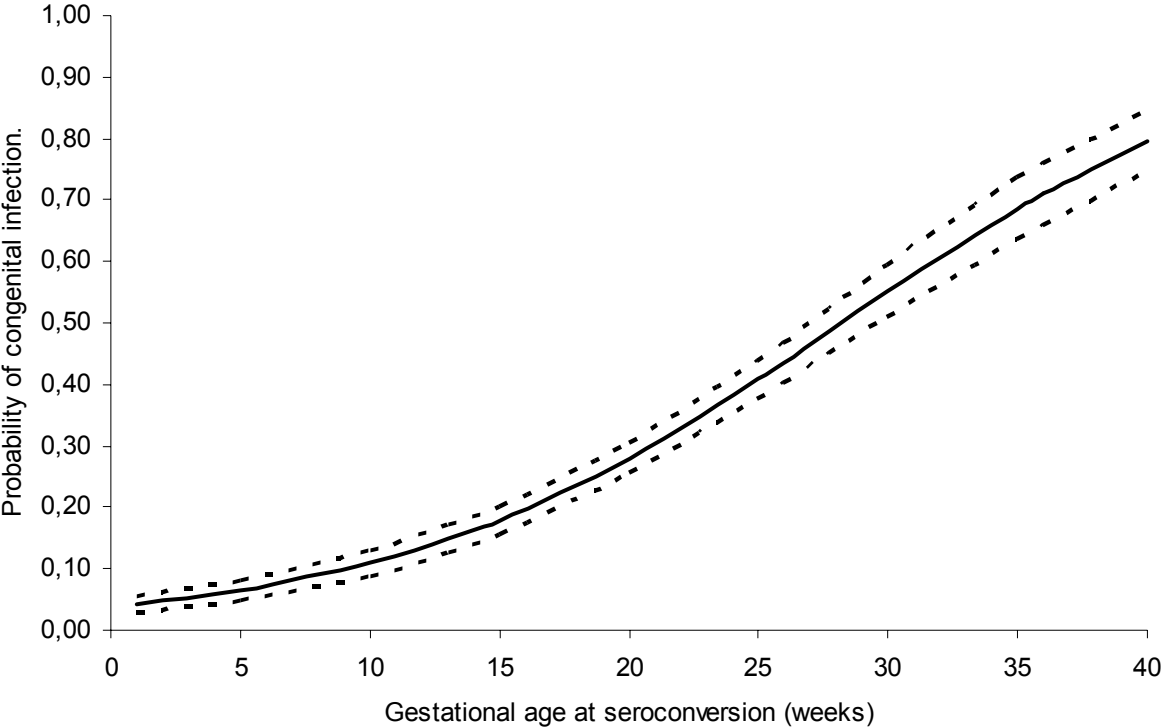
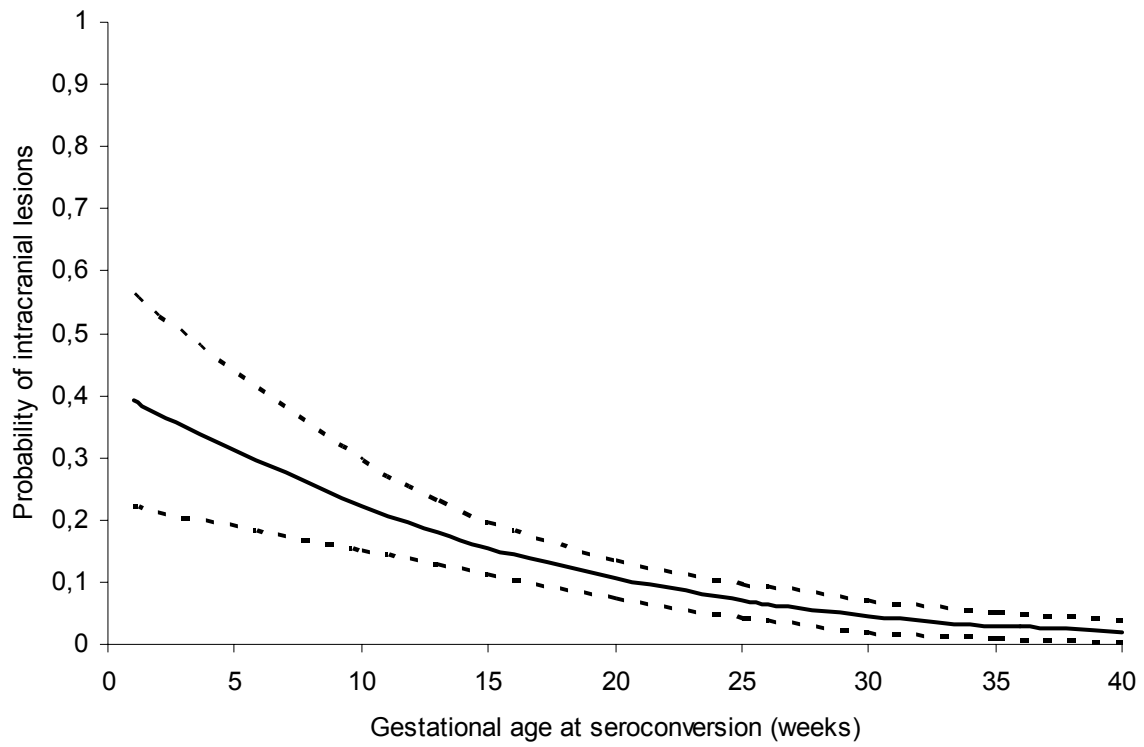


Figure 3. Risk of clinical manifestations in children infected by *T. gondii* according to gestational age at maternal seroconversion. Dotted lines are bounds of 95% confidence interval. SYROCOT Study. Europe only.

A. Risk of intracranial lesions (N=473)



B. Risk of eye lesions (N=526)

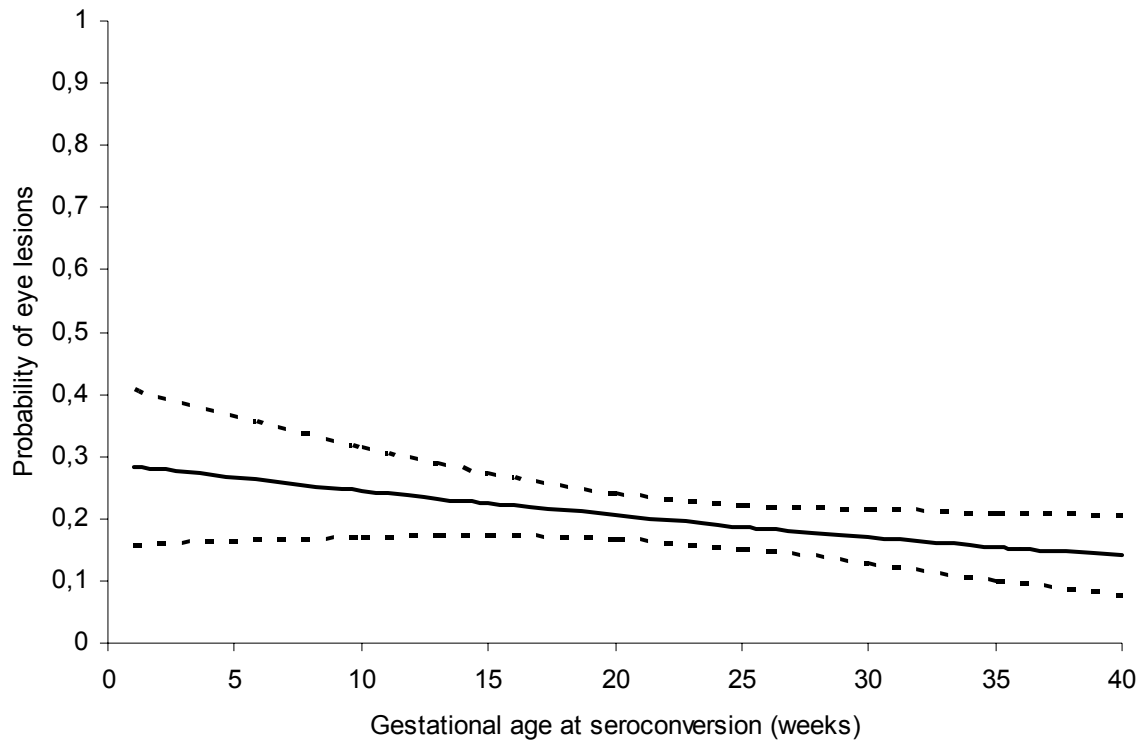




Table 1. Characteristics of cohorts included in the systematic review.

Country	Cohort region (study reference)	Recruitment Period	Screening §	Infected Mothers	Infected live born children (%)	Clinical manifestation (% of infected infants)					
						Any	Ocular	Intracranial			
Netherlands	South Netherlands <sup>13</sup>	1987-1988	PN 2m	52	12 (23.1)	3 (25.0)	2 (16.7)	1 (8.3)			
Norway	Oslo <sup>12</sup>	1992-1994	PN 3m	33	17 (51.5)	6 (35.3)	3 (17.7)	4 (23.5)			
Finland	Helsinki <sup>12</sup>	1988-1994	PN 3m	12	4 (33.3)	2 (50.0)	1 (25.0)	2 (50.0)			
Slovenia	Ljubljana <sup>31</sup>	1994-1996	PN 3m	19	3 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)			
Hungary	Hungary <sup>32</sup>	1987-1994	PN 3m	10	4 (40.0)	1 (25.0)	1 (25.0)	0 (0.0)			
France	Nice (unpublished) *	1998-2002	PN 1m	44	15 (34.1)	1 (6.7)	1 (6.7)	0 (0.0)			
	Grenoble <sup>7</sup>	1996-2000	PN 1m	24	6 (25.0)	2 (33.3)	1 (16.7)	1 (16.7)			
	Lyon <sup>7</sup>	1996-2000	PN 1m	167	43 (25.7)	10 (23.3)	9 (20.9)	3 (7.0)			
	Lyon <sup>13</sup>	1987-1995	PN 1m	583	179 (30.7)	33 (18.4)	23 (12.9)	16 (8.9)			
	Marseille <sup>7</sup>	1996-2000	PN 1m	67	20 (29.9)	2 (10.0)	2 (10.0)	0 (0.0)			
	Nice <sup>7</sup>	1996-2000	PN 1m	30	8 (26.7)	4 (50.0)	2 (25.0)	2 (25.0)			
	Paris <sup>7</sup>	1996-2000	PN 1m	197	65 (33.0)	8 (12.3)	8 (12.3)	1 (1.5)			
	Reims <sup>7</sup>	1996-2000	PN 1m	26	8 (30.8)	2 (25.0)	2 (25.0)	0 (0.0)			
	Toulouse <sup>7</sup>	1996-2000	PN 1m	68	22 (32.4)	3 (13.6)	2 (9.1)	1 (4.6)			

Austria	Austria <sup>13</sup>	1992-1995	PN 3m	129	33	(25.6)	3	(9.1)	3	(9.1)	2	(6.1)
	Austria <sup>7</sup>	1996-2000	PN 3m	108	24	(22.1)	5	(20.8)	5	(20.8)	2	(8.3)
Sweden	Stockholm <sup>7</sup>	1996-2000	NN RT	10	3	(30.0)	1	(33.3)	1	(33.3)	1	(33.3)
Italy	Naples <sup>7</sup>	1996-2000	PN 1m	35	11	(31.4)	3	(27.3)	3	(27.3)	3	(27.3)
	Milan <sup>7</sup>	1996-2000	PN 3m	8	4	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)
Denmark	Denmark <sup>7</sup>	1996-2000	NN	NA	14		4	(28.6)	3	(21.4)	1	(7.1)
	Denmark <sup>13</sup>	1992-1996	NN RT	123	26	(21.1)	5	(19.2)	4	(15.4)	3	(11.5)
Poland	Poznan <sup>7</sup>	1996-2000	NN	NA	29		7	(24.1)	3	(10.3)	6	(20.7)
Brazil	Campos <sup>33 *</sup>	1996-2000	NN	NA	8		3	(37.5)	2	(25.0)	3	(37.5)
	Porto Alegre <sup>10 *</sup>	1996-2003	NN	NA	22		17	(77.3)	13	(59.1)	14	(63.6)
USA	Massachusetts <sup>6</sup>	1986-1992	NN	NA	103		38	(36.9)	28	(27.2)	19	(18.5)
Colombia	Colombia <sup>34</sup>	2000-2004	PN/NN	NA	8		3	(37.5)	3	(37.5)	3	(37.5)
Total				1745	691		166	(24.0)	125	(18.1)	88	(12.7)

<sup>s</sup> Type of screening: PN = prenatal, NN= neonatal, m = monthly re-testing schedule (eg: 2m= two monthly), RT= retrospective testing of stored prenatal sera. \* Records based on standard prospective data collection form as used in the EMSCOT studies<sup>4,7</sup>

NA: Not applicable because study based on neonatal screening without retrospective measure of gestational age at seroconversion

Table 2. Adjusted effect of the timing and type of prenatal treatment on the risk of mother to child transmission in European prenatal screening centers in the sub-sample of treated mothers (N=1438 mothers, 398 infected fetus/children).

	OR	95% CI	p
Timing of prenatal treatment initiation*			0.05
< 3 weeks after seroconversion	(n=312) 0.48	[0.28 ; 0.80]	
3 ≤ and < 5 weeks after seroconversion	(n=442) 0.64	[0.40 ; 1.02]	
5 ≤ and < 8 weeks after seroconversion	(n=360) 0.60	[0.36 ; 1.01]	
≥ 8 weeks after seroconversion	(n=325) Ref		
Type of treatment (Spiramycin vs. P-S)	0.79	[0.55 ; 1.13]	0.19
Gestational age at maternal seroconversion (per week)	1.15	[1.12 ; 1.17]	<10 <sup>-4</sup>
Latitude (for 5° higher)	0.71	[0.53 ; 0.96]	0.03
Start of study period			0.14
After 1994	0.39	[0.15 ; 1.05]	
Between 1991 and 1994	0.46	[0.17 ; 1.21]	
Before 1991	Ref		

\* Contribution of woman time to each category given in brackets. Model adjusted for gestational age at maternal seroconversion estimated by the integrated maximum likelihood method

OR: Odds ratio, 95% CI: 95% confidence interval, P-S: Pyrimethamine-Sulphonamides

Table 3. Adjusted effect of the timing and type of prenatal treatment on the risk of clinical manifestations diagnosed during the first year of life in infected children identified by prenatal and neonatal screening in European centers.

	Any clinical manifestations (N=550)			Retinochoroiditis (N=524) <sup>§</sup>			Intracranial lesions (N=494) <sup>§</sup>		
	OR	95% CI	p	OR	95% CI	P	OR	95% CI	p
Gestational age at maternal seroconversion (per week)	0.96	[0.93; 0.99]	0.01	0.97	[0.93; 1.00]	0.04	0.91	[0.87; 0.95]	<10 <sup>-4</sup>
Prenatal treatment and timing of initiation after seroconversion*									
Not treated	(n=164)	Ref.	0.03	Ref.		0.03	Ref		0.41
Spiramycin started < 5 weeks	(n=112)	0.68	[0.31; 1.52]	0.86	[0.36; 2.09]		0.37	[0.09; 1.54]	
Spiramycin started ≥ 5 weeks	(n=143)	0.87	[0.41; 1.86]	0.98	[0.42; 2.32]		0.83	[0.28; 2.42]	
P-S (whatever the starting date)	(n=67)	0.66	[0.26; 1.69]	0.82	[0.30; 2.29]		0.73	[0.22; 2.48]	
Spiramycin then P-S	(n=64)	2.41	[1.15; 5.03]	2.89	[1.29; 6.49]		1.40	[0.46; 4.24]	

P-S: Pyrimethamine-Sulphonamides

<sup>§</sup> children not examined for each outcome were excluded from that analysis.

\* Contribution of patients in each category (based on quartiles) is provided in brackets for the analysis of the risk of any signs. These contributions were n=152 / 110 / 135 / 66 / 61, and n=149 / 101 / 131 / 64 / 49 for the analyses of the odds of retinochoroiditis only and

intracranial lesions only, respectively. Models were also adjusted for gestational age at maternal seroconversion, period of the study (<1991, 1991-1994, >1994), and latitude of centre.