Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data

*The SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group**

Summary

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

Methods We did a systematic review of cohort studies based on universal screening for congenital toxoplasmosis. We did a meta-analysis using individual patients' data to assess the effect of timing and type of prenatal treatment on mother-to-child transmission of infection and clinical manifestations before age 1 year. Analyses were adjusted for gestational age at maternal seroconversion and other covariates.

Findings We included 26 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 (adjusted odds ratio [OR] 0·48, 95% CI 0·28–0·80; p=0·05). In 550 infected liveborn infants identified by prenatal or neonatal screening, we found no evidence that prenatal treatment significantly reduced the risk of clinical manifestations (adjusted OR for treated *vs* not treated 1·11, 95% CI 0·61–2·02). Increasing gestational age at seroconversion was strongly associated with increased risk of mother-to-child transmission (OR 1·15, 95% CI 1·12–1·17) and decreased risk of intracranial lesions (0·91, 0·87–0·95), but not with eye lesions (0·97, 0·93–1·00).

Interpretation We found weak evidence for an association between early treatment and reduced risk of congenital toxoplasmosis. Further evidence from observational studies is unlikely to change these results and would not distinguish whether the association is due to treatment or to biases caused by confounding. Only a large randomised controlled clinical trial would provide clinicians and patients with valid evidence of the potential benefit of prenatal treatment.

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*Members listed at end of report

Correspondence to:
Dr Rodolphe Thiébaut, INSERM U875 and U593-ISPED, Université Bordeaux 2 Victor Segalen, 146 rue Léo Saignat, 33076 Bordeaux, France
rodolphe.thiebaut@isped.u-bordeaux2.fr

Introduction

Toxoplasma gondii is a common parasitic infection acquired by ingestion of oocysts excreted by cats and contaminating soil or water, or by eating tissue cysts that remain viable in undercooked meat of infected animals.[1,2] 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.[3] Overall, about a third of infected mothers give birth to an infant with toxoplasmosis.[3,4] Most children with congenital toxoplasmosis are developmentally normal[5] but up to 4% die or have evidence of permanent neurological damage or bilateral visual impairment during the first years of life.[6,7]

Toxoplasma infection in pregnancy is usually asymptomatic and can only be detected by serological testing. Prenatal testing for toxoplasmosis is routinely offered in many European countries so that infected mothers can be treated with antibiotics to reduce the risk of mother-to-child transmission and, if fetal infection has occurred, to reduce impairment in the child.[8] No consensus exists about the most effective screening strategy or the best type of treatment. Uncertainty about the benefits of prenatal treatment[9] and concerns about adverse treatment effects and the infrastructure and costs needed to implement prenatal screening have led to diverse policies including no screening, neonatal screening[6,10,11] and prenatal screening with monthly or 3-monthly re-testing schedules.[4,8,12,13] In countries where prenatal screening is done, recommendations for treatment can differ. In most centres, 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.[4] By contrast, in Austria, mothers are initially treated with pyrimethamine-sulphonamide (after 15 weeks of gestation), and changed to spiramycin if fetal diagnosis is negative.[4]

So far, two systematic reviews have evaluated the effect of prenatal treatment on mother-to-child transmission.[9,14] No randomised 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 because of differences between studies in analytical methods and the way aggregate data were presented. However, new observational data have been published since then: three analyses of retrospective cohort studies[12,13,15,16] and the results of a large prospective multicentre cohort study.[4,7] 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.[7,12,13,16]

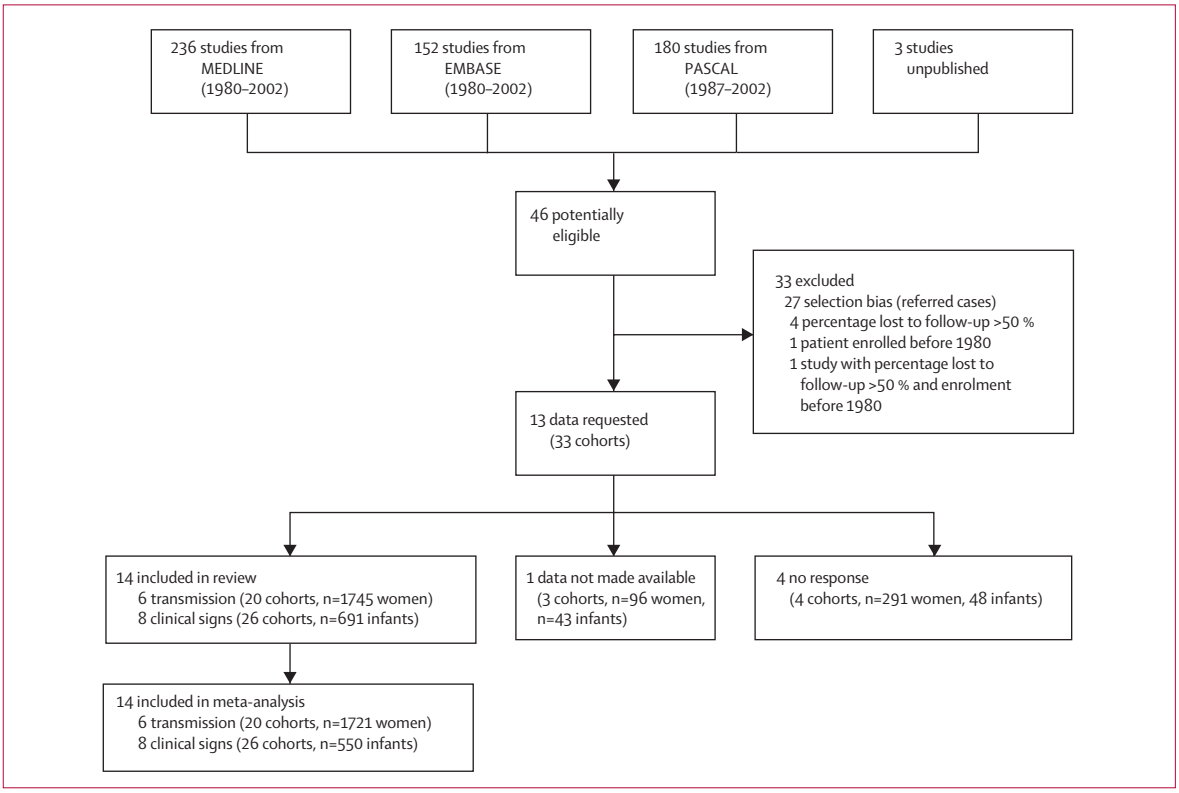

***Figure 1*: Results of searches and study selection**

Our aim was to estimate the effects of timing and different types of prenatal treatment on the risk of congenital toxoplasmosis and its clinical manifestations during infancy, with a systematic review using individual patients' 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 if the following data had been 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 on the basis of 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 if at least one ophthalmoscopy or intracranial imaging examination had been recorded during the first year of life. We excluded studies of mothers enrolled before 1985, because diagnosis with IgM was widely used only after this time.

We searched MEDLINE, EMBASE, and PASCAL from 1980 to 2002 (figure 1) and updated searches with Current Contents (based on MEDLINE) in November, 2005 (full details of searches reported elsewhere[17]). 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. No language restriction was used. 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 patients' data were examined by four reviewers (RT, SL, SDC, RG) before inclusion was confirmed.

Study population, clinical outcomes, and treatment effect

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

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 was less specific than in prenatal cohorts.[18] Primary analyses were further restricted to treated mothers because delayed detection of untreated mothers, who were clustered in late pregnancy, could have introduced a selection bias.

We compared the risk of transmission according to the time interval (categorised by quartiles) between seroconversion and the initiation of any type of prenatal treatment. We also assessed the effect of the type of this first treatment (spiramycin alone or pyrimethamine-sulphonamide combination). Patients who changed from spiramycin to pyrimethamine-sulphonamide 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 because of lack of power.

Congenital infection status was based on serological tests for IgG and IgM antibodies in liveborn infants. The presence of congenital infection was defined by the persistence of specific IgG antibodies beyond age 11 months. The absence of congenital infection was defined by undetectable IgG after age 2 months in the absence of treatment for toxoplasmosis.[18] 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.

We confined the analyses of clinical manifestations in children to European cohorts of liveborn 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.[19] Additionally, North and South American studies (mainly based on neonatal screening) were excluded because they used CT scans to screen for intracranial lesions. This method is more sensitive than cranial ultrasound scan.[20,21] 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.

We compared the effect of timing and type of prenatal treatment strategies, defined as: no treatment, spiramycin

	Cohort region (study reference)	Recruitment period	Screening	Infected mothers	Infected liveborn children (%)	Clinical manifestation (% of infected infants)		
						Any	Ocular	Intracranial
Netherlands	South Netherlands[13]	1987–1988	PN 2 m	52	12 (23%)	3 (25%)	2 (17%)	1 (8%)
Norway	Oslo[12]	1992–1994	PN 3 m	33	17 (52%)	6 (35%)	3 (18%)	4 (24%)
Finland	Helsinki[12]	1988–1994	PN 3 m	12	4 (33%)	2 (50%)	1 (25%)	2 (50%)
Slovenia	Ljubljana[27]	1996–1996	PN 3 m	19	3 (16%)	0 (0%)	0 (0%)	0 (0%)
Hungary	Hungary[28]	1987–1994	PN 3 m	10	4 (40%)	1 (25%)	1 (25%)	0 (0%)
France	Nice (unpublished)*	1998–2002	PN 1 m	44	15 (34%)	1 (7%)	1 (7%)	0 (0%)
	Grenoble[7]	1996–2000	PN 1 m	24	6 (25%)	2 (33%)	1 (17%)	1 (17%)
	Lyon[7]	1996–2000	PN 1 m	167	43 (26%)	10 (23%)	9 (21%)	3 (7%)
	Lyon[13]	1987–1995	PN 1 m	583	179 (31%)	33 (18%)	23 (13%)	16 (9%)
	Marseille[7]	1996–2000	PN 1 m	67	20 (30%)	2 (10%)	2 (10%)	0 (0%)
	Nice[7]	1996–2000	PN 1 m	30	8 (27%)	4 (50%)	2 (25%)	2 (25%)
	Paris[7]	1996–2000	PN 1 m	197	65 (33%)	8 (12%)	8 (12%)	1 (2%)
	Reims[7]	1996–2000	PN 1 m	26	8 (31%)	2 (25%)	2 (25%)	0 (0%)
	Toulouse[7]	1996–2000	PN 1 m	68	22 (32%)	3 (14%)	2 (9%)	1 (5%)
Austria	Austria[13]	1992–1995	PN 3 m	129	33 (26%)	3 (9%)	3 (9%)	2 (6%)
	Austria[7]	1996–2000	PN 3 m	108	24 (22%)	5 (21%)	5 (21%)	2 (8%)
Sweden	Stockholm[7]	1996–2000	NN RT	10	3 (30%)	1 (33%)	1 (33%)	1 (33%)
Italy	Naples[7]	1996–2000	PN 1 m	35	11 (31%)	3 (27%)	3 (27%)	3 (27%)
	Milan[7]	1996–2000	PN 3 m	8	4 (50%)	0 (0%)	0 (0%)	0 (0%)
Denmark	Denmark[7]	1996–2000	NN	n/a	14	4 (29%)	3 (21%)	1 (7%)
	Denmark[13]	1992–1996	NN RT	123	26 (21%)	5 (19%)	4 (15%)	3 (12%)
Poland	Poznan[7]	1996–2000	NN	n/a	29	7 (24%)	3 (10%)	6 (21%)
Brazil	Campos[29]*	1996–2000	NN	n/a	8	3 (38%)	2 (25%)	3 (38%)
	Porto Alegre[10]*	1996–2003	NN	n/a	22	17 (77%)	13 (59%)	14 (64%)
USA	Massachusetts[6]	1986–1992	NN	n/a	103	38 (37%)	28 (27%)	19 (19%)
Colombia	Colombia[30]	2000–2004	PN/NN	n/a	8	3 (38%)	3 (38%)	3 (38%)
Total	..	..	..	1745	691	166 (24%)	125 (18%)	88 (13%)

PN=prenatal. NN=neonatal. m=monthly re-testing schedule (eg, 2 m=2-monthly). RT=retrospective testing of stored prenatal serum samples. n/a=not applicable because study based on neonatal screening without retrospective measure of gestational age at seroconversion. *Records based on standard prospective data collection form as used in the EMSCOT studies.[4,7]

Table 1: **Characteristics of cohorts included in the systematic review by country**

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. Clinical manifestations were defined as ocular lesions (retinochoroiditis or microphthalmia), intracranial lesions (intracranial calcification or ventricular dilation), or both, 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.

See Online for webappendix

Statistical analyses

The gestational age at seroconversion was considered in all analyses. This variable was 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 *T gondii*) and the period of the study, (distinguishing studies predating PCR for prenatal diagnosis [before 1991], studies based on the same standard prospective data collection form as used in the EMSCOT studies[4,7] [after 1994], and studies in the interim period [1991—94]). We did not examine the effect of maternal age or sex of the child, because 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 webappendix).[7,22] 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.[7] 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.

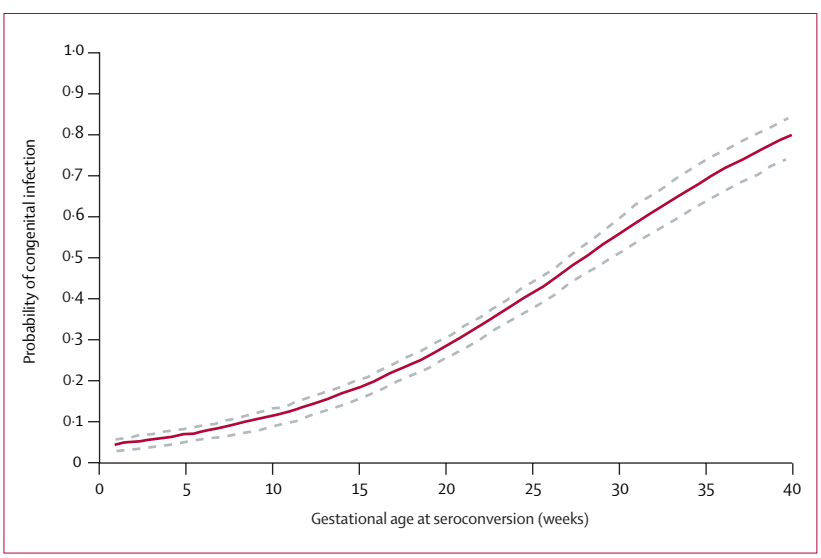

***Figure 2*: Risk of mother-to-child transmission of *T gondii* by gestational age at maternal seroconversion (n=1721)**
Dotted lines are bounds of 95% CI.

Results

We found no randomised controlled trials in our search. 26 observational cohorts were included in the review, including a total of 1745 infected mothers and 691 infected liveborn infants. Three cohorts from the same study[12] had relevant data but the investigators declined to participate (96 mothers, 43 infected infants). Investigators for four further studies,[23–26] accounting for 288 mothers and 49 infected infants, did not respond (figure 1). Studies of prenatal screening varied from a monthly to 3-monthly re-testing schedule for susceptible mothers (table 1). Details of the prenatal treatment regimens are published elsewhere.[4,7,12,13,15] The crude risk of mother-to-child transmission shown in table 1 varied between cohorts, mainly because of differences in gestational age at maternal seroconversion. In 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 (two in Brazil, one in Colombia, and one in Massachusetts, USA; n=141 infected infants), mainly based on neonatal screening, were excluded. The crude risk of ocular lesions diagnosed in the first year of life was much greater in the South American cohorts (47%, 18 of 38) than in Europe (14%, 79 of 550), and intermediate in the Massachusetts cohort (27%, 28 of 103). The crude risk of intracranial lesions detected by CT scan was much higher in the cohorts from North (19%, 19 of 103) and South America (53%, 20 of 38) than those from Europe (9%, 49 of 550), where cranial ultrasound was used.

Overall, there were 1721 infected mothers with 506 infected 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 estimated rate of mother-to-child transmission by gestational age at seroconversion was 15% (95% CI 13–17) at 13 weeks, 44% (40–47) at

	OR (95% CI)	p
Timing of prenatal treatment initiation		0·05
<3 weeks after seroconversion (n=312)	0·48 (0·28–0·80)	
>3 weeks and <5 weeks after seroconversion (n=442)	0·64 (0·40–1·02)	
>5 weeks and <8 weeks after seroconversion (n=360)	0·60 (0·36–1·01)	
≥8 weeks after seroconversion (n=324)	Ref	
Type of treatment (spiramycin *vs* PS)	0·79 (0·55–1·13)	0·19
Gestational age at maternal seroconversion (per week)	1·15 (1·12–1·17)	<0·0001
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	

Model adjusted for gestational age at maternal seroconversion estimated by the integrated maximum likelihood method. PS=pyrimethamine-sulphonamide.

Table 2: **Adjusted effect of the timing and type of prenatal treatment on the risk of mother-to-child transmission in European prenatal screening centres in subsample of treated mothers (n=1438 mothers, 398 infected children)**

26 weeks, and 71% (66–76) at 36 weeks. The odds of transmission increased by 12% (10–14) per week of maternal gestation at seroconversion (figure 2). The results were similar when we excluded data for mothers obtained by 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 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 (table 2). The type of prenatal treatment did not seem to have a significant effect (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 effect of the period when the study started was not significant (p=0·14). The odds of transmission decreased significantly with higher latitude.

We obtained data on clinical manifestations in infected infants for 26 cohorts (table 1). Overall, of 691 infected infants, almost a quarter developed intracranial lesions, ocular lesions, or both during the first year of life (table 1). When the analysis was restricted 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 (table 3). When the type of lesion was considered, we noted a marked reduction in the odds of intracranial lesions with gestational age at seroconversion, whereas the decline in odds with ocular lesions was less significant (table 3; figures 3A and 3B).

The adjusted odds of any clinical manifestations did not significantly differ between infants of treated mothers and those of untreated mothers (OR 1·11, p=0·74). We further analysed the treatment effect by type of treatment and 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 pyrimethamine-sulphonamide alone, compared with untreated mothers (all CIs including 1). However, infants born to mothers treated with spiramycin followed by pyrimethamine-sulphonamide had a higher odds of any clinical manifestations compared with those treated with pyrimethamine-sulphonamide alone (OR 1·29, 95% 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 intention-to-treat

	Any clinical manifestations (n=550)		Retinochoroiditis (n=524)*		Intracranial lesions (n=494)*	
	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)	<0·0001
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)	
PS, any starting date (n=67)	0·66 (0·26–1·69)		0·82 (0·30–2·29)		0·73 (0·22–2·48)	
Spiramycin then PS (n=64)	2·41 (1·15–5·03)		2·89 (1·29–6·49)		1·40 (0·46–4·24)	

PS=pyrimethamine-sulphonamide. *Children not examined for each outcome were excluded from that analysis. Models were adjusted for gestational age at maternal seroconversion, period of the study (<1991, 1991–1994, >1994), and latitude of centre.

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

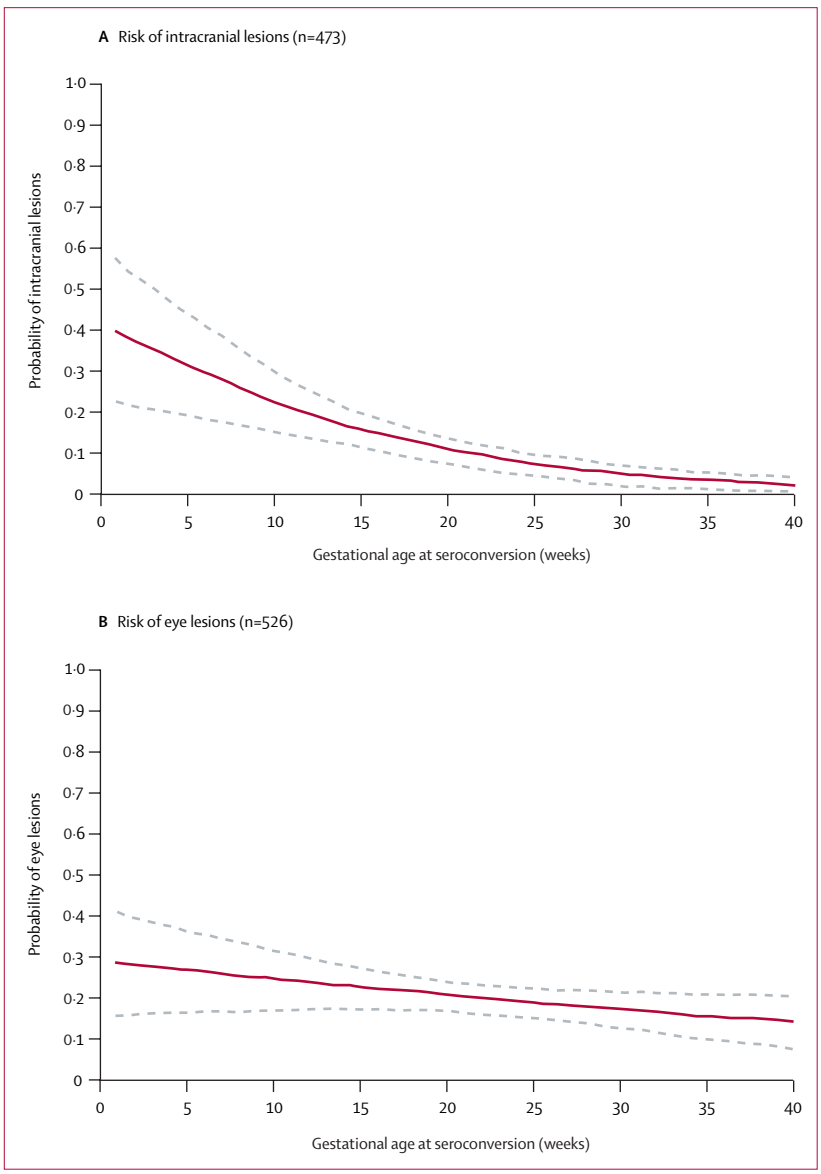

***Figure 3*: Risk of clinical manifestations in children infected by *T gondii* by gestational age at maternal seroconversion**
Dotted lines are bounds of 95% CI.

analysis of prenatal treatment (patients who switched treatment being included in the spiramycin group) did not show any significant effect of treatment (p=0·39). Compared with 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 (1·22, 0·63–2·38), or those treated with pyrimethamine-sulphonamide (0·63, 0·25–1·61). The odds of having clinical manifestations did not significantly differ by the period of the study (p=0·08) or the latitude of the centre (p=0·13).

Discussion

We found weak evidence for an increased risk of mother-to-child transmission the later prenatal treatment was started after maternal seroconversion. This result might be due to a true protective effect of early treatment, or to confounding caused by 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 liveborn 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.

We were unable to identify any previous meta-analysis of the effect of prenatal treatment for congenital toxoplasmosis. Almost all eligible cohorts were included in our meta-analysis, but three cohorts with appropriate data declined to participate. Four further cohorts were unlikely to have been eligible because of selection bias and enrolment before 1985.[23–26] Analysis of individual patients' 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 reduce bias and account for uncertainty due to the interval censored variables (gestational age at seroconversion and timing of prenatal treatment).

The main limitation of the study is that our results for prenatal treatment could be partly explained by biases in the way the cohort studies were designed and undertaken. Although we adjusted for the strong confounding effects of gestational age at seroconversion, we cannot exclude effects due to unmeasured confounders.[31] In the analysis of mother-to-child transmission, we included only prenatal screening cohorts because all used several tests on repeated samples to confirm maternal infection. We excluded neonatal screening cohorts because 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 a lower risk of mother-to-child transmission in untreated women than in 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.

Several sources of potential bias existed in the analyses of clinical manifestations, which included neonatal and prenatal screening cohorts. First, 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.[32] We minimised this problem by adjusting all analyses for gestational age at seroconversion. A second 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 underestimating or overestimating the treatment effect is difficult to predict. Third, bias could have been introduced if the accuracy of cranial ultrasound or ophthalmic examinations differed between neonatal and prenatal screened cohorts. Such a difference seems unlikely, since in European centres standard practice is to do a cranial ultrasound in early infancy (repeated if abnormalities are detected) and to do at least two ophthalmic assessments, one in early infancy and the other at 1 year. Prenatal centres stated that their protocol was to clinically examine children every 3–6 months, whereas neonatal centres reported 3-monthly assessments. We could not verify these practices because most datasets did not record every examination.

Fourth, indication bias probably explains the apparently harmful effect of changing treatment from spiramycin to pyrimethamine-sulphonamide compared with no treatment; clinicians might have undertaken 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 reduced this problem by doing an intention-to-treat analysis. Fifth, inclusion of ocular lesions detected at older ages could diminish the treatment effect if, as seems likely, the effect of prenatal treatment is greatest on lesions detected soon after birth. Sixth, treatment effects in both analyses could be diminished by poor compliance with treatment. Unfortunately, data on compliance were not recorded for any cohort. A major limitation of our study, and of all published cohort studies to date, is the absence of information on the clinical consequences of intracranial lesions for subsequent development.

We could not investigate the potential effect of missing data on these results. As is often 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.[4,7] Infection status was imputed for these cases based on prenatal PCR results, postnatal IgM tests, and the postnatal age when last IgG-positive.[4,7] In the remaining cohorts, we excluded only 21 mother-child pairs because of 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. In the rare cases where information on type or timing of treatment was missing (fewer than ten women) we assumed that treatment was as stated in the local protocol.

We excluded cohorts from America in the meta-analysis because of differences in the burden of the disease, the risk of clinical manifestations,[19] the parasite strain,[33,34] and the way in which intracranial lesions were measured (CT versus ultrasound scan).[20,21] Further studies are required to compare outcomes in treated and untreated mothers within South America and other endemic tropical areas.

From our results, whether prenatal treatment has any effect on transmission or the presence of clinical manifestations is unclear. 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 randomised controlled clinical trial.

The Systematic Review On Congenital Toxoplasmosis (SYROCOT) study group
Writing committee—Rodolphe Thiébaut, Sandy Leproust, Geneviève Chêne (INSERM, Bordeaux, France), Ruth Gilbert (Institute of Child Health, University College London, London, UK) on behalf of the SYROCOT investigators. Investigators of cohorts contributing to SYROCOT: A Prusa, M Hayde, A Pollak (University Children's Hospital, Vienna, Austria), M Wallon, F Peyron (Hôpital de la Croix Rousse, Lyon, France), S Romand, P Thulliez (Institut de Puericulture, Paris, France), W Buffolano, A Romano (Universita di Napoli, Naples, Italy), J Franck, H Dumon (Hôpital de la Timone, Marseille, France), P Bastien, E Issert (CHU de Montpellier, Montpellier, France); M-H Bessieres (Hôpital de Rangueil, Toulouse, France), N Ferret, P Marty (Hôpital de l'Archet, Nice, France), C Chemla, I Villena (Hôpital Maison Blanche, Reims, France), H Pelloux, H Fricker-Hidalgo, C Bost-Bru (Centre Hospitalier Universitaire de Grenoble, Grenoble, France), E Semprini, V Savasi (Milan, Italy), M Paul (University Medical Sciences, Poznan), G Malm, B Evengard (Huddinge Hospital, Stockholm, Sweden), E Petersen, D Schmidt (Statenseruminstitut, Copenhagen, Denmark), T Kortbeek (National Institute of Public Health and the Environment, Bilthoven, The Netherlands), J Logar (Medical Faculty, University of Ljubljana, Ljubljana, Slovenia), S Szenasi (Albert Szent-Györgyi Medical University, Szeged, Hungary), B Stray-Pedersen, P Jenum (University of Oslo, Rikshospitalet, National Insitute of Public Health, Norway), M Lappalainen (Helsinki University Central Hospital, Finland), E Lago, E Neto (Hospital Sao Lucas da PUCRS, Porto Alegre, Brazil), L Bahia-Oliveira (Universidade Estadual do Norte Fluminense, Campos, Brazil), R Eaton, H-W Hsu (Massachussets State Laboratory Institute, Boston, USA), J Gomez-Marin (Universidad del Quindio, Armenia, Colombia).
Study design and coordination—Geneviève Chêne, Ruth Gilbert, Luuk Gras, Rodolphe Thiébaut. Data management: Kathy Freeman, Tan Hooi Kuan (EMSCOT study), Sabrina Di Costanzo, Sandy Leproust, Rodolphe Thiébaut.
Statistical analysis—Sabrina Di Costanzo, Sandy Leproust, Rodolphe Thiébaut.

Conflict of interest statement
We declare that we have no conflict of interest.

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