Effect of timing and type of treatment on the risk of mother to child transmission of *Toxoplasma gondii*

European Multicentre Study on Congenital Toxoplasmosis*

Participants are listed on page 119

Objective To determine the effects on mother to child transmission of the timing and type of prenatal treatment, taking into account gestational age at maternal seroconversion.

Design Prospective cohort study.

Setting European centres offering prenatal screening for toxoplasmosis.

Population Children born to a cohort of pregnant women with toxoplasma infection.

Methods We determined the effects on mother to child transmission of the interval between seroconversion and start of treatment (treatment delay), and the type of treatment, taking into account gestational age at maternal seroconversion.

Main outcome measure Congenital infection status confirmed by toxoplasma IgG results at one year postnatal age.

Results Of 1208 women analysed, 72% were first prescribed spiramycin, 19% pyrimethamine–sulphonamide and 9% (mostly infected during the last trimester) were untreated. The odds ratios for mother to child transmission for all women treated after a delay of four to seven weeks was 0.77 (95% CI 0.34–1.69), and after eight weeks or more was 1.33 (0.56–2.89) compared with less than four weeks. The odds ratio per week of treatment delay was 1.01 (0.93–1.08). There was no evidence that transmission risk differed in women first treated with pyrimethamine–sulphonamide *versus* spiramycin: odds ratio 1.10 (0.63–1.91) or in untreated *versus* treated women: odds ratio 0.57 (0.27–1.17).

Conclusion We were unable to demonstrate a beneficial effect of the timing or type of prenatal treatment on the risk of mother to child transmission but we could not exclude a clinically important effect. Randomised controlled trials are required to determine the effect of prenatal treatment on mother to child transmission.

INTRODUCTION

Prenatal testing for toxoplasma infection aims to identify women who acquired infection for the first time during pregnancy and are at risk of transmitting infection to their fetus. Infected women are treated in order to prevent mother to child transmission of infection and, if fetal infection has occurred, to reduce the risk of intracranial and ocular damage. Although prenatal screening has operated for 30 years in some countries, no controlled trials have been conducted to evaluate prenatal treatment[1]. However, three recent studies, based on cohorts of treated and untreated women, have determined the effect of prenatal treatment on mother to child transmission after taking into account the strong effect of gestational age at maternal infection on transmission risk[2–4]. None of these studies reported a significant effect of treatment on mother to child transmission but none could exclude effects which might justify treatment. As these studies were retrospective, selection bias, for example, in the children lost to follow up, or due to inclusion of women tested because of suspected fetal abnormalities, may have distorted the results.

We prospectively studied a cohort of women and their children in 11 European centres. Serological testing to identify infection acquired during pregnancy varied from three monthly in Vienna to monthly in France. Compliance with testing was variable and the type of treatment differed between centres. Few women received no treatment. Such variation made it possible to investigate the effect of timing and type of treatment on mother to child transmission of toxoplasma infection.

METHODS

There were 11 study centres (Table 1); 10 routinely offered prenatal screening and provided a reference service for toxoplasmosis in pregnancy. In Stockholm, women were identified as part of a research study[5].

Infected women were prospectively enrolled between January 1996 and March 2000. Clinical and microbiological data were collected using standard proformas at the end of pregnancy and for infants at 1, 6 and 12 months of age.

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* **Correspondence**: Dr R. Gilbert, Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

Table 1. Prenatal testing and treatment protocols. PN = postnatal testing of seronegative women; RT = rising IgG titre; Avidity = low IgG avidity; HT= high IgG titre. All enrollments were consistent with the definite and probable criteria reported by Lebech et al.[15].

Centre	Method for detecting maternal infection		Prenatal treatment		
	Seroconversion: recommended testing schedule for IgG negative women	Tests of recent infection: IgM and IgG positive plus tests shown	After diagnosis of maternal infection for infection acquired in the:		After positive diagnosis of fetal infection
			First and second trimesters	Third trimester	
France					
Lyon	Monthly + postnatal[a]	Avidity <35%, RT	Spiramycin[b]	P–S[c,d]	P–S[c,d]
Paris	Monthly + postnatal[a]	None enrolled[e]	Spiramycin[b]	Spiramycin[b]	P–S[c,d]
Marseille	Monthly + postnatal[a]	RT	Spiramycin[b]	Fansidar[c,f]	Fansidar[c,f]
Grenoble	Monthly	RT	Spiramycin[b]	Fansidar[c,f]	Fansidar[c,f]
Nice	Monthly + postnatal[a]	RT	Spiramycin[b]	P–S[c,d]	P–S[c,d]
Toulouse	Monthly + postnatal[a]	RT, IgA	Spiramycin[b]	Spiramycin[b]	Fansidar[c,f]
Reims	Monthly + postnatal[a]	RT, IgA, IgE	Spiramycin[b]	Spiramycin[b]	Fansidar[c,f]
Austria					
Austria	12, 20, 32 weeks	RT, HT, low avidity	P–S[c,g]	P–S[c,g]	P–S[c,g]
Italy					
Naples	12, 20, 36 weeks	IgA, RT	Spiramycin[b]	Spiramycin[b]	P–S[c,d]
Milan	Monthly	RT, IgA	Spiramycin[b]	Spiramycin[b]	P–S[c,d]
Sweden					
Stockholm	10 weeks + postnatal[h]	Avidity <15%	Nil	Nil	Nil

[a] Routine postnatal testing of cord blood and/or postnatal sample in seronegative women.

[b] Daily dose in all centres = 3 g/day until delivery or until regimen changed after prenatal diagnosis.

[c] Pyrimethamine–sulphonamide combination with folinic acid alternates with spiramycin in three or four weekly cycles until delivery except in Paris, Reims, Marseille Toulouse and Grenoble where continuous pyrimethamine–sulphonamide is given.

[d] Pyrimethamine–sulphadiazine: Daily dose in Lyon, Paris and Nice = 50 mg/day pyrimethamine, 3 g/day sulphadiazine; in Naples = 25 mg/day pyrimethamine, 2 g/day sulphadiazine; in Milan = 50 mg/day pyrimethamine, 2 g/day sulphadiazine.

[e] Enrollment was restricted to women who seroconverted.

[f] Fansidar: dose in: Marseille, Toulouse and Grenoble = 50 mg/week pyrimethamine, 1 g/week sulphadoxine; Reims = 75 mg/2 weeks pyrimethamine, 1.5 g/2 weeks sulphadoxine.

[g] Daily dose in Austria = 25 mg/day pyrimethamine (50 mg for first dose), 0.75 g/day sulphadiazine (1.5 g for first dose), prescribed from 16 weeks of gestation onwards and continued until delivery. Spiramycin given before 16 weeks gestation.

[h] Neonatal screening for specific IgG antibodies in Guthrie card filter paper bloodspot followed by retrospective testing of stored prenatal sera.

Women were enrolled if the collaborating clinician considered that prenatal treatment for toxoplasmosis was justified. Infected women were identified using two methods. First, by detection of seroconversion during pregnancy (change from IgG negative to IgG positive specific antibodies). IgG negative women were re-tested monthly in France and approximately three monthly elsewhere (Table 1). In centres where seronegative women were routinely tested postpartum, we included infected women diagnosed after delivery. Second, we included women with IgG and IgM positive results at their first prenatal test and, in addition, a rising IgG titre, low IgG avidity, high IgG titre or positive IgA antibodies (referred to as 'tests for recent infection', Table 1). In Stockholm, infected women were identified by universal neonatal screening for specific IgG antibodies followed by retrospective testing of stored antenatal samples[5] to detect seroconversion during pregnancy or recent infection. All positive results were confirmed in subsequent samples. In order to minimise selection bias due to women who were investigated for fetal abnormalities associated with congenital toxoplasmosis, we excluded women in whom the dates for diagnosis of maternal infection, start of maternal treatment, amniocentesis for fetal diagnosis and detection of fetal or infant abnormalities were not sequential.

The case definition for congenital toxoplasmosis was the persistence of specific IgG beyond 12 months postnatal age: positive parasite culture or histology of the placenta or fetal tissue was accepted for perinatal or fetal deaths. Absence of congenital toxoplasmosis was defined by at least one IgG negative result in a child not receiving treatment.

Table 1 summarises the screening and treatment protocols for each centre. In the French and Italian centres, spiramycin was started immediately after confirmation of maternal infection and, following diagnosis of fetal infection, was changed to pyrimethamine–sulphonamide. In some centres, pyrimethamine–sulphonamide was prescribed immediately if maternal infection occurred in the third trimester as a result of a high risk of fetal infection. In Vienna, all infected women above 15 weeks of gestational age were prescribed pyrimethamine–sulphonamide unless fetal diagnosis was negative. None of the Stockholm women were treated.

The hypothesis that the earlier treatment is given, the more likely it is to be effective, underpins the rationale for the monthly re-testing of susceptible women. We determined the effect of the interval between seroconversion and the first date anti-toxoplasma treatment was prescribed. This interval is subsequently called 'treatment delay'. The odds ratio for each week of treatment delay and for four week categories (less than four, four to seven and eight or more weeks) was calculated taking into account the gestational age at maternal seroconversion. The effects of maternal age, parity, symptoms at diagnosis and country of study on the treatment effect were tested and as only maternal age was significant at the 5% level, this was included in the model. Reported P values (two-sided) are based on the assumption that the difference in deviance of nested models follows a χ^2 distribution.

In view of the hypothesis that transmission may be delayed in early pregnancy[6], we tested for an interaction between treatment delay and gestational age (both measured as continuous variables). We also examined the effect of the interval between seroconversion and amniocentesis and a positive diagnosis in infected children. An association would provide evidence of delayed transmission.

Additional analyses compared untreated with treated women and first treatment with pyrimethamine–sulphonamide *versus* spiramycin, allowing for treatment delay. Sensitivity analyses involved restriction of the data set to: (a) seroconverting women; and (b) mother–child pairs with complete data for congenital infection status.

To avoid bias, we used a statistical method[7] in which children with missing data on congenital infection status contributed to infected and uninfected groups. The contribution was determined by the likelihood ratios for test results in each child for: (1) PCR analysis of amniotic fluid; (2) specific IgM at any age in the child; and (3) specific IgG at any age. The likelihood ratios were calculated using data from children with complete data. (1) and (2) were adjusted for weeks of gestation at seroconversion. All equivocal results were considered to be negative and the three tests were assumed to be independent, given congenital infection status. Likelihood ratios were calculated for test results alone and in combination (defined as Z in calculations given below) and multiplied against the pre-test probability based on gestational age at maternal seroconversion to obtain the risk of congenital toxoplasmosis.

We avoided arbitrary assumptions about the timing of seroconversion (e.g. midpoint between the last negative and first positive test) by using an adaptation of a model described elsewhere[8]. This model considers all dates between the last negative and the first positive test (for seroconverting women) and between conception and the first positive test (for women identified by tests for recent infection). The distribution function for the interval between initial IgM and IgG positive results [defined as $R(t)$][8] describes the increased probability of seroconversion

close to the positive test date for women who were IgG negative at the first positive IgM test. For woman i, N_i denoted the duration of gestation at the last negative IgM test and P_i the duration of gestation at the first positive IgM test. $N_i = 1$ if woman i was identified by a test of recent infection and, if the first test was IgM positive but IgG negative, $N_i = -\infty$ [but $R(t)$ places a lower bound on the timing of seroconversion prior to P_i]. We defined $T(x)$ as the probability of mother to child transmission, given maternal seroconversion at time point x ($N_i \leq x \leq P_i$). We assumed that the effects of treatment and confounders on $T(x)$ were additive on a logistic scale.

The contribution to the likelihood function for a woman i who seroconverted at time point x was found by multiplying $R(P_i - x)$ if IgG antibody was found at the first positive IgM test or $1 - R(P_i - x)$ if no IgG antibody was found at the first positive IgM test with the appropriate term from the following list: for a woman identified through seroconversion and with an infected child $T(x)$, with an uninfected child $1 - T(x)$, and with a child with unknown infection status $P(Z \mid \text{infected child}) (T(x)) + P(Z \mid \text{uninfected child}) (1 - T(x))$. The corresponding terms for a woman identified by tests of recent infection with a probability θ that seroconversion occurred during pregnancy was: $\theta T(x)$, for an infected child, $1 - \theta T(x)$ for an uninfected child, $P(Z \mid \text{infected child}) (\theta T(x)) + P(Z \mid \text{uninfected child}) (1 - \theta T(x))$ for a child with unknown infection status. The complete contribution for woman i is found by taking the sum of all time points over the range N_i to P_i.

Table 2. Number of mother–child pairs enrolled and proportion of children with congenital toxoplasmosis.

Centre	Mother–child pairs enrolled		Overall proportion with congenital toxoplasmosis (%)*
	Number	Proportion identified by seroconversion (%)	
France			
Lyon	199	83	23.6
Paris	197	100	34.7
Grenoble	36	67	18.3
Marseille	95	72	22.8
Reims	34	79	23.8
Nice	47	64	15.4
Toulouse	79	87	29.7
Austria			
Vienna	315	34	7.8
Sweden			
Stockholm	40	28	10.1
Italy			
Naples	132	29	7.7
Milan	34	21	7.4
Total	**1208**	**62**	**18.5**

* Based on 1208 mother–child pairs. Percentages vary between centres due to differences in gestation at seroconversion of included women. Further details by centre are available from the authors.
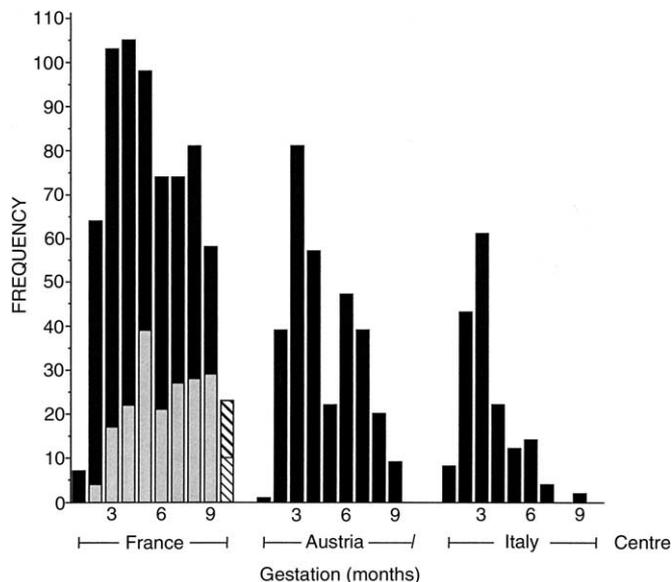
Fig. 1. The number of women is shown for each country according to month of gestation at their first positive test. In Paris, enrollment was restricted to seroconverting women (shown in grey). The diagonal shading depicts women whose first positive test was at or after delivery.

The study complied with research ethics requirements in all countries involved.

RESULTS

A total of 1260 mother–child pairs were enrolled between 1996 and 2000. Of these, 52 pairs were excluded: 15 were lost to follow up before pregnancy outcome, 11 were referred for fetal infection, 3 had inadequate con-

firmation of maternal infection, 1 was seropositive during a previous pregnancy and 22 had fetal diagnosis before starting treatment. Of the 11 twins, only the first born was included in the analyses. The total of 1208 mother–child pairs analysed included 16 miscarriages or stillbirths and 21 terminations (17 for toxoplasmosis). There were three postnatal deaths.

The mean maternal age was 28.4 years (SD 4.9). In 53% (601/1144) this was their second or subsequent delivery. Symptoms of lymphadenopathy were reported in 82 women (7%).

Table 2 shows that 62% (743/1208) of women seroconverted during pregnancy. Of these, 39% (288/743) were IgG negative and IgM positive at their first positive test indicating seroconversion close to the first positive IgM date. The remaining 38% (465/1208) were identified by tests for recent infection which included IgG and IgM positive results at the first pregnancy test and in descending order of priority: rising IgG titre in 252, low IgG avidity in 35 and other criteria in 178 (e.g. IgA positive, high IgG titre).

Figure 1 shows all mother–child pairs by country and gestational age at the first positive test. Except for Paris, where only seroconverting women were enrolled, the distribution was skewed towards early pregnancy. Figure 2 shows the number of women identified by seroconversion and tests for recent infection (rising IgG titre, low IgG avidity and other) by gestational age at first positive test, and child's infection status. Only seven infected children were born to the 465 (1.6%) women identified by tests for recent infection.

Figure 3a shows the estimated number of seroconverting women for each week of gestation. Women identified by
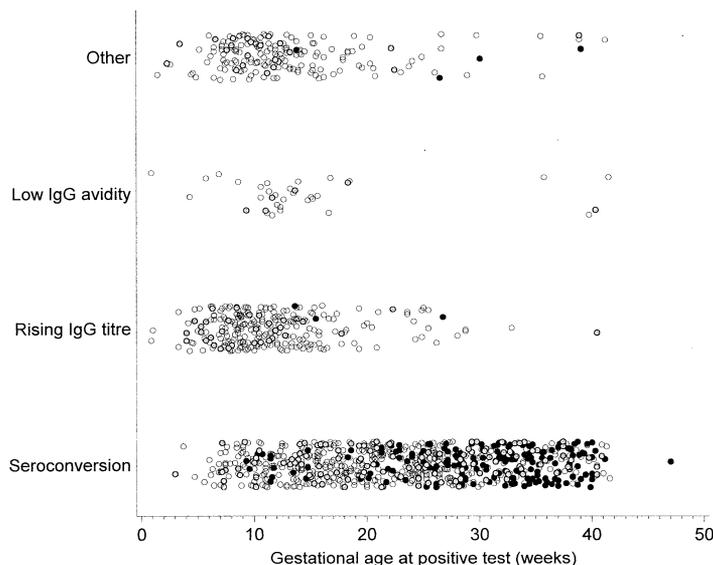

Fig. 2. The scatter plot shows mother–child pairs detected by seroconversion or by tests for recent infection defined as IgM and IgG positive and: rising IgG titre, low IgG avidity or other (e.g. IgA positive, high IgG titre). Categories are exclusive in order of priority (seroconversion excludes all others). Mother–child pairs are plotted against the weeks of gestation at the first positive test. Black dots indicate children with congenital toxoplasmosis, white dots uninfected children and grey dots children with missing data for congenital infection status.
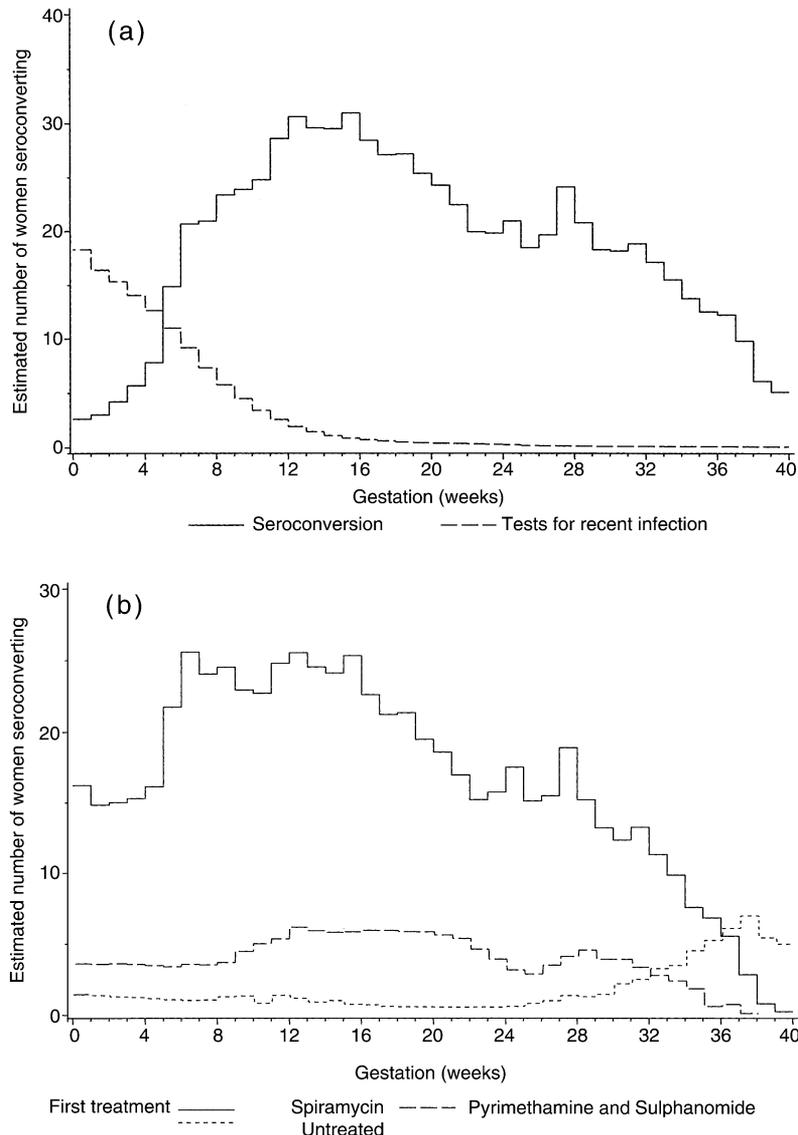
Fig. 3. The estimated number of women is plotted against the gestational age at seroconversion (weeks) according to: (a) criteria for detecting maternal infection (seroconversion or tests for recent infection); and (b) treatment status (spiramycin, pyrimethamine–sulphonamide or no treatment).

tests for recent infection largely contributed information in early pregnancy before the first prenatal test. Although the number of women enrolled by this method was large (465), their probability of postconceptional seroconversion was low. For women identified by this method before 21 weeks, the probability of postconceptional seroconversion was 27.8% (95% CI 7–75%).

Table 2 shows the proportion of children with congenital toxoplasmosis for each centre. Classification was based on complete data in 1027/1208 (85%) children of whom 17% (179/1027) had congenital toxoplasmosis. For the remaining 181 children (154 live born) with missing outcome data, the probability of congenital toxoplasmosis (24.4%, 44.2/181) was estimated using the pre-test probability of transmission based on gestational age at maternal seroconversion, combined with the likelihood ratios for PCR

results and/or postnatal serology in 159/181 children (in 22, only gestation at seroconversion was available). The risk of mother to child transmission is shown in Fig. 4: the risk was 9% (6.8–12.6) for women who seroconverted at 12 weeks of gestation and 83% (75.5–88.4) at 40 weeks of gestation. Transmission risk, adjusted for gestation at seroconversion, did not differ significantly between countries (Austria *vs* France, $P = 0.08$; Italy *vs* France $P = 0.13$).

Table 3 shows the number of women according to the type of prenatal treatment prescribed. Two-thirds were prescribed spiramycin alone and just over one-quarter were prescribed pyrimethamine–sulphonamide. There were few untreated women (8.8%, 106/1208). Similar proportions of treated and untreated women were identified by seroconversion. Of 89 women who changed from spiramycin to pyrimethamine–sulphonamide, most (77%, 69/89) had a
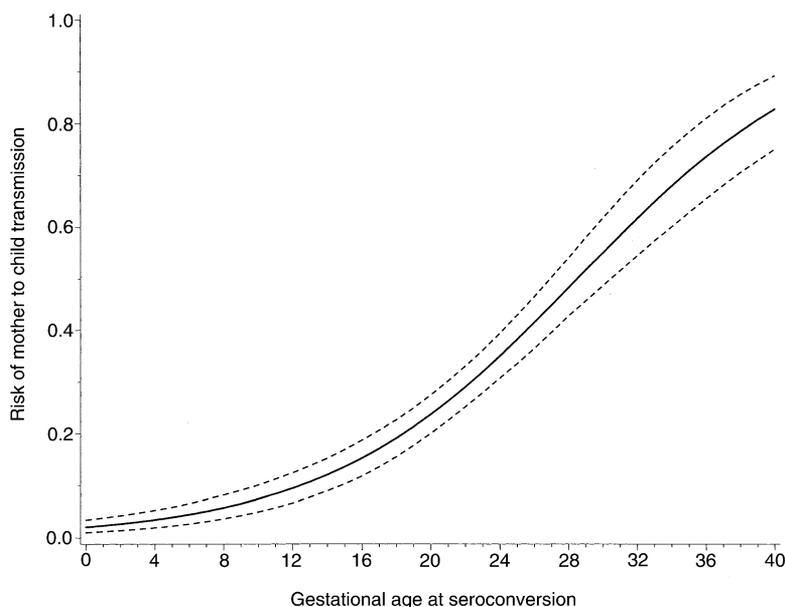
Fig. 4. The risk of mother to child transmission of toxoplasma infection is plotted against the gestational age (weeks) at maternal seroconversion (dotted lines show 95% confidence intervals). The risk of transmission for seroconverting women according to trimester was: 8% (<14 weeks), 25% (14 to <28 weeks) and 60% (28 weeks to delivery).

positive fetal diagnosis. Figure 3b shows that most of the untreated women seroconverted in the third trimester. In contrast, women first treated with spiramycin or pyrimethamine–sulphonamide seroconverted at all gestational ages with fewest in the last month of pregnancy. The median treatment delay (the interval between seroconversion and start of first treatment) for women first treated with pyrimethamine–sulphonamide was 55 days (interquartile range 33–88) and for spiramycin 29 days (interquartile range 17–46).

Women were treated promptly after their first positive serological test: median time six days (interquartile range 1–17). Treatment was stopped, at least temporarily, due to adverse effects in 24/1102 (2%) women of which 11 were on pyrimethamine–sulphonamide.

There was no evidence for an effect of treatment delay on the risk of mother to child transmission. Table 4 shows that compared with a delay of less than four weeks (between the start of treatment and seroconversion), the adjusted odds ratio for a treatment delay of four to seven weeks was 0.77 (95% CI 0.34–1.69), and for eight weeks or more was 1.33 (0.56 to 2.89). The odds ratio for mother to child transmission per week of treatment delay was 1.01 (0.93–1.08) for all women. Results were similar for seroconverting women (OR 1.01; 0.93–1.09) and for all women with complete outcome data (OR 0.99; 0.93–1.06). Tests for a range of parameterisations for the continuous effect of treatment delay (fractional polynomials)[9] found that none were significantly better than the linear model used (results not shown).

Table 3. Prenatal treatment regimen prescribed to infected pregnant women. Values are expressed as *n* or *n* (%). P–S = pyrimethamine–sulphonamide [sulphadiazine was prescribed to 293 women and sulphadoxine (Fansidar) to 29 women].

Prenatal treatment regimen	Amniocentesis performed for fetal diagnosis			Total (% seroconverting women)
	Yes		No	
	Positive	Negative		
Spiramycin *alone*	19	534	227	780 (63)
Pyrimethamine–sulphonamide				
Spiramycin *changed* to P–S	69	4	16	89 (84)
P–S prescribed first	11	111	111	233 (48)
No treatment	0	9	97	106 (58)
Total	99	658	451	1208 (62)

Table 4. Effect of prenatal treatment on mother to child transmission of toxoplasmosis. Tests for interaction between: (a) gestational age at seroconversion and treatment delay, $P = 0.84$; (b) type of treatment and treatment delay, $P = 0.23$.

Comparison	Number of women	Odds ratio	95% confidence interval
Effect of treatment delay*			
(5a) All treated women	750.3[†]		
<4 weeks	338.5[†]	1.0	
4–7 weeks	227.9[†]	0.77	0.34–1.69
≥8 weeks	183.9[†]	1.33	0.56–2.89
(5b) Seroconverting treated women	681.0[†]		
<4 weeks	323.2[†]	1.0	
4–7 weeks	241.8[†]	0.75	0.32–1.64
≥8 weeks	116.0[†]	1.45	0.57–3.24
Effect of type of treatment[§]			
(5e) All treated women			
Spiramycin	869[‡]	1	
Pyrimethamine–sulphonamide	233[‡]	1.10	0.63–1.91
(5f) Seroconverting treated women			
Spiramycin	567[‡]	1	
Pyrimethamine–sulphonamide	114[‡]	1.08	0.60–1.94
Effect of first treatment*			
(5c) All women			
Any treatment	1102[‡]	1.0	
No treatment	106[‡]	0.57	0.27–1.17
(5d) Seroconverting women			
Any treatment	681[‡]	1.0	
No treatment	62[‡]	0.59	0.26–1.25
(5e) Women who seroconverted after 27 weeks of gestation			
Any treatment	61[‡]	1.0	
No treatment	34[‡]	0.74	0.22–2.62

* Adjusted for gestational age at seroconversion and maternal age.
[†] Estimated numbers of women based on weekly probability of seroconversion.
[‡] Actual number of women.
[§] As for * and adjusted for treatment delay.

There was no difference in transmission risk between women first prescribed pyrimethamine–sulphonamide and those prescribed spiramycin. Nor was there evidence that transmission risk differed between untreated and treated women (Table 4). This last analysis mainly applies to women who seroconvert late in pregnancy (see also Fig. 3b). We failed to detect an association between the interval between seroconversion and prenatal diagnosis and a positive result ($P = 0.54$).

DISCUSSION

We found no evidence for an effect of the timing or type of prenatal treatment on the risk of mother to child transmission. The results were robust to sensitivity analyses and are consistent with previous studies that allowed for gestational age at maternal seroconversion[2–4].

The strengths of the study were prospective enrollment and data collection, the high rate of follow up and estimation of congenital infection status in mother–child pairs with missing data, all of which reduced selection bias[7].

There was also substantial variation in treatment delay for both types of treatment across all centres and gestational ages, which increased the power of the analysis and the applicability of the results. As the study was based in regional and national reference centres, it is unlikely that the lack of evidence for a treatment effect was due to suboptimal management.

Unlike previous studies[3,4,10], we included women identified by tests for recent infection to ensure that our results are applicable to the first trimester when few seroconversions occur. In routine practice, women identified in the first trimester are an important group accounting in some centres for up to 80% of all treated women (see Table 2). As many of these women acquired infection before conception, we used a statistical model to take account of the probability of seroconversion after conception.

The main limitation of the study was the failure to exclude benefits or harms which would be sufficient to change clinician practice. For example, the upper and lower confidence limits for the odds ratio for treatment delay of eight weeks or more compared with less than four weeks (OR 1.33; 95% CI 0.56–2.89) are consistent with clinically

important beneficial (a near threefold increase) and harmful (a 44% decrease) effects of treatment on mother to child transmission. Based on simulations, the study had approximately 85% power to detect an odds ratio for transmission of 1.5 in women treated after eight weeks *versus* women within four weeks of seroconversion (equivalent to an odds ratio of 1.1 per week of treatment delay). A further problem was that the comparison of treated with untreated women was limited to the few untreated women who seroconverted in the last trimester and the results may not be generalisable to the first and second trimesters. This problem was common to previous studies[2] based on routine practice[3,4,11]. Desmonts and Couvreur[12] reported a 50% reduction in transmission risk in women treated with spiramycin compared with untreated women but subsequently acknowledged that this crude comparison failed to take into account the fact that untreated women were infected later in pregnancy[11]. Given the steep rise in transmission risk, an apparent treatment effect can easily arise if women infected late in pregnancy are compared with those infected early in pregnancy without taking into account gestational age at seroconversion.

One explanation for our results is the lack of power to detect a significant effect. Another possible explanation is that transmission of parasites occurs early on before treatment is started[13]. Finally, although we found no evidence for an effect according to the type of antibiotic prescribed, the possibility remains that some antibiotics are more effective than others. We considered all women treated with pyrimethamine–sulphonamide combinations together and could not explore the effect of pyrimethamine–sulphadoxine (Fansidar) due to the small number of women treated.

CONCLUSION

We were unable to demonstrate a beneficial effect of the timing or type of prenatal treatment on the risk of mother to child transmission, but could not exclude a clinically important effect. There is now sufficient uncertainty to justify randomised controlled trials of treated *versus* untreated women[14]. In addition, studies are required to determine whether prenatal treatment has a beneficial effect on the severity of clinical manifestations of congenital toxoplasmosis and, more importantly, on the child's ability to function.

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