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Ecological comparison of the risks of mother-to-child transmission and clinical manifestations of congenital toxoplasmosis according to prenatal treatment protocol

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SUMMARY

We compared the relative risks of mother-to-child transmission of *Toxoplasma gondii* and clinical manifestations due to congenital toxoplasmosis associated with intensive prenatal treatment in Lyon and Austria, short term treatment in 51% of Dutch women, and no treatment in Danish women. For each cohort, relative risks were standardized for gestation at seroconversion. In total, 856 mother–child pairs were studied: 549 in Lyon, 133 in Austria, 123 in Denmark and 51 in The Netherlands. The relative risk for mother-to-child transmission compared to Lyon was 1·24 (95% CI: 0·88, 1·59) in Austria; 0·59 (0·41, 0·81) in Denmark; and 0·65 (0·37, 1·01) in The Netherlands. Relative risks for clinical manifestations compared with Lyon (adjusted for follow-up to age 3 years) were: Austria 0·19 (0·04, 0·51); Denmark 0·60 (0·13, 1·08); and The Netherlands 1·46 (0·51, 2·72). There was no clear evidence that the risk of transmission or of clinical manifestations was lowest in centres with the most intensive prenatal treatment.

INTRODUCTION

Routine testing of pregnant women to detect and treat toxoplasma infection is offered in many European countries [1–4]. However, evidence for the effectiveness of prenatal treatment on the risk of mother-to-child transmission of infection and on neurological or visual manifestations in infected children, is limited. Two systematic reviews showed that no controlled trials have been conducted [5, 6] and most of the observational studies lacked an untreated comparison group as almost all infected women identified prenatally were treated [7]. However, one recent study [8] showed no evidence for an effect of prenatal treatment on mother-to-child transmission but a reduction in the risk of neurological signs at 1 year of age.

As there are substantial differences in the screening and treatment protocols operating in different European centres, comparison of the risks of transmission and clinical manifestations between centres provides indirect evidence for the relative effectiveness of different testing and treatment protocols. We therefore sought cohort data from four centres operating different protocols. For example in Lyon, France, susceptible, IgG-negative women were retested monthly to detect seroconversion, compared with 3- monthly in Austria and The Netherlands (the

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Table 1. *Testing, treatment and examination protocols in study centres*

Centre and period of study	Re-test interval for IgG negative women*	Prenatal treatment		Postnatal treatment for congenital infection	Age at postnatal follow-up†
		After diagnosis of maternal infection	After diagnosis of fetal infection		
Lyon 1987–95	Monthly	(1) Spiramycin alone§¶ (2) P&S alternating 3 weekly with spiramycin if infection acquired after 32 weeks gestation§¶	P&S alternating 3 weekly with spiramycin§¶	P&S for 3 weeks; then spiramycin until weight >5 kg, then fansidar for 12 months‡‡	*Paediatric examinations*: at $\leqslant 2$ weeks, then at 2, 5, 8, 12, 15, 18, 24, 30, 36 months, then annually *Ophthalmoscopy* (direct/indirect): at $\leqslant 2$ weeks, 2, 5, 8 and 12 months, then annually. *Cranial imaging*: ultrasound and skull x-ray at $\leqslant 2$ weeks.
Austria 1992–5	12, 20, 32 weeks	(1) Spiramycin alone if <16 weeks gestation** (2) P&S alternating 4 weekly with spiramycin§**	(1) Fetal infection: P&S alternating 4 weekly with spiramycin§** (2) Fetus uninfected: spiramycin	*With manifestations*: P&S for 6 months, then P&S alternating 4 weekly with spiramycin for 6 months§§ *No manifestations*: P&S alternating 6 weekly with spiramycin for 12 months§§	*Paediatric examinations*: at birth, 3, 6, 9 and 12 months and then annually. *Ophthalmoscopy* (indirect): at $\leqslant 3$ months, 12 months, then annually. *Cranial imaging*: ultrasound $\leqslant 3$ months; skull x-ray if ultrasound abnormal.
Denmark 1992–6	8 to 12 weeks and 1 week postnatal‡	None	None	P&S alternating 4 weekly with spiramycin for 28 weeks¶¶	*Paediatric examinations*: at $\leqslant 6$ weeks, at 3, 6, 9 12 months then annually. *Ophthalmoscopy* (indirect): at $\leqslant 6$ weeks, 12 months, then annually. *Cranial imaging*: ultrasound and skull x-ray $\leqslant 6$ weeks.

Centre and period of study	Re-test interval for IgG negative women*	Prenatal treatment		Postnatal treatment for congenital infection	Age at postnatal follow-up†
		After diagnosis of maternal infection	After diagnosis of fetal infection		
The Netherlands 1987–8	18, 24, 32, 36 weeks and at birth	Spiramycin and sulfadiazine for 3 weeks†† then no treatment for 2 weeks (repeated once).	As for maternal infection	Not treated	*Paediatric examinations*: at birth, unspecified intervals during the first year, then annually. *Ophthalmoscopy* (indirect): at least once $\leqslant$ 12 months, then annually. *Cranial imaging*: ultrasound and skull x-ray at $\leqslant$ 12 months.

P&S, pyrimethamine and sulfadiazine.

* Recommended re-test interval for IgG negative women. Actual dates at last negative and first positive IgG test for each individual woman were included in the analysis.

† Recommended schedule for follow-up. Actual dates at examination used in the analysis.

‡ Based on neonatal filter paper sample.

Prenatal treatment:

§ Continued until delivery.

¶ Lyon: Pyrimethamine (50 mg/day); sulfadiazine (3 g/day) plus folinic acid. Spiramycin (3 g/day).

** Austria: Pyrimethamine (50 mg first dose, then 25 mg/day); sulfadiazine (1·5 g first dose, then 0·75 g/day) plus folinic acid. Spiramycin (3 g/day).

†† Netherlands: Spiramycin 3 g/day, sulfadiazine 3 g/day.

Postnatal treatment:

‡‡ Lyon: Pyrimethamine (3 mg/kg every 3 days), sulfadiazine (75 mg/kg per day) plus folinic acid. Spiramycin (125 mg/kg per day). Fansidar consists of pyrimethamine (6 mg/kg every 10 days) and sulphadoxine (125 mg/kg every 10 days).

§§ Austria: Pyrimethamine (1 mg/kg per day), sulfadiazine (85 mg/kg per day) plus folinic acid. Spiramycin (100 mg/kg per day).

¶¶ Denmark: Pyrimethamine (1 mg/kg per day), sulfadiazine (100 mg/kg per day) plus folinic acid. Spiramycin (100 mg/kg per day).

latter was a research study and is not routine practice) [9]. The type of treatment and dosage regimens also varied, and in Denmark, infected women were identified by retrospective testing of stored prenatal samples and received no prenatal treatment [10]. However, comparisons between centres may be misleading if they do not take account of differences in the distribution of gestation at maternal infection, a factor that strongly affects the risks of transmission and manifestations [11]. In addition, women referred due to suspected abnormalities in the fetus or neonate, should be excluded, because selection bias favouring inclusion of children with abnormalities due to congenital toxoplasmosis will exaggerate estimates of the risks of transmission and clinical manifestations for children of women identified by screening.

We analysed individual patient data for cohorts of infected women and their children from four centres (Lyon, Austria, Denmark, The Netherlands). All four centres achieved high rates of follow-up. Collection of precise dates of key events made it possible to minimize selection bias and to standardize the risk estimates for gestation at maternal seroconversion.

METHODS

For each centre, we included pregnant women diagnosed with toxoplasma infection during a time period when: (a) screening was routine; (b) standard treatment and follow up protocols were in operation; (c) the rate of follow up beyond 12 months postnatal age was consistently high (greater than 90%); and (d) local databases allowed identification of all women diagnosed with toxoplasma infection. Only women with evidence of seroconversion during pregnancy (change from undetectable to detectable toxoplasma-specific IgG antibodies) were included. The serological tests used varied between centres and have been summarized elsewhere [9–12]. In brief, in Lyon, diagnosis of seroconversion was based on a commercial ELISA run in parallel with an automated immunofluorescent assay for IgG [11]; in Austria, on the dye test or, if not performed, an immunofluorescent antibody test; and in The Netherlands, on an in-house ELISA [13, 14]. In Denmark, infected women were identified by retrospective testing of stored prenatal samples after detection of specific IgG on newborn filter paper blood samples. Negative prenatal samples were confirmed by the dye test [10]. In all centres, detection of specific IgG or IgM antibodies was confirmed by additional tests for these antibodies.

Criteria for diagnosing congenital toxoplasmosis were the persistence of specific IgG beyond 12 months, or in non-live births, a positive PCR or culture result based on the analysis of amniotic fluid or fetal products. Absence of congenital infection was defined by undetectable specific IgG antibodies in the absence of treatment. Clinical manifestations were defined as the detection of intracranial calcification, hydrocephalus or retinochoroiditis postnatally or, in non-live births, by fetal ultrasound or at autopsy.

In an effort to minimize selection bias, datasets were scrutinized for mother–child pairs referred due to infection or manifestations in the fetus or child. All mother–child pairs not meeting the local centre criteria for seroconversion, or with non-sequential dates for seroconversion, fetal diagnosis, and detection of fetal or neonatal anomalies, were excluded. Pregnancies terminated for toxoplasma infection were included. Terminations for other reasons, or pregnancies ending in spontaneous abortion or for which congenital infection status was not known were recorded but excluded from the analysis.

Protocols for testing, treatment and follow up of mother–child pairs are summarized in Table 1 and have been reported in detail [9–12]. Except for The Netherlands, all centres treated infected children for 6–12 months postnatally. Consequently, we could investigate the effect of prenatal but not postnatal treatment on the risk of clinical manifestations, as few infected infants were not treated.

Statistical analysis

It was necessary to adjust for the effect of gestation at maternal infection in the analysis since this varied between centres (Fig. 1) and has a strong effect on the risk of both transmission and clinical signs [11]. This was achieved by indirect standardization [15] using gestation-specific risk estimates previously derived from the Lyon cohort (reference population) [11]. With these estimates and the observed dates for the last negative and first positive IgG tests, the expected number (i.e. assuming Lyon-specific risk) of congenital infections and children with clinical manifestations was calculated for each centre. The ratio of observed to expected numbers of cases gives a relative risk, which is adjusted for gestational age at maternal infection. An additional complication is caused by the

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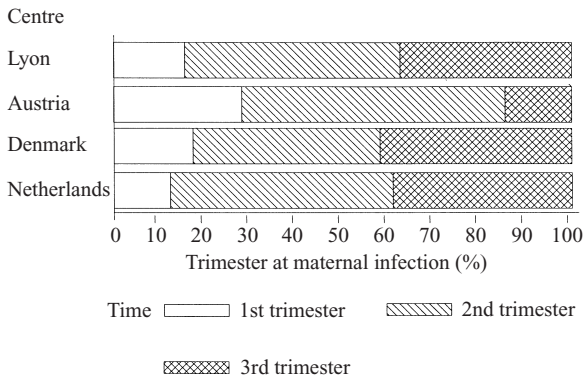

Fig. 1. Estimated proportions of women seroconverting during the first, second and third trimesters according to centre.

fact that maternal seroconversion could have occurred at any time between the last negative and first positive serological tests. We assumed that maternal infection was equally likely throughout this interval and estimated the expected numbers of cases based on an average of the risk for the period between the last negative and first positive test dates. Also, as the duration of clinical follow-up was variable, the risk of clinical signs was standardized to age 3 years using the Kaplan–Meier method. Confidence intervals (CIs) were derived by assessing statistical uncertainty in the observed number of events, using exact binomial methods or standard errors from the Kaplan Meier analysis.

If the assumption that maternal infection was equally likely throughout pregnancy was wrong, the resulting error would have been greatest in the Danish cohort, for which the interval between the last negative and first positive test was longest. For example, if the incidence of maternal infection decreased during pregnancy, the adjusted relative risk of transmission would have been underestimated and the risk of manifestations overestimated.

RESULTS

The number of mother–child pairs identified by seroconversion was 592 in Lyon, 135 in Austria, 133 in Denmark and 52 in The Netherlands. The number of infected pregnancies ending in a live birth or termination and for which data on congenital infection status were available was 549 (93%), 133 (99%), 123 (92%), and 51 (98%) respectively. The median postnatal follow-up ranged from 15 months in Austria to 54 months in Lyon and The Netherlands. Numbers of seroconverting women, infected children and infected children with manifestations are summarized in Tables 2 and 3. The proportion of women treated prenatally was 94% (516/549) in Lyon, 92% (122/133) in Austria, 0% in Denmark and 51% (26/51) in The Netherlands. In Lyon, where the type of treatment depended on gestation at infection and the results of fetal diagnosis, pyrimethamine-sulfadiazine was prescribed to 19% (108/564) of the seroconverting women and 39% (61/156) of the women with an infected child/fetus.

The crude transmission risk was similar in all centres, ranging from 21 to 28%. However, there were marked differences between the relative risks of transmission adjusted for weeks of gestation at maternal infection. In Denmark, where no women were treated, and in The Netherlands, where only 50% were treated, the adjusted risks of transmission were lower than in Lyon and Austria where over 90% of women were treated (upper limit 95% CI for Denmark and The Netherlands 0·81 and 1·01 respectively). The relative risk of transmission was similar in Austria and Lyon.

The estimated risk of clinical manifestations by age 3 years ranged from 9 to 33% and was significantly lower in Austria (adjusted relative risk 0·19 (0·04, 0·51) compared with Lyon. The adjusted relative risks of clinical manifestations in Denmark and The Netherlands did not differ significantly from Lyon, although in Denmark the upper confidence limit was 1·08. The adjusted relative risk estimates were imprecise, due to the small number of affected children and the fact that not all children were followed up to 3 years.

DISCUSSION

Unexpectedly, we found no evidence that the risk of mother-to-child transmission of infection was lower in centres operating the more intensive prenatal treatment protocols (defined by the frequency of serological testing of susceptible women, the use of pyrimethamine-sulfadiazine, treatment dosages and the proportion of women treated). The pattern of results was inconsistent. The risk of transmission was lowest in Denmark, where women were not treated and the risk of clinical manifestations was lowest in Austria, where women received intensive treatment.

The strengths of our study are that we attempted to minimize selection bias by excluding women identified on the basis of infected or affected fetuses or children, we allowed for the variation in gestation at maternal

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Table 2. *Centre-specific estimates of the risk of mother to child transmission of* Toxoplasma gondii

Centre	Number of seroconverting women	Proportion of seroconverting women treated (%)	Number of congenital infections (%)	Relative risk of transmission	
				Unadjusted	Adjusted (95% CI)
Lyon	549	94	156 (28)	1·00	1·00
Austria	131	90	34 (26)	0·92	1·24 (0·88, 1·59)
Denmark	123	0	26 (21)	0·74	0·59 (0·41, 0·81)
Netherlands	51	51	12 (24)	0·83	0·65 (0·37, 1·01)

seroconversion, and we adjusted for the differential length of follow up. One explanation for the lack of an effect of prenatal treatment is that transmission occurs predominantly during the parasitaemic phase of maternal infection which usually ceases as soon as the woman develops a serological response [16–19]. If true, treatment would inevitably be given too late to prevent transmission. However, interpretation of differences in the risk of transmission should be made with caution as differences attributable to prenatal treatment may have been masked by other centre-specific factors. For example, the lower risks of transmission in Denmark and The Netherlands compared with Lyon and Austria, may have been due to different cut-offs for serological tests, or to failure to exclude selection bias in Lyon and Austria. Alternatively, there may have been underlying geographical variations in the risk of transmission, possibly attributable to variation in the source of ingested tissue cysts (undercooked meat) or oocysts (excreted by cats and ingested in contaminated soil, food or water [20]). The parasite load released from a single tissue cyst is many fold higher than for oocysts [16], and in animal experiments, parasite inoculum has been strongly associated with the risk of mother-to-fetus transmission [16].

The significantly lower risk of clinical manifestations in children followed in Austria should be interpreted cautiously in view of the retrospective identification of this cohort and the small number of infected ($n = 34$) and affected ($n = 3$) children. The results may reflect a beneficial effect of prenatal treatment with pyrimethamine-sulfadiazine, prescribed to 90% of women, on the risk of clinical manifestations in infected children. Unlike spiramycin, pyrimethamine-sulfadiazine cross the placenta [16] and the fetal blood–brain barrier [21]. However, if prenatal treatment with pyrimethamine-sulfadiazine is effective, we would have expected to see the highest risk of clinical manifestations in Denmark, where no women were treated. An intermediate risk would have been expected in The Netherlands, where 51% of women were treated with spiramycin and sulfadiazine, and in Lyon, where 94% of women were treated prenatally but only 39% of women with infected fetuses received pyrimethamine-sulfadiazine.

Two other studies have analysed the effect of prenatal treatment after adjustment for gestation at maternal infection. The results are contradictory. Foulon et al. [8] reported that prenatal treatment had no effect on mother-to-child transmission of infection, but significantly reduced the risk of manifestations at 1 year of age. These findings, based on 144 consecutive, infected women referred to five fetal medicine centres, may represent a beneficial effect of treatment or may be explained by selection bias favouring inclusion of untreated women referred due to suspected abnormalities in the fetus or child. In a separate analysis of clinical manifestations in 181 infected children born to 704 women followed in Lyon (of whom 549 seroconverters are included in the present report), we found no evidence of a difference in the risk of clinical manifestation at 3 years in children whose mothers were treated prenatally with pyrimethamine-sulfadiazine compared with spiramycin or no treatment (adjusted odds ratio 0·89 95% CI: 0·41–1·88) [22]. Selection bias was minimized in the Lyon cohort by exclusion of women referred due to suspicion of infection or clinical manifestations in the fetus [11, 22].

There is no clear evidence from biological studies that treatment reduces the risk of clinical manifestation of congenital toxoplasmosis. Neither spiramycin nor pyrimethamine-sulfadiazine are effective against the encysted, bradyzoite form of the parasite [23, 24]. Fetal infection and tissue damage is caused by the tachyzoite form of the parasite, but transformation to bradyzoite starts to occur within days of infection [16, 25, 26], probably coinciding with serological and cell mediated responses [16, 25]. Consequently, as prenatal treatment is always given sometime after the maternal serological response, this

Table 3. *Centre-specific estimates of the risk of clinical manifestations in children with congenital toxoplasmosis*

Centre	Number of infected fetuses*	Median follow-up (months)	Number of children with clinical manifestations†				Estimated risk (%) of any manifestation by 3 years	Relative risk of any manifestation by 3 years	
			H	ICC	RC	Any sign*		Unadjusted	Adjusted (95% CI)
Lyon	156 (5)	54	4	15	33	42 (2)	22	1·00	1·00
Austria	34 (1)	15	0	2	3	3 (0)	9	0·41	0·19 (0·04, 0·51)
Denmark	26 (0)	38	1	2	4	5 (0)	21	0·95	0·60 (0·13, 1·08)
Netherlands	12 (0)	54	0	1	3	4 (0)	33	1·51	1·46 (0·51, 2·72)

* Figure in parenthesis denotes the number of planned terminations included in the total.
† H, hydrocephalus; ICC, intracranial calcification; RC, retinochoroiditis.

may be too late to prevent manifestations. A further possibility is that cell damage in the brain and eye is due principally to the immune response to infection [27–29] rather than destruction by the parasite. The effect of antibiotics on these immune processes in the fetus is unknown.

Finally, there is a lack of information on the association between intracranial lesions and subsequent developmental impairment. Further studies are required to determine the effect of prenatal treatment on visual and developmental impairment in childhood.

Our findings provide no evidence that prenatal treatment reduces the risks of mother to child transmission of toxoplasma infection. The low risk of clinical manifestations in Austria may be explained by the widespread use of pyrimethamine-sulfadiazine in infected women. However, further studies are needed to confirm or refute these findings.

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