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MAJOR ARTICLE

Efficacy of Rapid Treatment Initiation Following Primary *Toxoplasma gondii* Infection During Pregnancy

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Background. Treatment of *Toxoplasma gondii* infection acquired during pregnancy differs in many countries. In Germany, spiramycin is given until the 16th week of pregnancy, followed by at least 4 weeks of combination therapy with pyrimethamine, sulfadiazine, and folinic acid independent of the infection stage of the fetus. If infection of the fetus is confirmed by polymerase chain reaction or if fetal ultrasound indicates severe symptoms (hydrocephalus, ventricular dilation), treatment is continued until delivery with regular monitoring of pyrimethamine and sulfadiazine concentration in maternal blood and observation of possible adverse effects. In other European countries, such as France, only spiramycin is given unless infection of the fetus is proven.

Methods. To evaluate the effectiveness of the German treatment scheme, a retrospective analysis of 685 women who showed a serological constellation consistent with primary infection in pregnancy and their children was performed.

Results. We found an increased transmission rate to the fetus with increased time in gestation and a decreased risk of clinical manifestations. In comparison with studies performed in other countries, the overall transmission rate (4.8%) and the rate of clinical manifestations in newborns (1.6%) were lower.

Conclusions. Use of spiramycin from time of diagnosis of acute acquisition of infection by the pregnant woman until week 16, followed by pyrimethamine, sulfadiazine, and folinic acid for at least 4 weeks in combination with a standardized follow-up program is efficient in reducing transplacental transmission of the parasite and the burden of disease in the newborn.

Toxoplasma gondii, the causative agent of toxoplasmosis, infects a wide range of mammalian hosts, including humans. The consumption of meat contaminated with cysts of *T. gondii* or the ingestion of oocysts through contact with contaminated soil or water are the major risk factors for transmission of the parasite [1, 2]. Primary infection during pregnancy might result in congenital toxoplasmosis of the newborn. The burden of congenital toxoplasmosis has been estimated as 620 disability-adjusted life-years per year [3]. Diaplacental infection of the fetus occurs at a frequency of 5%–30% among pregnant women with primary infection in Europe, and this risk increases continuously with gestational age [4–7]. In contrast, the severity of clinical symptoms is worst when infection occurs during the first or second trimester, especially if untreated. To date, the impact of prenatal therapy on the outcome for the child has been controversial [6, 8]. A recent meta-analysis based mainly on French studies showed only a slight influence of early therapy for transmission of *T. gondii* to the fetus and no significant decrease of clinical manifestations in neonates [6]. In Germany, it is generally recommended to treat infection during pregnancy with spiramycin until the 16th week of pregnancy (WOP) and, thereafter, with the combination of pyrimethamine plus sulfadiazine plus folinic acid (PSF) for at least 4 weeks [9]. If ultrasound indicates severe symptoms in the fetus, therapy is continuously given until delivery, with regular drug monitoring and control of possible adverse effects. Here, we report the

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efficacy of this treatment scheme in a retrospective study that was performed with 685 pregnant women with primary infection during pregnancy.

METHODS

Study Population

A standardized follow-up program of toxoplasmosis acquired during pregnancy has been established at the Advisory Laboratory for Toxoplasmosis in Sindelfingen, Germany, and was the basis for this study. A total of 808 preselected women who voluntarily underwent toxoplasmosis screening and who presented from July 1995 through February 2008 with serological evidence of primary infection during pregnancy were initially enrolled (Figure 1). An in-house immunoblot assay was used for detection of *Toxoplasma* p18-specific immunoglobulin G (IgG) antibodies; this antigen is most common in bradyzoites and is therefore a marker for the chronic infection phase (p18 positivity: 0–3 months after infection, 0%; 3–6 months after infection, 51%; and 6–18 months after infection, 82% [11]). In the case of an ambiguous result in the in-house immunoblot that is not compatible with other results, the recomLine blot was used; by this test, high IgG avidity against SAG1 proved to be an infection for >6 months. Because prenatal amniocentesis and polymerase chain reaction (PCR) are not obligatory in the diagnosis of prenatal toxoplasmosis in Germany, they were only performed in 83 of 685 (12.1%) women and were based on the decision of the gynecologists and the affected women. Criteria for acute toxoplasmosis and inclusion or exclusion in the follow-up program are listed in Figure 1. An IgG seroconversion in pregnancy was observed in 94 of 685 (13.7%) women. A total of 123 of the original women were excluded from the study: 2 women decided to terminate their pregnancy because of (1) cancer with chemotherapy and (2) depression with a suicide attempt. In both cases, a possible fetal *T. gondii* infection was not determined. Seven additional women had spontaneous abortions. In 2 of these, the result of *Toxoplasma*-specific PCR performed on fetal tissue was negative. One additional woman was excluded because the final outcome was unclear; although the PCR result of amniotic fluid obtained at 20 WOP was positive for *T. gondii*, and the fetal ultrasound showed ventricular extensions, a further observation during pregnancy was not possible. Likewise, no data were available on the outcome of the child. Furthermore, 113 women were excluded because their serological findings suggested an infection that most likely occurred just before pregnancy, and it was not clear whether these infections were relevant for pregnancy.

Determination of congenital infection of the newborn followed the criteria of Lebech et al [12] (Figure 1).

Treatment

Prenatal therapy was conducted up to the beginning of the 16th WOP with spiramycin (3 × 3 million IU/day), followed by a combination of pyrimethamine (day 1: 50 mg; thereafter: 25 mg/day) plus sulfadiazine (<80 kg body weight: 3 g/day; ≥80 kg body weight: 4 g/day) plus folinic acid (10–15 mg/week) for 4 weeks. This treatment was extended (1) up to 6 weeks, when the maternal infection occurred after 16th WOP; (2) up to the beginning of 36th WOP, when results of PCR of amniotic fluid were positive, or (3) until delivery, when clinical manifestations were observed in utero (eg, hydrocephalus/ventricular extensions). Treatment of congenitally infected, asymptomatic newborns was given with pyrimethamine (1 mg/kg body weight/day) plus sulfadiazine (50 mg/kg body weight/day) plus folinic acid (2 × 3 mg/week) for 3 months. Newborns with discrete symptoms (minor ventricular dilatations or discrete intracranial calcifications with normal neurological state, retinal scars without inflammatory foci) received the PSF combination with a higher dose of sulfadiazine (100 mg/kg body weight/day) for 6 months. An extension of this scheme to 12 months is usually foreseen for children with severe symptoms (seizures, pathological neurological state, retinochoroiditis). However, none of the children in our study population were severely affected. In all cases, pyrimethamine and sulfadiazine blood concentrations were regularly determined and possible adverse effects were controlled.

Follow-up of Infected Children

Information about clinical symptoms and serological test results from infected children were collected at birth from the maternity hospitals and from pediatric examinations that were performed until at least 2 years of age. Clinical manifestations of the infected children were recorded by ultrasound in utero and at birth and by computed tomography (CT) scan at birth, especially when ultrasound was conspicuous. Additional CT scans and ophthalmologic examinations within the first year of life relied on the decision of the attending pediatrician. In about half of these cases, the ophthalmologic examination was repeated every 6 or 12 months by an ophthalmologist.

Analysis

On the basis of the serological information from sequentially obtained blood samples using the battery of serological methods (Figure 1), we determined the time of infection and classified the mothers and their children into the following groups: infection occurring during (1) 1–12 WOP, (2) 13–24 WOP, and (3) 25 WOP to birth.

RESULTS

Study Population and Congenital Infections

From the 685 women who were finally included in the study, 33 children (4.8%) were born with serological evidence of

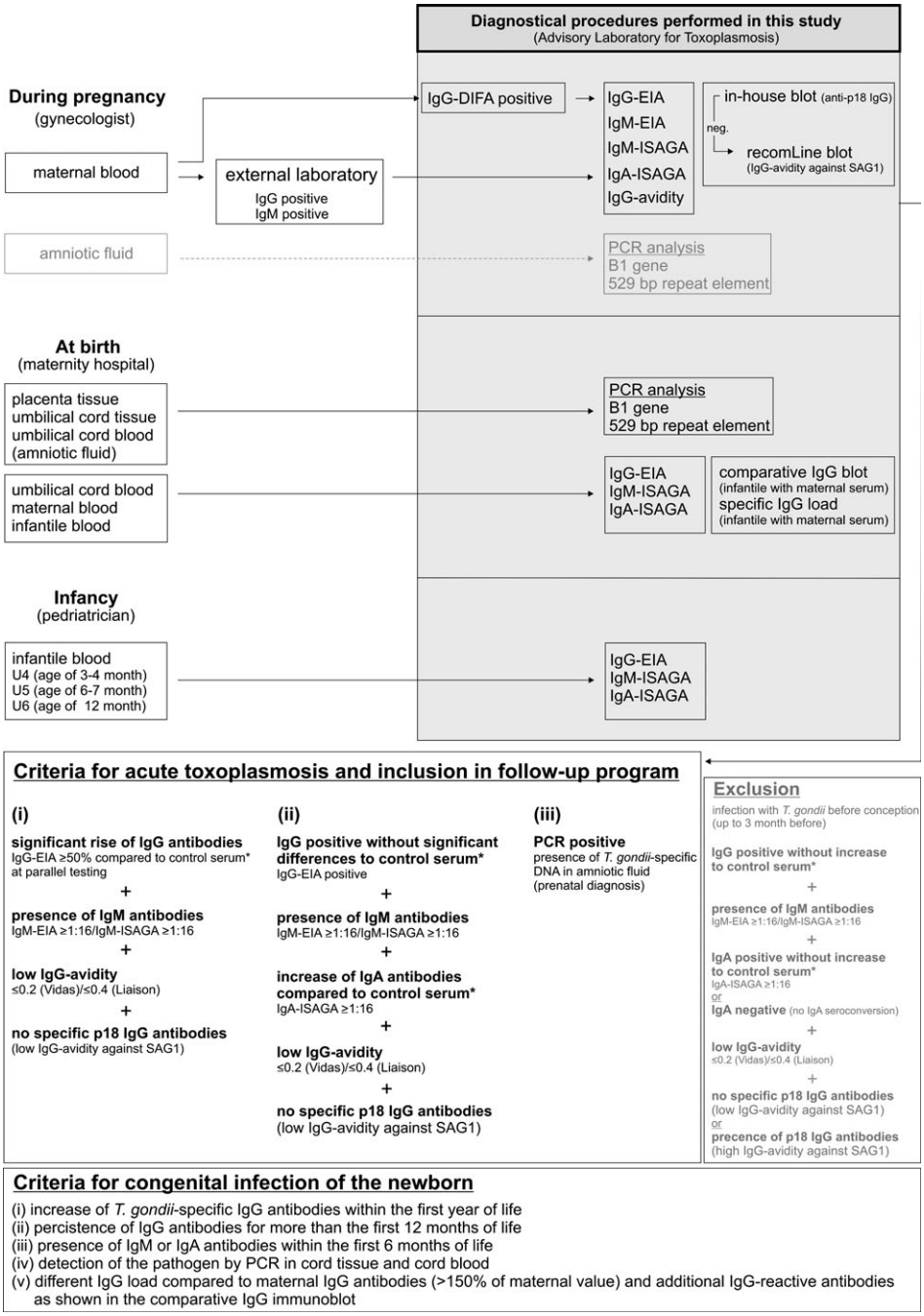

Figure 1. Follow-up program and diagnostic criteria for acute toxoplasmosis during pregnancy and for congenitally infected newborns. Diagostic tests: immunoglobulin G (IgG)–DIFA (direct immune agglutination-fixation assay; Ravo Diagnostika), IgG/IgM-EIA (enzyme immunoassay; Liaison, DiaSorin), IgM/IgA-ISAGA (immunosorbent agglutination assay; ToxoTool M-I/A-I ISAGA; Ravo Diagnostika), IgG-avidity (1995–September 2002: Mini Vidas, bioMérieux; since August 2002: Liaison, DiaSorin), recomLine blot (since July 2003: Mikrogen), comparative IgG blot (BioRépair), and specific IgG load, followed by the methods of Remington et al [10]. *The control serum sample was obtained during the previous 2–3 weeks. Abbreviation: PCR, polymerase chain reaction.

congenital *T. gondii* infection. All 685 mothers had received at least gestational PSF treatment. The tolerance of spiramycin and the PSF combination therapy was analyzed in 140 of 685 women (20%): 64 (45.7%) received spiramycin followed by PSF; the other 54.3% received only PSF. No adverse effects were observed for spiramycin, whereas 25 of 119 (21%) PSF-treated women experienced predominately nausea. Only 1 woman showed hypersensitivity against sulfadiazine, which consequently was exchanged with spiramycin.

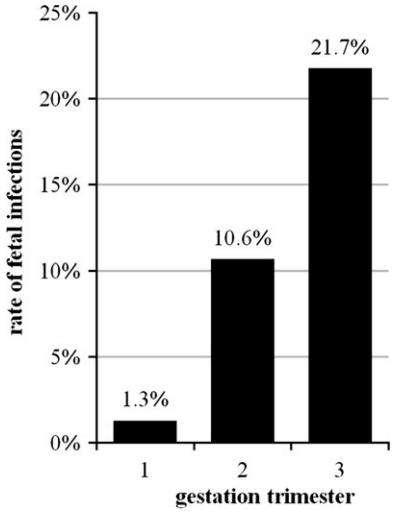

Figure 2. Rate of *Toxoplasma gondii* transmission to the child, by the gestational age at which the mother was infected (N = 685).

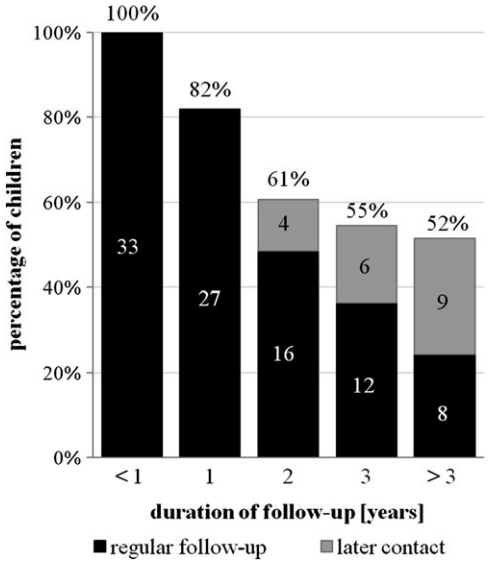

Figure 3. Duration of follow-up of children with congenital *Toxoplasma gondii* infection, by the regular follow-up program and after contacting the families for this study. At the time of the last contact (2010), 31 of 33 children were >3 years of age and 2 children were >2 years of age. Cumulative percentages are given. Numbers of affected children are shown in the columns.

In an analysis of the association between time of maternal infection and transmission of *T. gondii* to the fetus, the risk for congenital infection was 1.3% (6 of 479 children) during the first trimester and increased to 10.6% (17 of 160 children) during the second trimester and to 21.7% (10 of 46 children) when the mother was infected during the third trimester (Figure 2). When the transmission rates were determined only in the group of women with seroconversion (n = 94), similar results were obtained: 8.3% (2 of 24 children) during the first trimester, 9.6% (5 of 52 children) during the second trimester, and 22.2% (4 of 18) during the third trimester. Analyzing the transmission rate by detection of toxoplasma DNA in amniotic fluid samples from 83 of 685 (12.1%) women showed a similar risk for congenital infection of 1.6% (1 of 61) during the first trimester. However, when amniocentesis and PCR would have been used for calculations, the risk of transmission would be 20% (4 of 20 children) during the second trimester and 50% (1 of 2 children) during the third trimester. On the basis of the χ^2 test, we found no statistically significant differences in the results in all 3 trimesters ($P > .2$). Congenital infection occurred in 15 girls and 17 boys; the sex of 1 infected child was not documented.

All 33 congenitally infected children were examined in utero and at birth. During the first year of life, 6 (18%) of them were lost to follow-up. An additional 11 children were lost to follow-up between their first and second year of life (Figure 3). The median duration of regular follow-up was 2 years (95% confidence interval [CI], 0.0–9.0 years). In 9 of 25 (36%) cases that were lost during the total period of follow-up, we were able to contact the families during 2010 and obtain information about the child (Figure 3).

Clinical Manifestations

As mentioned above, all 33 congenitally infected children were examined in utero and during the first year of life, especially for typical signs of congenital toxoplasmosis, such as hydrocephalus or ventricular dilatation, intracranial calcifications, and ocular lesions indicative of retinochoroiditis or scars. A total of 11 children (1.6%) born to the 685 women with primary *T. gondii* infection acquired during pregnancy showed such typical clinical symptoms. Considering the number of 33 children with congenital infection, these 11 symptomatic children represented a rate of 33.3%. The probability for clinically symptomatic infection in the child was highest when the mother was infected during 13–24 WOP (7 of 17 [41.2%]). The least risk was when the mother acquired the infection during the third trimester (2 of 10 [20%]) (Figure 4).

The 33 women who had given birth to an infected child could be classified into 2 different treatment groups (Table 1). All children in the first group (n = 4), who were treated with spiramycin followed by the PSF combination, were asymptomatic. The second group of women (n = 29) had received only PSF because their infection was diagnosed later than the 16th WOP. All symptomatic children (n = 11) originated from this group. Of note, late initiation of therapy (>8 weeks after infection of the mother) had a negative impact on the rate of clinical manifestations in the respective children; their risk for developing clinical manifestations was 4 times higher compared

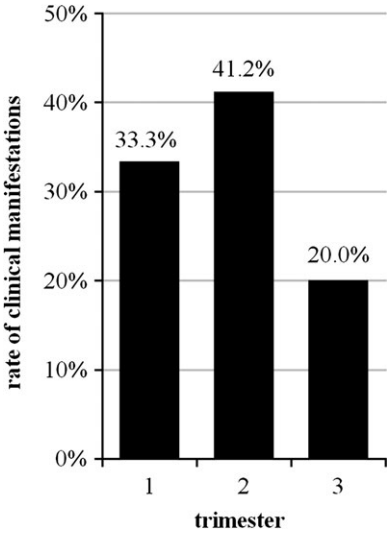

Figure 4. Rate of clinically symptomatic children, by the gestational age at which the mother was infected. The types of clinical manifestations are indicated in Table 2.

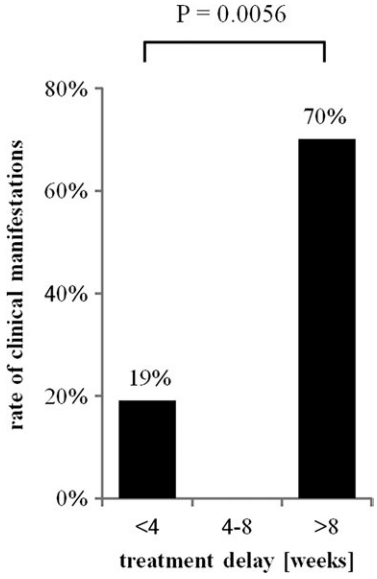

Figure 5. Treatment delay has a negative impact on the rate of clinical manifestations in infected newborns. The time from maternal infection to initiation of therapy was calculated on the basis of serological findings. The duration was divided in periods of 4 weeks each.

with children born to mothers who received the therapy within 4 weeks after infection (Figure 5).

Discrete abnormalities of the brain (hydrocephalus/ventricular dilatation) recorded by ultrasound examinations during pregnancy were identified in 3 of 11 (27.3%) children. All of them were born to women who had experienced a delay in initiation of therapy (>8 weeks) (Table 2). However, even under delayed prenatal treatment, a reduction of symptoms was observed by subsequent ultrasound examinations, and a CT scan of the brain within 6 months of life showed in all 3 children an involution of their clinical symptoms.

Most of the symptomatic children (4 of 11 [36.4%]) had only minor intracranial calcifications; in 3 of 11 children (27.3%), retinochoroiditis/scars were the sole clinical manifestation within the first year of life (Table 2, Figure 6). One of the 11 symptomatic children was asymptomatic at birth and during

Table 1. Impact of the Time From Maternal Infection to Initiation of Therapy on the Newborn

Trimester of Infection	1	2	3	1	2	3	1	2	3
Treatment Delay	<4 Weeks			4–8 Weeks			>8 Weeks		
Spiramycin followed by PSF (pyrimethamine + sulfadiazine + folinic acid)									
Total infections	4	0	0	0	0	0	0	0	0
	(2/2)								
		4			**0**			**0**	
Infections with clinical symptoms	0	0	0	0	0	0	0	0	0
		0			**0**			**0**	
Only PSF									
Total infections	0	9	8	0	1	1	2	7	1
		(3/6)	(4/4)		(1/0)	(0/1)	(2/0)	(3/4)	(0/1)
		17			**2**			**10**	
Infections with clinical symptoms	0	2	2	0	0	0	2	5	0
		(0/2)	(1/1)				(2/0)	(2/3)	
		4			**0**			**7**	

The numbers in parentheses indicate duration of follow-up: <1 y/>1 y. The numbers in bold represent the total numbers of children of each group (treatment delay <4 weeks, 4–8 weeks, >8 weeks).

Table 2. Clinical Manifestations in Children With Congenital Toxoplasmosis

	Case										
	1	2	3	4[a]	5	6	7	8	9	10	11
WOP with diagnosis of infection	25	26	32	35	22	24	25	27	28	30	30
Trimester of infection	2	2	3	3	1	2	1	2	2	2	2
Duration until therapy began (wks)		<4						>8			
Amniocentesis (WOP)	nd	26	34	nd	nd	26	27	nd	nd	31	nd
PCR result		+	−			+	−			+	
Duration of regular follow-up (y)	7.6	3	2.2	1	0.2	6.5	0	0.4	7	0	4
(with later contact) (y)	(8)	(10)		(14)		(10)			(10)		(6)
Sex	m	f	f	m	f	m	m	m	m	f	f
Clinical symptoms present in utero											
Hydrocephalus							+			+	
Ventricular dilatation							+	+		+	
Pericardial effusion										+	
Clinical symptoms present at birth/within first year of life											
Hydrocephalus							−[b]			(+)[b]	
Ventricular dilatation							−[b]	(+)[b]		(+)[b]	
Intracranial calcifications	+	+				(+)		+		+	+
Retinochoroiditis/scars			+	+	+				+		
Pericardial effusion										(+)[b]	+
Ascites											+
Blood-liquor barrier defect	+										

Clinical symptoms were regularly observed either in utero or at birth/within the first year of life.

Abbreviations: nd, not done; WOP, week of pregnancy; PCR, polymerase chain reaction.

+ Discrete clinical manifestation.

(+) Very discrete clinical manifestations with subsequent reduction.

[a] Identified at the age of 7 y.

[b] Reduction of symptoms under therapy examined by ultrasound (in utero) and computed tomography (at birth/within first year of life).

the regular follow-up but presented with retinochoroiditis with 1 small scar at the age of 7 years. It therefore can principally not be excluded that some of the congenitally infected children will later develop ocular lesions. Only 2 of the 11 symptomatic children (18.2%) presented with >1 neurological sign; however, none of them had all 3 symptoms that are typical for congenital toxoplasmosis. Two of 11 (18.2%) also developed pericardial effusions for which no other cause could be identified; indeed, *T. gondii* has also been shown to cause acute pericarditis [13]. None of the 11 symptomatic children developed severe disease with impairment to their life, as was assessed by the attending pediatrician. The median of the regular follow-up of these children was 2.6 years (95% CI, 0–7.6 years). Consulting of the respective families at later times was successful in 15 of 33 (45.5%) congenitally infected children and in 6 of 11 (54.5%) symptomatic children. Thereby, the median time of follow-up increased to 4.1 years (95% CI, 0–10 years).

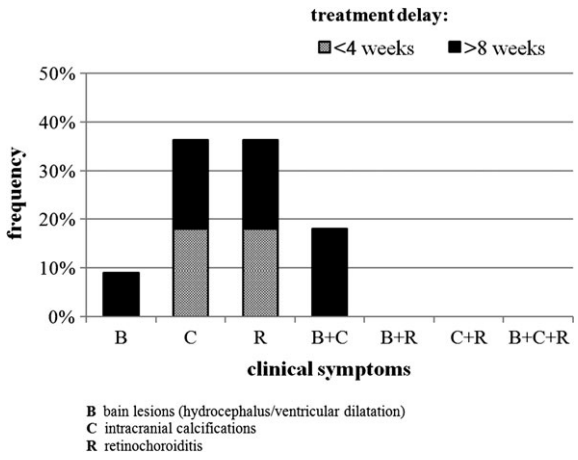

Figure 6. Frequency of clinical symptoms in infected newborns. B, brain lesions (hydrocephalus/ventricular dilatation); C, intracranial calcifications; R, retinochoroiditis. Cumulative percentages are given.

DISCUSSION

In our study, we observed an overall transmission rate of *T. gondii* from the infected mother to her child of 4.8%, which is consistent with the results of other studies in which

only a small study population was included [4, 5]. Screening during the second and third trimester of pregnancy occurs less frequently in Germany than during the first trimester because of the absence of a toxoplasmosis screening program during pregnancy. This aspect is reflected in our study, in which the vast majority of women were voluntarily tested during their first trimester (69.9%). Therefore, if the total number of women tested (n = 685) were equally distributed into each trimester (228 women per trimester) and the transmission rates as determined in our study were accordingly applied to each of these groups (first trimester: 3 infected children [1.3%]; second trimester: 24 [10.6%]; third trimester: 49 [21.7%]), the total number of infected children to this proportion would hypothetically be 76 of 685 children (11.1%). However, even this adjusted ratio is less than what has been shown in larger studies, in which a mean transmission rate of 29%–30% was reported [6, 7, 14]. Most of these studies were performed in France, where regular screening for *T. gondii* infection is performed in monthly intervals during pregnancy. Likewise, the overall rate of 1.6% of clinically symptomatic children born to mothers with primary infection acquired during pregnancy was low in our study, compared with 5.4% in a meta-analysis that was mainly based on French studies [6]. In France, for example, PSF is only administered when fetal infection has been proven by PCR of amniotic fluid in or after the 18th WOP and when the infection has lasted >8 weeks. With this algorithm, there is a longer period when spiramycin is given without PSF. In Germany, PCR of amniotic fluid plays only a minor role for diagnosis. Instead, spiramycin is given at the time of serological diagnosis and is automatically switched to PSF at the 16th WOP without a PCR test. The advantage is that, with this algorithm, there is substantially less waiting for procedures and results, compared with the French algorithm. However, the 13%–20% rate of clinical symptoms of congenitally infected children was lower in the French studies [6, 11, 15], compared with our observations (33.3%). This difference might be explained by the fact that, in our study, most clinically symptomatic infections occurred during the first half of pregnancy. However, a direct comparison between studies performed in France and in Germany is rather difficult because of the absence of a screening program for toxoplasmosis during pregnancy in Germany and different treatment schemes. As a consequence, most women in Germany decide to have a voluntary serological examination at the beginning of pregnancy and later only in case of suspicion or at intervals >1 month.

Our study indicates that rapid initiation of therapy (within 4 weeks after infection of the mother) is important to be efficient in the fetus, because the infection of the fetus follows shortly after the mother has been infected [6, 16]. In Germany, however, control examinations are recommended only at 8- to 12-week intervals in seronegative cases and not every 4 weeks as in France. Because these examinations are not covered by the health insurance funds, a screening interval of 4 weeks would not be accepted by most women. Nevertheless, even a short delay of treatment initiation of up to 8–12 weeks seems to be beneficial for the fetus, because the respective children in our study showed only minor clinical symptoms at birth that improved during the follow-up study phase. We postulate that during this time, inflammatory reactions induced by the parasite can still be controlled by adequate antiparasitic treatment, whereas later intervention would result in no benefit. Indeed, recently, it was shown that the risk for serious neurological sequelae of congenital toxoplasmosis is reduced by three-quarters when prenatal treatment was started early in pregnancy [17]. Furthermore, a delay of >8 weeks between maternal infection and treatment initiation increased the risk for retinochoroiditis in congenitally infected children during the first 2 years of life [18].

Furthermore, our observation suggests an efficacy of prenatal PSF combination therapy by reducing the rate of clinical symptoms as determined in utero. These findings are in line with the infection biology of the parasite and efficient antiparasitic treatment of immunocompromised patients with reactivated cerebral toxoplasmosis: only the rapidly dividing tachyzoite stage of *T. gondii* is susceptible to treatment with pyrimethamine and sulfadiazine, but not the dormant bradyzoite stage that is found in persisting cysts. Spiramycin seems to be not as efficient against the tachyzoite stage as are pyrimethamine and sulfadiazine [19]. For this reason, after reactivation of latent infection and its resulting stage conversion from the dormant bradyzoites into the aggressive tachyzoites, treatment with pyrimethamine and sulfadiazine is efficient by inhibiting the tachyzoite stage and forcing reconversion into the cyst stage [20].

In addition to the efficacy of early initiation of treatment, our study reveals the usefulness of an adequate follow-up program for children with congenital toxoplasmosis. It has recently been shown that treatment of infants with severe neurologic disease at birth resulted in a beneficial outcome for >70% of them when long-term follow-up was applied [21].

In conclusion, the data from our study reveal that the German treatment scheme for toxoplasmosis acquired during pregnancy in combination with a general follow-up program is efficient in reducing the transmission from the infected mother to the fetus and the burden of disease in the newborn.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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