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Congenital toxoplasmosis in twins: a report of fourteen consecutive cases and a comparison with published data

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Background. **The combined influence of congenital toxoplasmosis and twin pregnancy on the duration of gestation has not been previously examined. Similarly little is known about the influence of genetic factors on the clinical course of this disease. The present study addresses these issues.**

Patients and methods. **Fourteen consecutive twin pairs born of mothers with *Toxoplasma* seroconversion during pregnancy were monitored after birth and during childhood, and the relevant data were compared with those already documented for similar cases.**

Results. **The presumed time of gestation at which women became infected was earlier in noninfected than in infected twins ($P = 0.007$). Congenital infection did not influence the duration of pregnancy either in our own cases [36.2 ± 4.3 weeks for noninfected children and 37.4 ± 1.8 weeks for infected ones ($P = 0.45$)] or in previously published ones [35.4 ± 3.6 weeks ($P = 0.69$)]. The infection status was identical for monozygotic twins (with one exception) but different for dizygotic ones (19 of 20 *vs.* 35 of 45 cases). The clinical course through childhood corresponded more closely for monozygotic twins than for dizygotic ones [17 of 20 *vs.* 20 of 45 cases ($P = 0.007$)].**

Conclusion. **Twin pregnancy is not a risk factor either for premature birth or for *Toxoplasma* infection in contaminated twins. The closely corresponding infection status between monozygotic twins highlights the crucial role played by the placenta in disease transmission. However, the substantial proportion of discrepant clinical courses suggests that other, as yet unknown, factors may be involved.**

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Key words: Congenital toxoplasmosis, twins, pregnancy, zygosity, placenta, prematurity.

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INTRODUCTION

Congenital toxoplasmosis may be extremely harmful to the fetus and developing child.[1–4] It has also been

assumed to contribute to premature delivery.[5] Given that twin pregnancy is likewise a risk factor for prematurity,[6] these two factors combined might have an important impact on pregnancy and therefore be of clinical concern to obstetricians. Nevertheless we still do not know whether the combination of twin pregnancy and congenital toxoplasmosis is an aggravating risk factor for premature birth. We have little information concerning mother-to-child transmission of the disease, the severity of fetal infection or the clinical sequelae in such cases. The recognition of an influence of twin pregnancy on these aspects might further contribute to our understanding of the role played by the placenta in mother-to-child transmission of infectious diseases and especially of congenital toxoplasmosis. Moreover several publications indicated the role of host genetic factors in the control of *Toxoplasma* infection.[7–10] Studies with twins afford a unique opportunity of determining the respective roles played by genetic and environmental factors.[11] If concordance in terms of both the transmission and the evolution of the disease proved to be higher among monozygotic twins (who share the same genes) than among dizygotic ones (who share on average 50% of genes), then genetic factors would be more important than environmental ones in influencing its progression.[12]

Only a few studies relating to congenital toxoplasmosis in twins have been published, and these deal with only a few cases. However, the prevailing view is that concordance is good for monozygotic twins and poor for dizygotic ones,[13] thus favoring the predominance of the genetic over the environmental influence.

In this study we describe the evolution of the disease in a cohort of twins born of mothers who seroconverted for toxoplasmosis during pregnancy and who were followed up in our department. We also conducted a literature survey to assess the putative aggravating role of twin pregnancy on congenital toxoplasmosis and the influence of genetic factors on its course of development after birth.

PATIENTS AND METHODS

Our cases. Between January 1988 and August 2002, all consecutive twin pairs whose mothers had been followed in our center for seroconversion during pregnancy underwent analysis for fetal contamination.

Maternal infection was assumed to have occurred if specific IgM was detected in previously seronegative women and if their titers of specific IgG rose significantly. After confirmation of maternal infection, all women were offered a standard course of treatment with spiramycin (3 g/day) until delivery. If infection occurred after the 32nd week of pregnancy and in confirmed cases of fetal infection, 3-week courses of spiramycin were alternated with 3-week courses of sulfadiazine (3 g/day) and pyrimethamine (50 mg/day).

Twin children were considered to be infected if at least one of the following criteria was satisfied: (1) positive mouse inoculation or parasite DNA amplification from amniotic fluid and fetal blood, or positive specific IgM and IgA in fetal blood for children born before 1994 when cordocentesis was performed for prenatal diagnosis of toxoplasmosis; (2) positive IgM (index, >2) after birth (detected by ISAGA; BioMérieux, Marcy l'Étoile, France); (3) a rise in IgG (detected by indirect immunofluorescence) during the first year of life; (4) persistent specific IgG (>5 IU/ml; detected by indirect immunofluorescence) after the first year of life; and (5) ultrasonographic evidence of toxoplasmic damage to the brain, including ventricular dilatation and cerebral calcifications or chorioretinal lesions, in combination with one of the four abovementioned criteria. The detection of specific IgG at birth alone did not meet the definition criteria for congenital *Toxoplasma* infection, in that it can be of maternal origin. After confirmation of congenital infection, children were subjected to clinical, ophthalmologic and serologic evaluations every 3 months until the end of their first year of life, then every 6 months until the age of 3 years and annually thereafter. Each child underwent 3-week courses of peroral treatment with pyrimethamine (3 mg/kg every 3 days), sulfadiazine (25 mg/kg every 8 h) and folinic acid (50 mg every 7 days), until a body weight of 5 kg had been attained (with regular controls of the blood and urine, i.e. for proteinuria). This therapy was then replaced by Fansidar (1 tablet/20 kg every 10 days which is equivalent to 1.25 mg of pyrimethamine/kg, 25 mg of sulfadoxine/kg every 10 days) and folinic acid (50 mg every 7 days) until the age of 15 months, with hematologic evaluations every month.

Cases reported in the literature. A Medline survey using the key words "toxoplasmosis" AND "twins" revealed most of the publications. A manual search in specialized text books and a cross-literature search for related articles completed the list. Studies were further considered only if they documented maternal seroconversion and furnished precise information respecting the zygotic type of twin pregnancy and the status of the children at birth and up to at least 1 year of age. Stillborn and early childhood death cases and pregnancy termination were consequently also not considered.

RESULTS

In our own series of cases, no deliveries occurred before 32 weeks of amenorrhea. No difference was found between the gestational age at birth ($P = 0.45$) for our monozygotic (mean, 36.2; range, 32 to 41; median, 36; SD 4.3 weeks) and dizygotic twins (mean, 37.4; range, 34 to 39; median, 38; SD 1.8 weeks) or for the published cases [mean, 35.4; range, 29 to 42;

TABLE 1. Own cases; clinical course of twins born of mothers who seroconverted for toxoplasmosis during pregnancy and were followed at the Hôpital de la Croix Rousse (Lyon, France) between 1988 and 2002.

	Gestational Age at Contamination (wk)	IgM at Birth	IgA at Birth	Confirmation of Congenital Toxoplasmosis	Clinical Signs	Follow-up (mos)
Monozygotes	24 [37]*	Negative	Negative	NI		
		Negative	Negative	NI		
	16 [32]	Negative	Negative	AI	No	31
		Negative	Negative	AI	No	31
	21 [32]	Positive	Positive	SI	Retinochoroiditis i.e. (34)† and r.e. (95)	98
		Positive	Positive	SI	Retinochoroiditis r.e. (95)	98
Dizygotes	Periconceptional [39]	Negative	Negative	NI		
	3 [38]	Negative	n.d.	NI		
		Negative	n.d.	NI		
	6 [35]	Negative	Negative	NI		
		Negative	Negative	NI		
	6 [39]	Negative	Negative	NI		
		Negative	Negative	NI		
	9 [39]	Negative	Negative	NI		
		Negative	Negative	NI		
	12 [34]	Negative	Negative	NI		
		Negative	Negative	NI		
	12 [38]	Negative	Negative	NI		
		Negative	Negative	NI		
	23 [38]	Negative	Negative	NI		
		Negative	Negative	NI		
	25 [40]	Positive	n.d.	AI	No	56
		Negative	n.d.	NI		
	28 [36]	Negative	Negative	NI		
		Negative	Negative	AI	No	75
	29 [41]	Positive	Positive	SI	Retinochoroiditis r.e. (9)	69
		Negative	Negative	SI	Retinochoroiditis r.e. (39) and i.e. (52)	69

* Numbers in brackets, term at delivery.

† Numbers in parentheses, age at detection in months.

NI, not infected; AI, asymptomatic infection; SI, symptomatic infection; intracranial calcifications; r.e., right eye; l.e., left eye; n.d., not done. Symptomatic infection was defined as the presence of any signs or symptoms of organ involvement. Asymptomatic infection was assumed if the diagnosis was only supported by laboratory tests.

median, 35; SD 3.6 (n = 18) weeks; P = 0.69]. The literature survey revealed only three exceptions, one twin pair having been delivered during the 29th week of gestation[14] and the other two during the 30th.[13, 15]

Mothers who had given birth to at least one infected child had seroconverted significantly later than mothers whose children both had not been infected, the gestational age at mother's seroconversion being 23.8 (range, 16 to 29; median, 25; SD 4) weeks and 10.8 (range, 2 to 24; median, 9; SD 8) weeks, respectively (P = 0.007).

Monozygotic twins. *Our cases.* From the 1558 pregnancies followed in our department for pregnancy-acquired toxoplasmosis between January 1988 and August 2002, 3 pairs of monozygotic twins were born.

Two of the mothers delivered after 32 weeks of amenorrhea, the third at 37 weeks (Table 1).

In all three cases the serologic analyses performed at and after birth were identical for each cotwin. One pair of twins whose mother had seroconverted during the 24th week of pregnancy was not infected, specific IgG titers being correspondingly negative at 5 months of age. The two other twin pairs whose mothers had been contaminated during the 16th and 21st week of gestation were born without clinical signs of congenital toxoplasmosis, although parasites had been detected in each amniotic sac after amniocentesis.

In the first of the two pairs, the infection remained subclinical throughout the follow-up period of 31 months. IgM and IgA antibody titers were negative at birth. The evolution of specific IgG was similar in each child, there being a transient drop in the concentrations of specific antibodies below detectable values (negation) during the course of treatment, which was followed by a serologic rebound at 22 months of age, 11 months after the discontinuation of therapy (Fig. 1).

In the second pair parasites were recovered from the placenta and cranial calcifications as well as specific IgM and IgA were detected at birth in both infants.

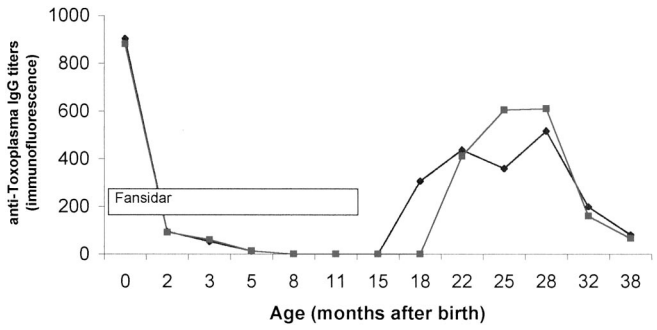

FIG. 1. Evolution of specific serum antibodies in one pair of monozygotic twins. ♦, Twin 1; ■, Twin 2.

TABLE 2. Cases described in literature: *congenital toxoplasmosis in twins*

Authors	No. of Twin Pairs Investigated	No. of Pairs, Infection State and Follow-up of Children (mo)	
		Monozygotes	Dizygotes
Zuelzer[16]	1	1 SI-SI: 7 (1 child)	
Bamater[17]	1		1 SI-SI (both children died before 1 mo of age)
Abbott and Camp[18]	1	1 SI-SI:	-
Tolentino and Bucalossi[19]	1		1 SI-SI: 22
Farquhar[20]	1		1 SI-SI:8
Granström and Magnusson[21]	1	1 SI-SI: 30	-
Michaux et al.[22]	1		1 SI-SI: 84
Murphy and Flannery[23]	1	1 SI-SI:48	-
Hoppeler and Sodoun[24]	1	1 SI-SI: ?	-
Benjamin et al.[25]	1	1 SI-SI: 14	-
Rieger[14]	1		NI-SI: 120
François[26]	1	1 SI-SI: 60	-
Carbonnel-Estrany and Mestre E.[27]	1		1 SI–SI: 15
Glasser and Delta[28]	1	1 SI-SI: 18	-
Yukins and Winter[29]	1		1 SI-SI:7
Guffanti and Carones[30]	1		1 SI-SI: ?
Miller et al.[31]	2	1 AI-SI: 24 (1 child)	1 AI-SI: 16 (1 child)
Calvi Zampetti et al.[32]	1		1 SI-AI: 5
Couvreur et al.[13]	7	1 AI-SI:144	2 SI-SI:4, 84 2 AI-SI: 21; 96 2 NI-SI: 10, 90
Statz et al.[15]	1		1 AI-SI: ?
Couvreur[33]	1		1 AI-SI: 22
Sibalic et al.[34]	17	3 AI-AI: ? 3 SI-SI: ?	1 NI-SI: ? 8 AI-SI: ? 2 NI-AI: ?
Daffos et al.[35]	1		1 NI-SI: stillborn*
Couvreur et al.[36]	4	1 NI-AI : 108	1 AI-SI : 18 2 AI-AI: 25, 24
Tjalma et al.[37]	1		1 NI-SI: 1 (intrauterine death)†
Total	51	1 NI-AI 3 AI-AI 2 AI-SI 11 SI-SI	2 NI-AI 6 NI-SI 2 AI-AI 15 AI-SI 9 SI-SI

* Cordocentesis and amniocentesis-positive; lesions detected by ultrasonography.
† Stillborn; severe malformations, mouse inoculation positive, parasite isolated.
NI, not infected; AI, asymptomatic infection; SI, symptomatic infection; ?, data not furnished; (1 child), 1 of the twins died before 1 month of age.

Serologic rebounds were observed after 10 and 34 months, respectively. In one of the twins, a chorioretinal scar, three disc diameters in size, was detected in the superonasal portion of the retina at 34 months. At 95 months a small macular lesion was detected in each child. Psychomotor development was normal during follow-up.

Cases described in the literature. Seventeen reported pairs of monozygotic twins fulfilled the inclusion criteria (Table 2).

In 16 of the 17 pairs, both children had been contaminated *in utero*. In the remaining pair, one child was not infected; whereas in the cotwin, infection remained subclinical until the age of 108 months after which there was no follow-up.[36] In most of the reported cases, the pattern of disease development was similar for each cotwin and had manifested either in asymptomatic infection[34] or in identical ocular lesions[26] and cerebral calcifications.[18] However, pairs of twins have been described, where severity and evolution of the clinical disease had been different for each cotwin. For three independently reported pairs of monozygotic twins, one of the pair died within 1 month of birth as a result of severe organ involvement of the infection, whereas the likewise congenitally infected cotwin survived.[16, 25, 31] In the first case the cotwin had bilateral ocular lesions with normal psychomotor development after a follow-up period of 7 months.[16] In the second, well-developing child, bilateral retinochoroiditis was detected at 14 months, but with no gross impairment of vision.[25] In the third case infection remained asymptomatic after a follow-up period of 24 months.[31] Couvreur et al.[13] have also reported a case in which one child presented with bilateral chorioretinitis and strabismus whereas the other remained asymptomatic for 28 months.

Dizygotic twins. *Our cases.* Of the 11 pairs of dizygotic twins delivered, 8 were not contaminated. Seven of them were born of mothers who had seroconverted during the first trimester of pregnancy (Table 1). Serologic findings at birth were identical for each pair of twins. In six cases serum antibody titers became simultaneously negative in each cotwin; in the other two pairs they turned negative within <3 months of each other.

Of the three remaining pairs, at least one twin was contaminated. In one pregnancy the mother became infected during the 29th week of gestation. However, the clinical and serologic situation of each child at birth

differed markedly. One (a girl) had specific IgM and IgA at birth as well as intracranial calcifications. With therapy there was a transient serologic drop of specific antibodies below the detection level, but rebound occurred at the age of 23 months. The twin brother was negative for specific IgM and IgA at birth, but IgG remained positive after the age of 1 year. He also developed a serologic rebound in parallel with his sister. The ophthalmologic situation was different in each twin. The girl developed retinochoroiditis in the right eye at 9 months, whereas her brother manifested bilateral lesions after 39 and 52 months, respectively. In the two remaining pairs, maternal contamination had occurred within the final trimester of pregnancy. For each pair, in one child serum antibody titers were negative between one and 9 months, ruling out a congenital infection. In the infected cotwin infection remained subclinical up to, respectively, 69 and 75 months (age at last visit).

Cases described in the literature. Of the 34 reported pairs of dizygotic twins, 26 were congenitally infected (Table 2). In 11 of these pairs, similar patterns of disease were reported for each cotwin, there being no clinical manifestations in 2 pairs and patent infection in 9. In the other 15 pairs, clinical findings differed for each cotwin. In all cases one of the pair had subclinical toxoplasmosis, whereas the other had patent (13 cases) or even lethal (2 cases) infection.[31, 36] The age at which ocular lesions were diagnosed sometimes differed for each cotwin, the interval spanning several years in some instances. Noncoincidental infection status was observed in 8 pairs. In each of these, 2 infected child presented a subclinical infection and 6 were symptomatic, with symptoms ranging from bilateral involvement of the eyes to either severe organ involvement and death at birth (within 48 h[31]) or hydrocephalus and death at 10 months of age.[13]

DISCUSSION

Congenital toxoplasmosis in twins has not been frequently reported. This is because the manifestation depends on the coincidence of two independent and rare events, fetal toxoplasmic infection and twin births (estimated at 8‰ for dizygotic pregnancies and 3.5‰ for monozygotic ones in Caucasians[6]). In addition to our own 14 cases, we thus found only 51 other published instances, 45 of these having been reported between 1945 and 1990. In the literature information concerning the time of gestation at which maternal seroconversion had taken place or at which the women had delivered was not always furnished, but gross prematurity was not frequently reported. In our series of cases, no severe prematurity was observed, none of the women having delivered before 32 weeks of amenorrhea and only 4 before 36 weeks. Taking our own and previously published data together, we were unable to confirm the presumed, but never proven, tenet that twins with congenital toxoplasmosis are at greater risk of being delivered preterm.

In our nine uninfected twin pairs, maternal infection occurred at or before the 24th week of gestation. Fetal infection occurred only in mothers who seroconverted during the second and third trimesters. This finding does not differ from that observed for singletons[38] and hence does not favor the view that the risk of maternal-fetal disease transmission is heightened in twin pregnancies. Moreover the severity of disease in our cohort of patients was not obviously greater than that in congenitally infected singletons (our personal data), although the case numbers were not sufficient to allow a definite conclusion. Interestingly fetal infection rate in twins has not been documented hitherto.

We then addressed the question as to whether the clinical pattern of disease development is similar in pair of monozygotic twins and dissimilar in dizygotic ones. When our own and previously published cases are pooled, in 19 of 20 monozygotic twins, children were either both contaminated (18 pairs) or both not infected (1 pair). The only exception has been reported by Couvreur et al.,[36] and in this case the twining was monochorial biamniotic. These data highlight the well-recognized role of the placenta in fetal infection. Indeed in both of the infected pairs presented by ourselves, parasitic infection was demonstrated in each amniotic sac. Because of vascular anastomoses in a monochorionic placenta,[39] toxoplasmic infection is highly likely to occur in both children. Moreover serologic analyses at birth and the evolution of antibody titers thereafter support the expression of identical disease patterns in monozygotic twins and a similar reaction of the immune system. In our series both twins simultaneously presented a transient serologic drop of specific IgG below the detection level followed by a rebound (Fig. 1), a common phenomenon observed after drug discontinuation.[40] Eighty-five percent of monozygotic twins reported in the literature likewise manifested a similar infection pattern (Table 3). However, in three pairs clinical findings were different. In one case[31] one twin died 1 month after birth, whereas the other presented with subclinical infection and a normal psychomotor development after 24 months. Whether the cause of death was solely toxoplasmic infection is debatable, because the child also presented with bacterial peritoneal infection. Nevertheless postmortem examination revealed proliferative forms of *Toxoplasma* in the myocardium, adrenals, kidneys, testis and brain. In another case[13] one of a pair of monozygotic twins presented with strabismus and bilateral ocular lesions, whereas the other remained asymptomatic. In one of our cases the course of disease was also different, one child having an early ocular lesion and the other no such manifestation, even though both were congenitally infected. The reasons for these discrepancies are

TABLE 3. Comparison of the clinical profiles of mono- and dizygotic twins with congenital toxoplamosis. Fisher's exact test (monozygote/dizygote); $P = 0.007$

Subjects	Identical Clinical Profiles			Different Clinical Profiles		
	Profile	*n*	%	Profile	*n*	%
Monozygotes: 20 pairs	SI-SI	12	85	AI-SI	2	15
	AI-AI	4		NI-AI	1	
	NI-NI	1				
Dizygotes: 45 pairs	SI-SI	10	44	NI-AI	4	56
	NI-NI	8		NI-SI	6	
	AI-AI	2		AI-SI	15	

NI, not infected; AI, asymptomatic infection; SI, symptomatic infection.

unexplained. In all previously published cases, it was stated only that children were monozygotic or, in one instance, monochorial biamniotic. Because it is difficult to differentiate monochorionic from fused dichorionic placentas in children of the same gender, information concerning membrane structures or other markers of monozygosity, such as blood group or HLA typing, would have been valuable. The monozygotic twins presented by ourselves shared the same blood group. Because the course of congenital toxoplasmosis is characterized by recurrences and relapses, individual environmental factors, such as viral infection, might account for the differences in ocular lesions. However, our discrepant pair of twins was reared together, which suggests that the environment does not have a significant impact on the course of the disease.[41]

In contrast to monozygotic twins, dizygotic ones may behave like nontwin siblings, with a heterogeneous maternofetal transmission in 10 cases (22%) compared with 1 (5%) in instances of monochorial pregnancy. Discrepant clinical patterns were observed in 56% of cases (Table 3).

On the basis of our literature analysis and our own experience, we can confirm that transmission of congenital toxoplasmosis is concordant in monozygotic twins and is discordant in dizygotic ones. Although the course of disease may be strikingly similar in monochorial twins, substantial differences have nevertheless been described in terms of the severity or occurrence of new retinal lesions. Hence the relative importance of genetic and environmental factors remains enigmatic. The analysis of published cases is sometimes hampered by uncertainties concerning the type of zygosity and by short follow-up periods. Future reports should systematically include information concerning the placental structure, and biologic markers, such as blood group and HLA typing, be furnished. Moreover the high resolution of ultrasonography now should facilitate the detection of monochorionic placentae. If this more meticulous approach is adopted in the future, we may be afforded a better insight into the mechanisms underlying recurrence and relapse in ocular toxoplasmosis.

Thus whatever their zygosity, each child should be individually monitored, because the course of disease, and especially the occurrence of new ocular lesions, may differ between the mono- and dizygotic twins.

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Safety and immunogenicity of *Shigella sonnei*-CRM_9 and *Shigella flexneri* type 2a-*r*EPA_{succ} conjugate vaccines in one- to four-year-old children

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***Background and objective. Shigella* conjugate vaccines have been shown to be safe, immunogenic and efficacious in adult volunteers. We have now investigated the safety and immunogenicity of investigational *Shigella sonnei* and *Shigella flexneri* 2a conjugate vaccines in 1- to 4-year-old children, the age group at greatest risk for shigellosis.**

***Methods.* The O-specific polysaccharides of *S. sonnei* and *S. flexneri* 2a, the two most common shigellae from patients in Israel, were bound to medically useful carrier proteins to form conjugates. Eighty healthy 1- to 4-year-olds were ran-**

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Key words: Shigellosis, immunization, conjugate vaccines.

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