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Toxoplasma Seroconversion with Negative or Transient Immunoglobulin M in Pregnant Women: Myth or Reality? A French Multicenter Retrospective Study

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Classically, *Toxoplasma* infection is associated with high levels of specific IgM antibody and a rise in specific IgG levels 1 to 3 weeks later. Atypical IgG seroconversion, without IgM detection or with transient IgM levels, has been described during serologic follow-up of seronegative pregnant women and raises difficulties in interpreting the results. To evaluate the frequency and the characteristics of these atypical cases of seroconversion, an investigation was conducted within the French National Reference Center for Toxoplasmosis, from which 26 cases collected from 12 laboratories belonging to the network were identified. The aim of this work was to retrospectively analyze the results of serologic testing, the treatments administered, and the results of prenatal and postnatal follow-up for these women. In each case, IgG antibodies were detected using both screening and confirmatory tests. IgM antibodies were not detected in 15 cases, and the levels were equivocal or low-positive in 11 cases. The IgG avidity results were low in 16 cases and high in one case. Most of the pregnant women (22/26) were treated with spiramycin from the time that IgG antibodies appeared until delivery. Amniotic fluid was analyzed for *Toxoplasma gondii* DNA by PCR in 11/26 cases, and the results were negative in all cases. Congenital toxoplasmosis was ruled out in 12/26 newborns. There was no abnormality observed at birth for 10 newborns and no information available for 4 newborns. In conclusion, when the interpretation of serological results is so difficult, it seems cautious to initiate treatment by spiramycin and to follow the pregnant women and their newborns.

Primary toxoplasmic infection during pregnancy can induce transplacental transmission of *Toxoplasma gondii*, resulting in a congenital infection (1, 2, 3) with possible severe outcomes for the fetus, including chorioretinitis, intracranial calcifications, hydrocephalus, and even stillbirth, especially if the congenital infection is acquired early in the pregnancy (4, 5, 6). The majority of infants infected later in pregnancy are asymptomatic at birth, with sequelae potentially occurring later in life (7, 8, 9, 10). Since pregnant women are generally asymptomatic during the course of *Toxoplasma* infection, the diagnosis is based on serological methods (11). Thus, seronegative women can be monitored during pregnancy and prophylactic recommendations provided (2). Consequently, serological tests for IgM and IgG antibodies against *T. gondii* are commonly used as initial screening approaches in the laboratory diagnosis of toxoplasmosis. IgM anti-*T. gondii* antibodies are known to be a marker of acute infection and appear earlier and decline faster than IgG antibodies. IgM is frequently the first antibody isotype to be detected after the primary infection. The diagnosis of recently acquired toxoplasmosis is usually based on the detection of specific IgM antibodies, followed by the detection of specific IgG antibodies 1 to 3 weeks later with confirmatory tests as described by the French National Reference Center for Toxoplasmosis (12). *Toxoplasma* seroconversion is thus defined by specific IgG antibodies appearing after IgM antibodies (2).

However, the serological diagnosis of toxoplasmosis is very complex and has been discussed extensively in the published literature (13, 14, 15). The interpretation of serological tests is complicated by the long-term persistence of specific IgM and the detection of either IgM alone or IgG alone in a previously seronegative pregnant woman (2). Consequently, serological tests other than those for IgG and IgM detection have been developed to facilitate the diagnosis of seroconversion. The measurement of IgG avidity was developed almost 20 years ago to exclude any infection acquired in the preceding 3 or 4 months using most commercial tests (16, 17, 18, 19, 20, 21, 22, 23, 24). Immunoblot analysis (Toxo II IgG test; LDBio, Lyon, France) may promptly confirm the seroconversion when IgG concentrations detected with routine tests are negative or equivocal (25, 26, 27). In fact, the

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appearance of IgM alone might be difficult to interpret because it can be due to nascent toxoplasmosis seroconversion or a nonspecific IgM reaction (28).

Detection of anti-*Toxoplasma* IgG antibodies alone in previously seronegative pregnant women is so rarely observed that the serological interpretation is difficult. With the exception of cases of immunoglobulin injections and blood transfusions reported in the literature (29), no data have been published showing the presence of anti-*Toxoplasma* IgG antibodies with negative or borderline IgM titers in a pregnant woman known to be negative for *Toxoplasma* antibodies shortly before that test. Laboratories of the French National Reference Center for Toxoplasmosis have been confronted with this situation in their routine work. To investigate these complex situations, we surveyed these laboratories. Twelve of them submitted 26 cases of atypical results obtained in the serodiagnosis of toxoplasmosis over a 10-year period (2001-2011). Thus, the aim of our work was to analyze the serological results as they have been presented in real practice, the treatments prescribed to the pregnant women, and the results of prenatal and postnatal follow-up of these cases of atypical seroconversion with negative or borderline IgM titers.

MATERIALS AND METHODS

Patients. We analyzed the results of serological testing in the 48 laboratories of the French National Reference Center for Toxoplasmosis in order to evaluate their difficulties in interpreting atypical seroconversions in their daily practice of toxoplasmosis diagnosis. Ten towns (twelve medical centers) responded to our request, and we collected 26 cases of seroconversion with negative or low-positive IgM titers among approximately 4,500 seroconversions detected between 2001 and 2011 (0.58%). The number of exceptional serological patterns (IgG seroconversion in the absence of an IgM response) was low at 26 of approximately 800,000 patients tested (1,200,000 serologies). The serological results of 120 serum samples collected from the 26 patients were analyzed. These 26 cases had been selected because the IgG tests were negative with at least one sample and then positive with two to six samples, thus demonstrating a real seroconversion. The IgM tests were negative or borderline by screening or confirmatory techniques.

Serological methods. A battery of serological tests, including screening and confirmatory tests, and reference methods were performed by the different laboratories for specific IgG, IgM, and IgA antibodies and IgG avidity detection. IgG and IgM antibodies were detected by Vidas Toxo IgG and IgM assays (bioMérieux, Marcy l'Etoile, France), Vidia Toxo IgG and IgM assays (bioMérieux), AxSYM IgG and IgM assays (Abbott Diagnostic, Wiesbaden, Germany), Architect Toxo IgG and IgM assays (Abbott Diagnostic), Cobas Toxo IgG and IgM assays (Roche Diagnostics, Basel, Switzerland), Enzygnost Toxo IgG and IgM assays (Siemens Healthcare Diagnostics, Deerfield, IL), Advia Centaur Toxo IgG and IgM assays (Siemens), Platelia Toxo IgG and IgM assays (Bio-Rad, Marnes-la-Coquette, France), and Liaison Toxo IgG and IgM assays (DiaSorin, Saluggia, Italy). Confirmatory tests for IgG detection were the immunofluorescence antibody test (IFAT) (produced in-house [29] or by bioMérieux), the Toxo-Screen test (bioMérieux), the Toxo II IgG Western blot test (LDBio, Lyon, France), the high-sensitivity direct agglutination (HSDA) test (house-made) (30, 31), and the Toxo indirect hemagglutination test (Fumouze Diagnostics, le Malesherbes, Levallois Perret, France). The dye test, first described by Sabin and Feldman 60 years ago, is still the reference method for specific antibody (IgG) detection. However, this assay, based on live *T. gondii*, is now used in only a few laboratories (two laboratories in our study). Confirmatory tests for IgM detection were the immunosorbent agglutination assay (ISAGA-M from bioMérieux or ICT-M, an in-house immunocapture test for IgM [31]) and the immunofluorescence antibody test (IFAT, produced by bioMérieux or in-house).

TABLE 1 Equivocal zone or cut-off values recommended by the manufacturer of the Toxo IgG, Toxo IgM, Toxo IgG avidity, and Toxo IgA assays

Technique	Equivocal zone[a] or cutoff value for indicated assay			
	Toxo IgG (IU/ml)	Toxo IgM (index)	Toxo IgG avidity (index)	Toxo IgA (index)
Advia Centaur	10	0.9		
Architect	1.6–2.9	0.50–0.59	0.50–0.59	
AxSYM	2–2.9	0.5–0.6		
Cobas	6			
Dye test	2			
Elecsys	1–2.9	0.8–0.9		
Enzygnost	4	Determined for each plate		
HSDA	1–5			
ICT		3		2
Immunofluorescence	6 or 8	1/40		
ISAGA		6–8		6–8
Liaison	7.2–8.7	6.0–7.9		
Platelia	6–8.9	0.8–0.9	0.40–0.49	6–8.9
Toxo screen DA	1–3			
Vidas	4–7.9	0.55–0.64	0.20–0.29	
Vidia	3–4.9	0.68–0.99		

[a] In the equivocal zone, the lower value is the limit of negativity, and the higher value is the limit of positivity.

The IgA antibodies were detected by the ISAGA-A (bioMérieux), the ICT-A (in-house) (31), or the Platelia Toxo IgA (Bio-Rad). In addition, the level of IgG avidity was determined using the Vidas Toxo IgG avidity assay (bioMérieux) in 4/10 laboratories, the Platelia Toxo IgG avidity assay (Bio-Rad) in 2/10 laboratories, and the Architect Toxo IgG avidity assay (Abbott Diagnostic) in 1/10 laboratories. All commercially available assays were performed according to the manufacturers' recommendations, and the results are expressed in IU/ml or as indices. The cutoff or equivocal zone values of IgG, IgM, IgA, and IgG avidity are summarized in Table 1.

Maternal treatment and prenatal diagnosis. When maternal infection acquired during pregnancy was highly suspected, the mother was treated with spiramycin at the standard dosage of 9×10^6 units per day until delivery (2). In some cases, amniocentesis was performed after 16 weeks of gestation and at least 4 weeks after maternal infection (32). Prenatal diagnosis was based on the detection of *T. gondii* DNA by PCR (33, 34) and, in most reference centers, on the detection of the parasite after mouse inoculation. Moreover, ultrasound surveillance was scheduled every month to carefully monitor fetal development as recommended by the French National Reference Center for Toxoplasmosis (see http://www.perinat-france.org/guide/cnr-centre-national-de-reference-de-la-toxoplasmose-16-386.php).

Follow-up of newborns. At birth, each neonate underwent thorough clinical and neurological check-ups (transfontanellar ultrasound examination) and an examination of the ocular fundus. Parasitological and immunological tests were used during the first year of life to diagnose potential congenital toxoplasmosis (see reference 8 and http://www.perinat-france.org/guide/cnr-centre-national-de-reference-de-la-toxoplasmose-16-386.php). Standard methods for the detection of specific *Toxoplasma* antibodies (IgG, IgM, and IgA), such as the enzyme immunoassay (EIA) and immunosorbent agglutination assay (ISAGA or ICT), were combined with Western blotting or an enzyme-linked immunofiltration assay (ELIFA) (31) to distinguish maternal antibodies, transmitted either passively (IgG) or by leakage (IgM), and neonatal neosynthesized antibodies. The parasitological examination of placental tissue was sometimes used to diagnose a congenital *Toxoplasma* infection at birth. However, serological follow-up of the in-

TABLE 2 Fifteen cases of atypical toxoplasmic seroconversion with negative IgM results by screening tests and the ISAGA test[a]

Case	Date	Result for indicated assay									
		Toxo IgG				Toxo IgM				IgG avidity	Toxo IgA
		Advia Centaur	Vidia	Vidas	AxSYM/ Liaison	Advia Centaur	Vidia	AxSYM	ISAGA-M		
1	06/20/09	<2[b]				0.6					
	07/25/09	**120**[c]				0.6					
	08/20/09	**270**		**204**	**212/159**	0.6		0.2	4		
	09/16/09		**64**				0.17				
		Architect	Vidas	Platelia	Elecsys	Architect	Vidas/IFAT	Platelia/Elecsys	ISAGA-M	Vidas/Platelia/Architect	ISAGA-A
2	12/07/09	0			<0.125	0		/0			
	03/30/10	**8.8**	3			0.25	0.08/0		1	—/—/0.32	
	05/26/10	**93.5**	**82**	**48**	**238.2**	0.21	—/0	0.5/0	1	0.07/0.17/—	**12**
	05/31/10	**90.3**	**103**			0.22	0/0		1	0.07/0.2/—	
	06/15/10		**87**	**44**	**331.9**			0.5/0		0.15/—/—	
	06/29/10	**386.5**		**37**	**362.5**	0.23	—/0	0.4/0	1	—/0.18/0.45	**12**
		AxSYM	Vidas	HSDA		AxSYM			ICT-M	Vidas	ICT-A
3	09/12/05	0		0					0		0
	10/08/05	0		0					0		0
	11/11/05		4[d]	**6**		0.24			0		0
	11/18/05	**35**	**9**	**10**		0.24			0		0
	12/09/05		**43**	**25**		0.25			0	0.02	0
		AxSYM	Vidas	HSDA		AxSYM			ICT-M	Vidas	ICT-A
4	11/05/07	0		0		0.081			0		0
	12/19/07	0		0		0.081			0		0
	01/26/08	**7.8**		1		0.081			0		0
	02/20/08	**19**	**25**	**10**		0.081			0	0.08	0
	02/28/08								0		0
		Elecsys	Platelia	IFAT		Elecsys	Platelia		ISAGA-M		ISAGA-A
5	04/30/11	0.6				0					
	05/11/11	0.95	1.8	3		0	0.7				
	05/24/11		5.7	**10**			0.5		3		0
	06/27/11	**30.8**	**30**	**20**		0	0.4		5		0
	07/15/11		**34**	**60**			0.5				
		Vidas	IFAT			Vidas	IFAT	AxSYM	ISAGA-M	Vidas	
6	12/01/01	0	0			0.07	0		0		
	12/28/01	**10**	**8**			0.1	0		0	**0.55**	
	01/07/02	**85**	**8**			0.14	0		0	**0.54**	
	01/26/02	**135**	**8**			0.16	0		0		
	02/28/02	**83**	**8**			0.16	0		0		
	04/15/02	**39**	**8**			0.12	0		0		
		Vidas	IFAT			Vidas	IFAT		ISAGA-M	Vidas	
7	05/17/03	0	0			0.47	0		0		
	06/20/03	**26**	**8**			0.49	0		0	0.06	
	07/10/03	**44**	**8**			0.43	0		0		
		AxSYM	Vidia			AxSYM	Vidia		ISAGA-M		
8	06/22/06	<2				0.28					
	09/27/06	<2				0.39					
	10/16/06	**5.1**				0.42					
	12/01/06	**35.7**				0.36					
	01/18/07		**168**				0.34		0		
		AxSYM	Toxo-Screen			AxSYM			ISAGA-M	Vidas	ISAGA-A
9	01/07/06	0				0.11			0		0
	02/18/06	0	Negative			0.18			4		0
	03/18/06	**31**	**Positive**			0.18			4		0
	03/29/06	**48**	**Positive**			0.17			4	0.02	0

(Continued on following page)

TABLE 2 (Continued)

Case	Date	Result for indicated assay					
		Toxo IgG		Toxo IgM		IgG avidity	Toxo IgA
		AxSYM	Toxo-Screen	AxSYM	ISAGA-M	Vidas	ISAGA-A
10	03/27/07	0	Negative	0.10	0		0
	04/05/07	1	Negative	0.11	0		0
	04/25/07	**34**	**Positive**	0.12	1	0.07	0
	05/03/07	**47**	**Positive**	0.113	1		0
	05/18/07	**75**		0.116	0		0
		AxSYM	HSDA	AxSYM	ISAGA-M	Vidas	ISAGA-A
11	12/08/10	0	Negative	0.09	0		0
	01/22/11	**4**	**Positive**	0.17	3		0
	02/09/11	**7**	**Positive**	0.21	3		0
	03/07/11	14		0.178	3	0.046	0
		Vidas	HSDA	Vidas	ICT-M	Vidas	ICT-A
12	08/14/08		0	0	0		0
	09/11/08	2	**10**	**0.55**	0		0
	10/09/08	**65**	**100**		0	0.02	0
	10/22/08		**200**		0		0
		Vidas	HDSA	Vidas	ICT-M	Vidas	ICT-A
13	03/11/11	0	0	0	0		0
	07/08/11	**4**	**50**	0.41	0		0
	08/24/11	**64**	**100**	0.40	0	0.05	0
		Platelia	IFAT	Platelia	ISAGA-M	Platelia	ISAGA-A
14	01/18/10	0	0	0.2	0		
	03/08/10	1.6	2	0.5			
	04/08/10	**11**	**6**	0.3	3		0
	04/20/10	**29**	**10**	0.2	5	0.12	**7**
		Enzygnost		Enzygnost	ISAGA-M		ISAGA-A
15	09/29/03	<2.7		0.145	0		**12**
	10/25/03	**12**		0.136	3		**12**
	11/07/03	**26**		0.124	3		**12**

[a] Detailed results of IgG, IgM, IgA, and avidity index on 15 atypical seroconversion panels.
[b] Nonreactive results are not highlighted.
[c] Reactive results are in bold type and highlighted in gray.
[d] Equivocal zone results are in bold type.

fants remained necessary to definitely diagnose the presence or absence of congenital toxoplasmosis in a few cases. When the results of specific IgG (detection) tests became negative before the infants reached the age of 1 year, congenital toxoplasmosis was definitively ruled out.

RESULTS

Anti-*Toxoplasma* IgG results. Results of the anti-*Toxoplasma* IgG tests concerning the 26 cases in this study are detailed in Tables 2, 3, and 4. In all cases for all techniques, the results of the first serum samples were negative. Then, IgG antibodies appeared in the second or third serum sample. In three cases (Table 2, case 15, and Table 4, cases 21 and 26), anti-*Toxoplasma* IgG antibodies were analyzed with only one technique, but two (case 15) or four successive serum samples (cases 21 and 26) tested positive. In the other 23 cases, analysis of anti-*Toxoplasma* IgG revealed positive results using two to six different techniques on two to six successive serum samples. The confirmatory tests for IgG detection were always positive (IFAT [7 cases], Toxo-Screen [3 cases], Toxo II IgG Western blot [1 case], HSDA [7 cases], hemagglutination [1 case], and dye test [2 cases]). The average IgG levels were quite low regardless of which technique was used. In three cases (11.5%), IgG antibodies were detected during the first trimester, in 15 cases (57.7%) during the second trimester, and in eight cases (30.8%) during the last trimester (Table 5). In all cases, the IgG test results remained positive in the successive samples collected over a time period ranging from 2 weeks to 6 months, depending on the patient. Moreover, in one case (Table 4, case 18), not only did varicella symptoms appear during the same time period as the anti-*Toxoplasma* IgG, but IgG antibodies against the varicella-zoster virus also appeared in the second serum sample, as did *T. gondii*-specific IgG antibodies. The Vidas IgG results were very low in this case; consequently, IgG avidity was not tested, as recommended by the manufacturer.

Anti-*Toxoplasma* IgM results. Between two and six tests for IgM antibodies against *T. gondii* were performed on each serum sample. An immunocapture test (ISAGA-M or ICT-M) was the confirmatory test in all 26 cases. The patients were classified into three categories according to the IgM results (Tables 2, 3, and 4).

TABLE 3 Two cases of atypical seroconversion with weakly positive IgM by two techniques (Platelia and Liaison) and negative by three other techniques (ISAGA, immunofluorescence, and Elecsys)[a]

Case	Date	Toxo IgG assay					Toxo IgM index					Toxo IgG avidity
		Platelia	Elecsys	Liaison	HSDA/dye test	IFAT	Platelia	Elecsys	Liaison	ISAGA-M	IFAT	Platelia
16	04/02/09	2[b]	<0.125	0	1/2	Negative	0.44	0.270	10	0	0	
	05/11/09		**6**		**8/10**			0.32		0		
	05/25/09	**52**[c]	**16.47**	**70**	**64/100**	**Positive**	0.72	0.370	**18**	0	0	0.13
	05/29/09	**57**		**84**		**Positive**	**0.81**[d]		**9**	0	0	0.14
	06/18/09	**131**		**122**		**Positive**	**1.16**		**15**	0	0	0.15
17	09/29/08	0	0.13	0		Negative		0	4	1	0	
	11/03/08	1	3	**7.6**		**Positive**	**1.39**	0	**7**	3	0	
	11/29/08	**30**	**15**	**43.3**		**Positive**	**0.85**	0	**7**	1	0	0.27
	12/10/08	**46**	**43**	**55.6**		**Positive**	0.73	0	**8**	1	0	0.22
	12/29/08	**13**		**56.3**		**Positive**	0.52		**8**	1	0	0.2
	01/30/09			**37.3**		**Positive**			**8**		0	

[a] Detailed results of IgG, IgM, IgA, and avidity index on 2 atypical seroconversion panels.
[b] Nonreactive results are not highlighted.
[c] Reactive results are in bold type and highlighted in gray.
[d] Equivocal zone results are in bold type.

For 15 patients in the first category, the IgM results were negative with the screening tests and the ISAGA-M or the ICT-M assay (Table 2, cases 1 to 15). In the second category, including two cases, the IgM results were slightly positive with two techniques (Platelia and Liaison) and negative by three other techniques (ISAGA-M, immunofluorescence, and Cobas) (Table 3, cases 16 and 17). In case 16, the Liaison IgM and Platelia IgM tests were positive after IgG antibodies had appeared. In case 17, the Platelia IgM test was positive in the first serum sample, with equivocal IgG titers using the Liaison test. The Platelia IgM test results were then equivocal and negative in the successive serum samples. The Liaison IgM test results were equivocal in the next five samples. In the third category, including nine cases, although the results of IgM screening tests were negative or equivocal by one technique, the ISAGA-M or ICT-M results were equivocal or positive in one to four successive serum samples (Table 4, cases 18 to 26). In two of nine cases (Table 4, cases 21 and 26), the ISAGA-M tests gave positive or equivocal results for the first serum sample, and in the remaining cases, IgM and IgG antibodies were observed simultaneously in the second serum samples. In 8/11 cases, decreasing IgM values were observed.

Anti-*Toxoplasma* IgA results. The IgA assay (ISAGA-A, ICT-A, or Platelia) was performed on 54 serum samples collected from 16 patients. The results were negative for 10 patients, equivocal for one patient, and positive for five patients. The patient with equivocal IgA results (case 14) had negative IgM results. Of the five patients who tested positive for IgA, two (cases 2 and 15) tested negative for IgM, two (cases 20 and 21) had positive IgM results by only the ISAGA-M test, and one (case 24) had equivocal IgM titers in the ISAGA-M test.

IgG avidity results. The IgG avidity assay was performed on 26 samples collected from 17 patients. Two serum samples from one patient (case 6) revealed high avidity, while 24 samples from 16 patients exhibited low avidity. High avidity excluded a *Toxoplasma* infection acquired less than 4 months earlier.

Maternal treatment and prenatal diagnosis. Of the 26 patients, 22 (84.6%) were treated with spiramycin from the time that the IgG appeared until delivery, and one received no treatment (Table 5). We had no information about the treatment for three patients. PCR analysis of the amniotic fluid was performed on samples from 11 patients, and the results were always negative. Amniotic fluid was not collected in 13 cases, and no information was available in two cases.

Follow-up of newborns. The postnatal serological follow-up period was long enough to definitively exclude congenital toxoplasmosis in 12 cases (Table 5). In 10 cases, biological, clinical, radiological, and ophthalmological examinations of the newborns at birth did not reveal any abnormalities, but no information was available concerning their follow-up. In four cases, we had no information about the newborns.

DISCUSSION

Serological diagnosis is the main approach for defining the risk of primary *Toxoplasma* infection in a pregnant woman. The measurement of IgG, IgM, IgA, and IgG avidity by different methods usually allows doctors to establish the immunologic status of a patient and to diagnose seroconversion (2). The difficulties encountered in the serodiagnosis of toxoplasmosis in pregnant women have been underlined for a long time (15, 29, 35, 36). The absence of IgG antibodies before, or early in, pregnancy enables the identification of women at risk for acquiring infection (12). Thus, in the daily routine, serological results showing positive IgG and negative or transient IgM in previously negative pregnant women can create interpretation difficulties.

Different factors might explain such results: (i) the injection of gamma globulins or a blood transfusion, which might have led to the appearance of exogenous anti-*Toxoplasma* IgG (29); (ii) immune disorders or other pathologies in the patient, which might have led to the presence of unusual antibody subsets; (iii) toxoplasmosis serological reactivation or reinfection in chronically infected patients with previously very low IgG residual titers below the detection threshold; and (iv) toxoplasmosis that was recently acquired but with a very unusual serological profile. The first two factors should be investigated before the others, and if appropriate, the pregnant women should be considered seronegative and

TABLE 4 Nine cases of atypical seroconversion with negative IgM by the screening techniques and positive or equivocal IgM by ISAGA[a]

Case	Date	Result for indicated assay									
		Toxo IgG				Toxo IgM				IgG avidity	Toxo IgA
		Vidas	IFAT	AxSYM		Vidas	IFAT	AxSYM	ISAGA-M		
18	04/27/10	0[b]	0			0.05	0		0		
	06/03/10	**8**	**80**[c]			0.13	0	0.3	**9**		
	06/21/10	**6**[d]	**80**	**171**		0.09	0	0.25	**6**		
	07/21/10	**5**	**8**	**137**		0.08	0	0.24	0		
	08/26/10	**5**	**8**	**61**		0.08	0	0.17	0		
	09/22/10	3	**8**	**42**		0.06	0	0.15	0		
	10/19/10	2	**8**			0.08	0		0		
		AxSYM	Vidia	Dye test/HSDA		AxSYM	Vidia		ISAGA-M		
19	01/26/08	<0.4		<2/<1		0.06			0		
	03/01/08	**3**		**5/1**		**0.56**			**9**		
	03/22/08	**21**		**80/8**		**0.53**			**9**		
	08/28/08		**28**				0.45				
		Architect	Vidia	Western blot	Hemagglutination	Architect	Vidia		ISAGA-M		ISAGA-A
20	07/30/11	0.1	0	Negative	Negative	0.04	0.08		0		
	09/06/11	**27**	**3.0**	**Positive**	**Positive**	0.20	**0.76**		**9**		**6**
	09/30/11	**114.2**			**Positive**	0.22			5		**9**
		Enzygnost				Enzygnost	Vidas		ISAGA-M		ISAGA-A
21	03/03/06	<2.1				0.043	0.32		**12**		**12**
	04/05/06	**11**				0.091	0.32		**12**		**12**
	05/15/06	**64**				0.076			**12**		**12**
	06/17/06	**84**				0.028			**9**		**12**
	08/04/06	**37**				0.036			3		**12**
		Advia Centaur	Platelia			Advia Centaur	Platelia	Vidas/IFAT	ISAGA-M	Platelia	
22	01/25/10	<0.5				0.72					
	02/22/10	**4**				0.85					
	03/18/10	**46**				0.87		0.51/<10	**12**	0.01	
	04/08/10		**48**				0.95		**6**		
	06/02/10		**33**				0.919				
		Vidas	IFAT			Vidas	IFAT		ISAGA-M	Vidas	
23	03/22/01	0	0			0.12	0		0		
	05/02/01	**73**	**160**			0.37	0		**7**	0.03	
	05/28/01	**195**	**320**			0.32	0		**6**		
	09/03/01	**157**	**320**			0.18	0		3		
		Liaison	Vidia			Liaison	Vidia		ISAGA-M		Platelia-A
24	08/02/07	<2				<8			ND		
	09/05/07	**20**				<6			**6**		**41**
	09/26/07	**40**				<6			**6**		**59**
	12/20/07		**42**				0.17		3		
		AxSYM	Toxo-Screen			AxSYM			ISAGA-M	Vidas	ISAGA-A
25	09/24/04	0	Negative			0.125			0		
	10/28/04	0	Negative			0.145			0		0
	01/04/05	**8**	**Positive**			0.224			**6**		0
	01/10/05	**14**	**Positive**			0.285			**6**	0.05	0
	02/11/05	**60**				0.314			**7**		0
	03/04/05	**68**				0.323			**7**		0
		Cobas				Vidas			ISAGA-M	Vidas	ISAGA-A
26	03/23/06	0				**0.6**			**7**		
	04/28/06	**148**				0			**6**	0.06	
	05/08/06	**175**				0			**6**		
	05/30/06	**264**				0			**6**		
	10/27/06	**42**				0			0		0

[a] Detailed results of IgG, IgM, IgA, and avidity index on 9 atypical seroconversion panels.
[b] Nonreactive results are not highlighted.
[c] Reactive results are bold type and highlighted in gray.
[d] Equivocal zone results are in bold type.

TABLE 5 Gestational age at time of IgG conversion, maternal treatment, prenatal diagnosis, and infant follow-up of the 26 women with atypical seroconversion

Patient no.	Gestational age at IgG conversion (wk of amenorrhea)	Maternal treatment by spiramycin	Prenatal diagnosis[a]	Infant follow-up[b]
1	29	ND[c]	No	No abnormality at birth
2	5	Yes	ND	ND
3	33	No	No	No congenital toxoplasmosis
4	20	Yes	No	No congenital toxoplasmosis
5	40	ND	No	No abnormality at birth
6	25	Yes	Negative result	No congenital toxoplasmosis
7	18	Yes	Negative result	No abnormality at birth
8	25	Yes	No	No abnormality at birth
9	18	Yes	ND	ND
10	10	Yes	Negative result	No congenital toxoplasmosis
11	28	Yes	No	ND
12	28	Yes	Negative result	No congenital toxoplasmosis
13	24	Yes	Negative result	No congenital toxoplasmosis
14	15	Yes	Negative result	ND
15	16	Yes	Negative result	No abnormality at birth
16	15	Yes	No	No abnormality at birth
17	16	Yes	No	No abnormality at birth
18	21	Yes	No	No abnormality at birth
19	14	Yes	Negative result	No congenital toxoplasmosis
20	36	Yes	No	No congenital toxoplasmosis
21	23	Yes	Negative result	No abnormality at birth
22	26	Yes	Negative result	No congenital toxoplasmosis
23	22	Yes	Negative result	No congenital toxoplasmosis
24	25	ND	No	No abnormality at birth
25	32	Yes	No	No congenital toxoplasmosis
26	12	Yes	No	No congenital toxoplasmosis

[a] Prenatal diagnosis was based on the PCR results and, in a few centers, results of inoculation of amniotic fluid into mice.
[b] Infant follow-up was based on clinical, radiological, ophthalmological, and biological examinations at birth. Serological results during the first year of life definitively excluded congenital toxoplasmosis.
[c] ND, no data.

be given hygiene recommendations to avoid toxoplasmic infection.

The first factor (injection of gamma globulins) was ruled out for the 26 cases described in our study. Concerning immune disorders or other pathologies, only varicella symptoms were observed in 1/26 cases (Table 2, case 18). To our knowledge, while viral infections are known to generate positive anti-*Toxoplasma* IgM, no data can be found in the literature about the influence of viral infection on the detection of anti-*Toxoplasma* IgG. The third factor is toxoplasmic serological reactivation or reinfection. In chronic toxoplasmosis, IgG could exceptionally decrease to below the cutoff level of the IgG assay, and in such cases, the reappearance of IgG is not a real seroconversion but a serological reactivation. An additional exposure to *T. gondii* could trigger an immediate IgG response without an (or with a weak) IgM response (37).

In the case of high IgG avidity results and the absence of gamma globulin injection or transfusion, IgG detection is the paramount criterion of chronic toxoplasmosis (12). In one case of our study (Table 2, case 6), the result of the avidity assay was high, but it is necessary to stress that high avidity has been observed, if rarely, in serum sampled 1 month after infection (21). However, the determination of IgG avidity in an atypical serological profile is necessary (12).

The fourth factor (recently acquired toxoplasmosis with an atypical serological profile) is often considered by medical biologists and clinicians. In fact, in our study, 22/26 patients were treated with spiramycin as a consequence of such serological results, and an amniocentesis was performed for 11/26 patients. However, the results of the infants' follow-ups did not reveal any case of congenital toxoplasmosis. In fact, our results definitively excluded toxoplasmosis in 12 cases and revealed no neonatal abnormality or any serological argument for congenital toxoplasmosis at birth in 10 cases.

The results of IgG confirmatory techniques were always the same as those of the IgG screening techniques, i.e., negative on the first serum sample and positive on the other successive serum samples. Moreover, the avidity findings were low in 16/17 cases. Typically, in seroconversion, the appearance of IgG is linked to high IgM and rising IgM levels (15). In our study, IgM antibodies were not detected in 15 cases, although we cannot totally exclude that they could have been transient and present during only a very short time period so that they were not observed in the samples at the time they were collected. If the interval between two samplings had been shorter, IgM might have been observed. In the 11 other cases, IgM antibodies were detected at low levels by the Liaison and Platelia tests (2 cases) or with a positive index by only the ISAGA test (9 cases). The detection of IgM usually indicated toxoplasmosis. However, considering that these IgM antibodies were

detected at low levels for a very short time period and that they tested positive with only one or two techniques (only the ISAGA test or only the Platelia and Liaison tests) and quickly disappeared, anti-*Toxoplasma* IgM antibodies were probably not specific. The persistence of IgG in all successive serum samples might have suggested a life-long immunity to toxoplasmosis, but the follow-up time of these women was not long enough (from 2 weeks to 6 months, depending on the patient).

A pregnant woman who tests negative requires regular check-ups for seroconversion (2). In fact, the frequency of check-ups varies from one country to another, depending on the screening programs adopted in each country. Seroconversion without IgM can be observed in France thanks to monthly routine testing of seronegative pregnant women. Such results in a previously seronegative pregnant woman should motivate further exploratory tests, as defined by the French National Reference Center for Toxoplasmosis (12). Double-checking IgG results using other techniques is necessary to confirm the first results, and the avidity test is necessary. It is also important to collect all information about the clinical history of a pregnant woman (immune disorders, other pathology, transfusion, and previous results of toxoplasmosis serology). The interpretation of seroconversion with either no IgM or transient IgM levels may then be discussed.

It is noteworthy that the pathophysiology of toxoplasmosis is far from being fully understood, and the pathogenesis mechanism is complex because the parasite and host specificities are interrelated (38). In such complex serological cases as those that we analyzed, we think that it is cautious to initiate treatment and to follow up with pregnant women (4). Puncture and analysis of the amniotic fluid may also be discussed, depending on the gestational age at which the IgG antibodies appeared.

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