

Table S1. Demographic characteristics of the historical cohort of 344 pregnant women who seroconverted for *Toxoplasma gondii* during pregnancy and underwent amniocentesis for molecular detection of congenital toxoplasmosis

Maternal age (year)	
Mean	28
Median	28
Standard deviation	5.4
Range	17-41
Characteristics of children	
Female	168
Male	176
Sex ratio	1,05
Twins	3

Table S2. Distribution of cases and clinical forms of congenital toxoplasmosis, overall and by dates of maternal infection

Date of maternal infection	Nb	Lost to F-up		Nb with F-up	Congenital Toxoplasmosis			MTP/ IUFD*	Clinical form									
		Nb	%		Total	PND			Symptomatic forms *			Subclinical forms			Unknown			
						Neg	Pos		Total	PND		Total	PND		Total	PND		
										Neg	Pos		Neg	Pos		Neg	Pos	
0-6 WA	81	16	20	65	2	0	2	2	2	0	2	0	0	0	0	0	0	0
7-15 WA	108	19	18	89	9	1	8	6	4	0	4	3	1	2	2	0	0	2
16-28 WA	122	8	7	114	27	6	21	6	11	0	11	15	6	9	1	0	0	1
>29 WA	25	2	8	23	12	0	12	0	0	0	0	12	0	12	0	0	0	0
Imprecise	8	1	13	7	1	0	1	0	0	0	0	1	0	1	0	0	0	0
All	344	19	13	298	51	7	41	14	17	0	17	31	7	24	3	0	0	3

WA: weeks of amenorrhea, Nb: number, F-up: follow-up, PND: prenatal diagnosis, Neg: negative, Pos: positive, MTP: medical termination of pregnancy, IUFD: intra-uterine fetal death. * all these cases were PCR-positive on amniotic fluid.

Table S3. Overall data: detailed comparison of all biological results in the entire cohort (n=344)

	Date of maternal infection	AF	Placenta		Cord blood		Neonatal serology			Commentaries with respect to postnatal follow-up
		MI	PCR	MI	PCR	MI	IC (IgG)	Ig M	IgA	
Congenital toxoplasmosis pos PND Pos (n=44)	0-6 WA (n=2)	Pos 1 Nd 1	Pos 1 Nd 1 ^a	Pos 1 Nd 1 ^a	Pos 1 Nd 1 ^a	Nd 2 ^b	NA 2	NA 2	NA 2	No follow-up: MTP/IUFD
	7-15 WA (n=8)	Pos 2 Neg 3 Nd 3	Pos 3 Nd 5 ^a	Pos 3 Nd 5 ^a	Pos 1 Nd 7 ^c	Pos 1 Neg 1 Nd 6 ^a	Neg 1 Nd 1 NA 6	Pos 1 Neg 1 NA : 6	Neg 2 NA 6	No loss to follow-up
	16-28 WA (n=21)	Pos 3 Neg 5 Nd 13	Pos 14 Neg 2 Nd 5 ^a	Pos 12 Neg 5 Nd 4 ^a	Pos 1 Neg 12 Nd 8 ^c	Neg 13 Nd 8	Pos 3 Neg 11 Nd 1 NA 6	Pos 9 Neg 6 NA : 6	Pos 9 Neg 6 NA 6	No loss to follow-up
	> 29 WA (n=12)	Pos 2 Neg 2 Nd 8	Pos 7 Neg 4 Nd 1	Pos 7 Neg 5 Nd 0	Pos 2 Neg 8 NI 1 Nd 1	Neg 10 Nd 2	Pos 1 Neg 11	Pos 10 Neg 2	Pos 8 Neg 4	No loss to follow-up
	Imprecise (n=1)	Nd	Pos	Pos	Neg	Neg	Neg	Pos	Pos	No loss to follow-up
	<i>Total (n=44)</i>	<i>Pos 8</i> <i>Neg 10</i> <i>Nd 26</i>	<i>Pos 26</i> <i>Neg 6</i> <i>Nd 12^a</i>	<i>Pos 24</i> <i>Neg 10</i> <i>Nd 10^a</i>	<i>Pos 5</i> <i>Neg 21</i> <i>NI 1</i> <i>Nd 17^d</i>	<i>Pos 1</i> <i>Neg 25</i> <i>Nd 18^d</i>	<i>Pos 4</i> <i>Neg 24</i> <i>Nd 2</i> <i>NA 14</i>	<i>Pos 21</i> <i>Neg 9</i> <i>NA 14</i>	<i>Pos 18</i> <i>Neg 12</i> <i>NA 14</i>	<i>No loss to follow-up</i>
Congenital toxoplasmosis pos PND neg (n=7)	0-6 WA (n=0)	0	0	0	0	0	0	0	0	
	7-15 WA (n=1)	Nd	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Delayed diagnosis on the basis of serologic follow-up
	16-28 WA (n=6)	Neg 3 Nd 3	Pos 4 Neg 2	Pos 5 Neg 1	Pos 2 Neg 3 Nd 1	Pos 2 Neg 4	Pos 2 Neg 4	Pos 3 Neg 3	Pos 4 Neg 2	No loss to follow-up
	> 29 WA (n=0)	0	0	0	0	0	0	0	0	
	Imprecise (n=0)	0	0	0	0	0	0	0	0	
	<i>Total (n=7)</i>	<i>Neg 3</i> <i>Nd 4</i>	<i>Pos 5</i> <i>Neg 2</i>	<i>Pos 5</i> <i>Neg 2</i>	<i>Pos 2</i> <i>Neg 4</i> <i>Nd 1</i>	<i>Pos 2</i> <i>Neg 5</i>	<i>Pos 2</i> <i>Neg 5</i>	<i>Pos 3</i> <i>Neg 4</i>	<i>Pos 4</i> <i>Neg 3</i>	<i>No loss to follow-up</i>

Congenital toxoplasmosis neg PND neg (N=293) (220 'not infected', 27 'unlikely fetal infection' and 46 lost to follow-up)	0-6 WA (n=79)	Neg 34 Nd 45	Pos 3 Neg 52 NI 1 Nd 23 ^f	Pos 1 Neg 63 Nd 15 ^f	Neg 44 Nd 35 ^f	Neg 47 Nd 32 ^f	Neg 63 Nd 16 ^f	Neg 68 Nd 11 ^f	Neg 64 Nd 15 ^f	Neg 63 (9) Lost to follow-up 16 ^f
	7-15 WA (n=99)	Neg 33 Nd 66	Pos 4 Neg 81 Nd 14 ^f	Neg 86 Nd 13 ^f	Neg 69 Nd 30 ^f	Neg 67 Nd 32 ^f	Neg 84 Nd 15 ^f	Neg 88 Nd 11 ^f	Neg 86 Nd 13 ^f	Neg 80 (10) Lost to follow-up 19 ^f
	16-28 WA (n=95)	Neg 40 Nd 55	Pos 8 Neg 61 Nd 26 ^g	Pos 2 Neg 69 Nd 24 ^g	Neg 59 Nd 36 ^g	Neg 59 Nd 36 ^g	Neg 75 Nd 20 ^g	Neg 82 Nd 13 ^g	Neg 78 Nd 17 ^g	Neg 87 (7) Lost to follow-up 8 ^g
	> 29 WA (n=13)	Neg 3 Nd 10	Pos 1 Neg 11 Nd 1	Neg 13	Neg 8 Nd 5	Neg 8 Nd 5	Neg 12 Nd 1	Neg 12 Nd 1	Neg 12 Nd 1	Neg 11 Lost to follow-up 2
	Imprecise (n=7)	Neg 6 Nd 1	Neg 4 NI 1 Nd 2 ^h	Neg 5 Nd 2 ^h	Neg 3 Nd 4 ^h	Neg 5 Nd 2 ^h	Neg 6 Nd 1 ^h	Neg 6 Nd 1 ^h	Neg 6 Nd 1 ^h	Neg 6 (1) Lost to follow-up 1 ^h
	<i>Total (n=293)</i>	<i>Neg 116 Nd 177</i>	<i>Pos 16 Neg 209 NI 2 Nd 66ⁱ</i>	<i>Pos 3 Neg 236 Nd 54ⁱ</i>	<i>Neg 183 Nd 110ⁱ</i>	<i>Neg 186 Nd 107ⁱ</i>	<i>Neg 240 Nd 53ⁱ</i>	<i>Neg 256 Nd 37ⁱ</i>	<i>Neg 246 Nd 47ⁱ</i>	<i>Neg 247 (27) Lost to follow-up 46ⁱ</i>
All (n=344)	Pos 8 Neg 129 Nd 207	Pos 47 Neg 217 NI 2 Nd 78ⁱ	Pos 32 Neg 248 Nd 64ⁱ	Pos 7 Neg 208 NI 1 Nd 128ⁱ	Pos 3 Neg 216 Nd 125ⁱ	Pos 6 Neg 269 Nd 69ⁱ	Pos 23 Neg 254 Nd 67ⁱ	Pos 22 Neg 255 Nd 67ⁱ	Congenital toxoplasmosis 51 Neg 247 (27) Lost to follow-up 46ⁱ	

PND: prenatal diagnosis, AF: amniotic fluid, MI: mouse inoculation, IC: immune charge, MTP: medical termination of pregnancy, IUFD: intra-uterine fetal death, WA: week of amenorrhea, Pos: positive, Neg: negative, Nd: not done, indeed, depending on the date of maternal infection and on the high/poor quality of the maternal, hence neonatal and pediatric, follow-up, certain tests were not performed. NA: not applicable due to fetal loss (MTP and/or IUFD), NI: non interpretable. In the follow-up column, Neg means IgG titers undetectable, using screening methods, during the first year of life; rare cases of almost complete negativity (titers < 5 UI/mL) are indicated into brackets (see Materials and Methods). The figures in the columns indicate the number of cases. Results that affirmed the diagnosis of congenital toxoplasmosis are shown in bold. With respect to the analyses that were not performed (Nd) and the follow-up (last column): ^a all were IUFD/MTP, ^b one was IUFD/MTP, ^c six of them were IUFD/MTP, ^d 14 were IUFD/MTP; follow-up was lost during pregnancy for six ^f, three ^g, one ^h and 16 ⁱ cases.

Table S4: Three by three tables of results of cord blood and placental PCR tests in case of positive and negative PND

		Positive PND			Negative PND		
		PCR on cord blood			PCR on cord blood		
		Positive	Negative	Not done	Positive	Negative	Not done
Placental PCR	Positive	5	17	4	2	17	2
	Negative	0	5	0	0	165	46
	Not done	0	1	12 (9 MTP/IUFD)	0	5	63

PND: prenatal diagnosis, MTP/IUFD: medical termination of pregnancy / intra uterine fetal death. Only positive PCR result on AF and/or cord blood (in bold) are considered to allow affirming the diagnosis of congenital toxoplasmosis.