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RESEARCH ARTICLE

The prevention of congenital toxoplasmosis using a combination of Spiramycin and Cotrimoxazole: The long-time experience of a tertiary referral centre

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Abstract

Background: Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii* and is responsible for gestational and congenital infections worldwide. The current standard therapy is based on the administration of Spiramycin to prevent trans-placental transmission. Other therapies are being studied to reduce the rates of foetal transmission and symptomatic congenital infection.

Objectives: We report our long-standing experience in maternal toxoplasmosis infection treatment using a combination of Spiramycin–Cotrimoxazole, assessing its effectiveness in preventing vertical transmission compared to the expected incidence of congenital infection.

Methods: We retrospectively collected cases of pregnant women referred to our centre for suspected toxoplasmosis infection according to Lebech criteria, treated with Spiramycin–Cotrimoxazole.

Results: Of 1364 women referred to our centre, postnatal follow-up of primary toxoplasmosis was available in 562 cases (73.9%). The overall vertical transmission rate was 3.4% in women treated immediately with Spiramycin–Cotrimoxazole after the diagnosis of infection. In comparison, it was 7.7% in women undergoing the same therapy but late or with poor compliance. The foetal transmission rate was 71.4% in untreated cases. All the infected newborns of mother treated adequately with Spiramycin–Cotrimoxazole were asymptomatic afterbirth, while 6/21 infected infants of the inadequate Spiramycin–Cotrimoxazole therapy group had postnatal sequelae (28.5%). The incidence of transmission after appropriate Spiramycin–Cotrimoxazole therapy was significantly lower than the expected rate reported in literature.

Conclusions: A combination of Spiramycin and Cotrimoxazole is safe and effective in preventing foetal congenital toxoplasmosis and reducing sequelae in case of in-utero infection. The timing and adherence to the therapy are crucial to lowering the risk of congenital infection and neonatal morbidity.

KEYWORDS
Cotrimoxazole, pregnancy, Spiramycin, toxoplasma, toxoplasmosis

Rosaria Santangelo and Lucia Masini share the last authorship.

Sustainable Development Goals (SDGs): Good Health and Well-being

INTRODUCTION

Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii* and is responsible for gestational and congenital infection worldwide [1]. While immunocompetent pregnant women who contract the parasite usually do not develop symptoms, if the infection is not diagnosed or inadequately treated, it can result in severe disease with relevant morbidity and mortality in foetuses and newborns [2]. Toxoplasmosis infection has been associated with an increased risk of spontaneous abortion in the first trimester [3]. Countries with lower income levels and higher mean temperatures present a significantly higher prevalence of toxoplasmosis infection [4]. In Italy, the reported incidence of primary maternal infection approaches 0.08%–0.2% [5] while the incidence of congenital toxoplasmosis (CT) is estimated 1–2/10.000 [6]. The risk of vertical transmission depends on gestational age at the time of maternal seroconversion, growing from 5% in the first trimester, 13% in the second trimester and 32% in the third trimester [7]. Conversely, the incidence of symptomatic disease in the foetus and the newborn follows a relatively opposite trend [8]. In addition, pre-conceptional and peri-conceptional infections can cause congenital transmission, as well as reactivations and reinfection with a different strain or in immunodeficient patients [9]. CT is often asymptomatic and the risk of symptoms at birth and in the first 3 years of life depends on the trimester of maternal infection. The most common signs are intracranial lesions and ocular lesions, present overall in about 19% of infected infants [10]. Ocular lesions seem to be less predictable and less influenced by the timing of maternal infection or maternal treatment and often occur as late sequelae, usually between 5 and 10 years of age in infants asymptomatic at birth [11]. Some countries apply screening programs during pregnancy to promptly detect maternal infection. In Italy, a serological screening is offered to all pregnant women, repeated monthly since the first trimester in seronegative ones. The monthly blood test screening in pregnancy is aimed at starting the prenatal treatment immediately to reduce the rate of vertical transmission. The current standard therapy is based on the administration of Spiramycin at the time of the diagnosis of maternal seroconversion [12]. In many centres, Pyrimethamine–Sulphonamide, a combination of anti-toxoplasma drugs crossing the placenta and foetal blood–brain barrier, is prescribed to obtain a therapeutic concentration in the infected foetus [13]. However, due to the concern about the potential teratogenic effect of folic acid antagonist drugs and the risk of hematologic toxicity, Pyrimethamine–Sulphonamide is currently recommended only in cases of high risk of transmission, such as documented primary maternal infection, foetal infection diagnosed at amniocentesis or with clear ultrasound signs of infection [14]. These drugs have proved to be safe when administered in the second and third trimesters, and some retrospective studies have reported a variable efficacy in the prevention of transmission and foetal and neonatal complications [15, 16]. The efficacy of Pyrimethamine–Sulphonamide in reducing transmission and symptoms in infected infants has been questioned by many authors [17]. Recently, according to the Austrian [18] and TOXOGEST [19] study groups, Pyrimethamine–Sulphonamide administration showed encouraging results in reducing the risk of transmission and incidence of prenatal cerebral ultrasound anomalies.

For some difficulties in supplying Pyrimethamine–Sulphonamide in our country and the concerns about the possible side effects, since the 1980s, our academic hospital developed an alternative regimen based on the association of Spiramycin plus Cotrimoxazole, a Trimethoprim–Sulphamethoxazole association [20]. Cotrimoxazole is effective against *T. gondii* and has shown greater activity in pre-clinical models than Spiramycin against the parasite [21]. Its efficacy in human infection has been proven in the treatment and prophylaxis of toxoplasma encephalitis and, like Pyrimethamine–Sulphonamide, it reaches therapeutic concentrations in foetal tissue, including the foetal central nervous system [22]. Promising results have been already reported in previous retrospective studies [23], comparing Spiramycin plus Cotrimoxazole to Spiramycin alone or to Pyrimethamine–Sulphonamide.

The present study aims to report the long-time experience of our institution in the treatment of Toxoplasma infection in pregnant women using a combination of Spiramycin plus Cotrimoxazole, evaluating its safety and efficacy in preventing foetal transmission and symptomatic congenital infection.

METHODS

We retrospectively collected all cases of toxoplasma infection during pregnancy referred to the Prenatal Diagnosis Service of the Obstetrics and Gynecology Department of 'Fondazione Policlinico Universitario Agostino Gemelli IRCCS', Rome, between 1978 and 2020. Our unit is considered a nationwide referral centre for prenatal diagnosis and infectious diseases in pregnancy, and the high number of cases during the last four decades allowed us to increase our experience in toxoplasmosis infection treatment and to build up a multidisciplinary team dedicated to high-risk cases during pregnancy and after birth. The suspect of toxoplasmosis maternal infection was based on a positive serological test during the pregnancy screening program or in case of structural foetal anomalies suggestive of in-utero infections. All serum tests were reassessed in our centre after referral, where a complete serologic panel including anti-toxoplasma IgG, IgM and IgG avidity was executed at the first visit. Since 2000, the serologic panel has been completed with IgA testing. All patients were followed up monthly by serological tests and ultrasound, according to the most recent recommendations [24].

An invasive prenatal diagnosis has been offered in the second trimester to all the patients. Until 2000, a mouse bioassay was performed to detect the presence of viable *T. gondii* intra-amniotic localisation [25]. After, according to the innovation of molecular biology, a polymerase chain

reaction (PCR) was performed on amniotic fluid in the second trimester in women who gave consent, according to the time of first diagnosis. Therapy was continued even in case of negative amniocentesis.

The maternal infection has been classified considering anamnesis, symptoms and serology in:

- Primary/active toxoplasma infection in pregnancy.
- Pre-conceptional infection (up to 6 months before conception).
- Peri-conceptional infection (up to 6 weeks before conception).
- Non-primary infection (reactivation/reinfection).

Maternal infection in pregnancy has also been classified according to the Lebech classification in definite, probable, possible and improbable [26]. The trimester of maternal infection was assessed where feasible. In some cases, patients did not repeat serological tests each month and seroconversion has been demonstrated by two serological tests repeated after a long interval, so it has not been possible to identify the certain trimester of maternal infection and these infections have been classified as 'undatable'. A patient was defined as 'IgM chronic carrier' in case of positive IgM and IgG with high IgG avidity and a history of previous positive IgG and/or IgM [27]. In the case of IgM positivity in the absence of IgG seroconversion at serologic follow-up, 'IgM false positivity' due to natural IgM was diagnosed [28]. All women with primary/active toxoplasma infection in pregnancy were prescribed a combination therapy with Spiramycin plus Cotrimoxazole or Pyrimethamine–Sulphonamide; all the patients with a diagnosis of pre-conceptional, peri-conceptional, improbable or non-primary infection were prescribed Spiramycin alone. Our Spiramycin plus Cotrimoxazole treatment protocol provided for the following therapeutic scheme:

- Spiramycin: 3×10^6 IU (870 mg) four times daily, from diagnosis until delivery.
- Cotrimoxazole: 960 mg (Sulphamethoxazole 800 mg + Trimethoprim 160 mg) twice a day in 30 days cycles with 7 days off, from the 14th week of gestational age (GA) to the 36th or 37th (depending on the end of the last cycle of therapy).
- Folinic acid: 4 mg per day during the treatment period.

Some patients were referred to our institution late in pregnancy or only at the time of delivery and received different treatments or no treatment at all. Pyrimethamine–Sulphonamide treatment was administered in case of therapy already started in another centre, in case of a confirmed diagnosis of foetal infection or when the infection was acquired in late pregnancy. Concerning the type and adequacy, maternal treatment for primary/active toxoplasma infection was classified in:

- 'Adequate maternal treatment' (aSp/C): women who started the treatment according to our protocol within 4 weeks from the time of first positive serology.
- 'Inadequate maternal treatment' (iSp/C): in case of treatment with Spiramycin plus Cotrimoxazole started more than 4 weeks from the time of diagnosis of presumed maternal infection or who interrupted the treatment or who did not complete a cycle of Cotrimoxazole or who did not follow the treatment scheme taking lower drug doses or discontinued it for more than 10 days.
- 'Pyrimethamine and Sulphadiazine treatment' (pyrimethamine–sulphonamide): mothers who received medical prescriptions from other centres, including Pyrimethamine–Sulphonamide alone or Spiramycin plus Pyrimethamine–Sulphonamide.
- No treatment (NT).

All neonates from mothers with a confirmed diagnosis of toxoplasmosis during pregnancy underwent a follow-up including clinical paediatric evaluation, fundus oculi examination, routine blood tests, serology tests for anti-toxoplasma IgG, IgM and IgA, assessment of auditory brainstem response, cerebral ultrasound. Clinical paediatric examinations followed by serology were repeated at 1, 2, 3, 4, 5, 6, 9 and 12 months of life.

Diagnosis of neonatal CT, symptomatic or asymptomatic, was made in the presence of:

- Positivity of anti-toxoplasma IgM and/or IgA in the first 6 months of life.
- Increased levels of anti-toxoplasma IgG in the first 12 months of life, with or without clinical signs.
- Persistent anti-toxoplasma IgG up to 12 months of life, with or without clinical signs.
- Placental culture or pathological examination in case of perinatal deaths positive for toxoplasma.

Congenital infection was excluded when newborn's serologic tests (specific IgG) became negative within 12 months of life or earlier in untreated infants. In the case of CT, a postnatal treatment was provided by our paediatricians, according to their protocols [23].

In case of delivery in other institutions, data were retrieved via phone calls interviewing the mothers about:

- Any neonatal health problem at birth.
- Any actual health problem of the infant.
- Neonatal serology at birth, during the first year of life and/or later.
- Neonatal brain imaging (ultrasound, computed tomography, or x-ray) after birth.
- Infant ophthalmologic evaluation at birth and/or later.
- Any therapies for toxoplasma infection administered to the newborn.

We considered as infected cases of miscarriage or termination of pregnancy with a pathological examination documenting CT or positive isolation of the parasite from foetal tissues, membranes, or amniotic fluid. We considered symptomatic for CT those infected infants with any clinical or

instrumental findings compatible with the infection: brain calcifications, hydrocephalus, microcephaly, fundus oculi abnormality, microphthalmia, sensorineural hearing loss, signs of progressive generalised disease (respiratory distress, hepato-splenomegaly, rash, anaemia, purpura). Since in all twin pregnancies with a complete follow-up infection status of the foetuses was concordant, we considered these foetuses as one. At the admission, all the patients gave written informed consent to the therapy, advised about the possible pharmacological approaches to the infection and the relative risks. All the patients consented for themselves and for their offspring to participate in the research and consented for the publication of anonymised clinical data.

Statistical analyses

Statistical analysis has been performed using the Statistical Package for Social Sciences (SPSS) v. 22 (IBM Inc., Armonk, NY, USA). Chi-square or Student's *t*-test have been used as appropriate, and the results are presented as number (percentage) or mean ± standard deviation (SD). Not-normally distributed data have been compared using the Mann–Whitney *U*-test and presented as median (interquartile range, IQR). The effects of different therapies or no therapy in preventing CT have been analysed by univariate and multivariate logistic regression. In depth, we computed odds ratio (OR) and 95% confidence intervals (CIs) by univariable logistic regression models. Only p-values <0.05 have been considered statistically significant.

RESULTS

Between 1978 and 2020, 1364 pregnant women were referred to our centre for suspected toxoplasma infection during pregnancy (Figure 1). After the exclusion of false positives, chronic carriers, reinfections, pre- and peri-conceptional infections, 760 cases (750 single and 10 twin pregnancies) were diagnosed as primary infections during pregnancy. A total of

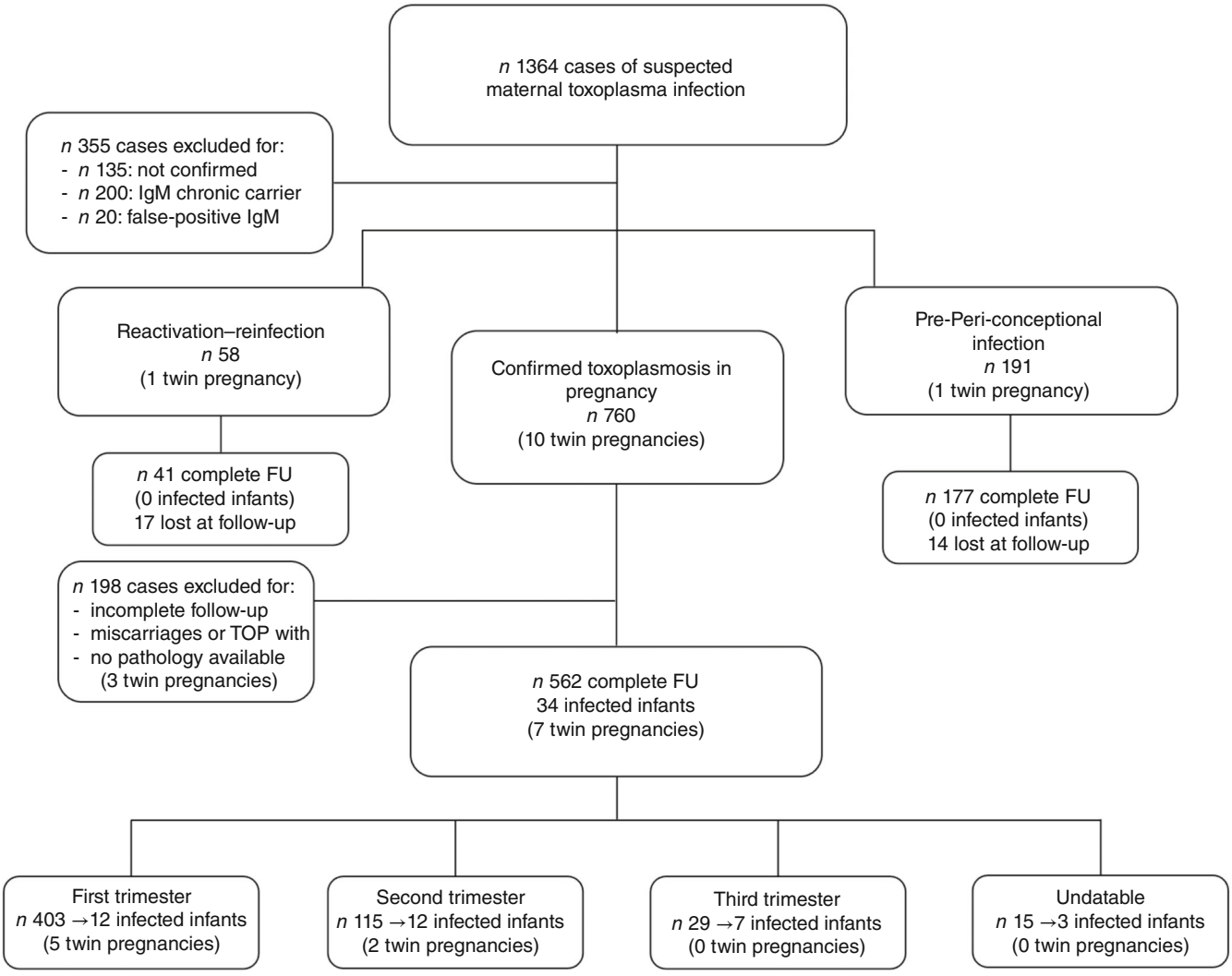


FIGURE 1 Population selection for the study.

TABLE 1 Cases of confirmed toxoplasma infection in pregnancy with complete paediatric follow-up.

Trimester of maternal infection (no. of cases)		I (403)	II (115)	III (29)	Undatable (15)	Total (562)
GA at diagnosis, weeks (SD)		10.7 (±3.64)	22.49 (±4.37)	31.5 (±3.82)	18.57 (±10.75)	15.18 (±8.05)
Diagnosis of maternal infection, cases (%)	Definite	56/403 (13.9%)	77/115 (67%)	23/29 (79.3%)	3/15 (20%)	159/562 (28.3%)
	Probable	257/403 (63.8%)	31/115 (27%)	5/29 (17.2%)	9/15 (60%)	302/562 (53.7%)
	Possible	31/40 (7.7%)	0/115 (0%)	0/29 (0%)	1/15 (6.7%)	32/562 (5.7%)
	Improbable	59/403 (14.6%)	7/115 (6.1%)	1/29 (3.4%)	2/15 (13.3%)	69/562 (12.3%)
Treatment groups, cases (%)	aSp/C	182/403 (45.2%)	42/115 (36.5%)	9/29 (31%)	0/15 (0%)	233/562 (41.5%)
	iSp/C	192/403 (47.6%)	59/115 (51.3%)	13/29 (44.8%)	8/15 (53.3%)	272/562 (48.4%)
	Pyr/Sul	14/403 (3.5%)	5/115 (4.3%)	3/29 (10.3%)	0/15 (0%)	22/562 (3.9%)
	No treatment	2/403 (0.5%)	2/115 (1.7%)	1/29 (3.4%)	5/15 (33.3%)	10/562 (1.8%)
	Missing data	13/403 (3.2%)	7/115 (6.1%)	3/29 (10.3)	2/15 (13%)	25/562 (4.4%)
Symptomatic maternal infection, cases (%)[a]		20/390 (5.1%)	9/108 (8.3%)	3/26 (11.5%)	0/13 (0%)	32/537 (5.9%)
Positive IgA, cases (%)[b]		80/168 (47.6%)	19/51 (37.3%)	5/20 (25%)	0/7 (0%)	104/246 (42.3%)
Mode of delivery, cases (%)[a]	Vaginal	248/390 (63.6%)	68/108 (62.9%)	15/26 (57.7%)	10/13 (76.9%)	341/537 (63.5%)
	Caesarean section	140/390 (36.4%)	42/108 (37.1%)	13/26 (42.3%)	4/13 (23.1%)	199/537 (36.5%)
GA at delivery, weeks (SD)[a]		39.69 (±1.99)	39.61 (±2.06)	39.89 (±1.37)	39.29 (±0.83)	39.7 (±1.96)
Birth weight, grams (SD)[a]		3362.15 (±529.87)	3264.35 (±573.64)	3525.7 (±491.43)	3166.43 (±405.7)	3346.12 (±536.64)
Infected infants, cases (%)[a]		12/390 (3.1%)	12/108 (11.1%)	7/26 (26.9%)	3/13 (23.1%)	34/537 (6.3%)

Abbreviations: aSp/C, appropriate maternal treatment with Spiramycin–Cotrimoxazole; GA, gestational age; iSp/C: Inappropriate maternal treatment with Sp/C; Pyr/Sul: Pyrimethamine and Sulphadiazine treatment.
[a]Excluded cases of missing data.
[b]Data on maternal IgA are available for a subgroup of women with clinical records from 2003.

TABLE 2 Severely symptomatic infected foetuses/newborns.

N.	Maternal infection	Prenatal ultrasound findings	Postnatal findings and outcome	Prenatal treatment group
1	I trimester	Anasarca, hydrocephalus, hepatomegaly at 34 weeks scan	Hydrocephalus, hydrops, hepatomegaly. Neonatal death	iSp/C
2	II trimester	Hydrocephalus at 37 weeks scan	Hydrocephalus, brain periventricular calcifications, chorioretinitis, microphthalmia. Surgery required	No treatment
3	Uncertain	Hydrocephalus at 37 weeks scan	Hydrocephalus, brain ependymal calcifications, microphthalmia, low-set ears. Death at 28 days	iSp/C
4	II trimester	Peritoneal calcifications, ascites, polyhydramnios at 34 weeks scan	Meconium peritonitis, ileal perforation. Surgery required	No treatment
5	II trimester	No abnormal findings	Peripheral right unilateral chorioretinitis	iSp/C
6	III trimester	Hydrocephalus at 31 weeks scan	Hydrocephalus, brain calcifications, porencephaly, splenomegaly	No treatment
7	Uncertain	Hydrocephalus, brain calcifications at 32 weeks scan	Hydrocephalus, brain calcifications. Surgery required	No treatment
8	I trimester	No abnormal findings	Right unilateral chorioretinitis (late onset)	iSp/C
9	I trimester	Hydrocephalus at 29 weeks scan	Hydrocephalus, chorioretinitis. surgery required	iSp/C
10	II trimester	No abnormal findings	Right ventriculomegaly, frontal horn periventricular calcifications. Normal development at 3 years	iSp/C

Abbreviations: iSp/C, inappropriate maternal treatment with Sp/C.

198 cases were excluded due to a dropout (170/760, 22.4%), or in case of spontaneous miscarriage/termination of pregnancy with no microbiological or pathological examinations available (28/760, 3.7%). A complete paediatric follow-up is available for 562 women with confirmed infection (Table 1): in 403 cases (71.7%), infection occurred in the first trimester; in

115 (20.5%) during the second trimester; and in 29 (5.2%) in the third trimester. Due to misleading information about the time of first positivity, 15 cases (2.7%) were considered 'undatable'. We observed 34 (6.3%) cases of confirmed CT afterbirth, 12 from a first-trimester infection (3.1%), 12 from a second-trimester infection (11.1%), 7 from a third-trimester infection (26.9%) and 3 from undatable maternal infections (23.1%). In 10 cases, foetuses showed relevant ultrasound abnormalities suspicious of severe in-utero CT, and their characteristics are shown in Table 2. Among the symptomatic infected foetuses, six cases occurred in pregnancies subjected to iSp/C (60%) while four cases (40%) in mothers who did not receive the therapy. The most frequent prenatal feature was hydrocephalus (6/10, 60%), while in 3 cases (30%) no prenatal structural abnormalities were found. In our big population, only a small percentage of patients were treated with Pyrimethamine–Sulphonamide (22/562, 3.9%), mostly referred to our institution already in treatment. According to our protocol, only 41.5% (233/562) of the cases have been treated adequately with Spiramycin plus Cotrimoxazole (aSp/C) while 48.4% (272/562) started the same treatment after 4 weeks from the diagnosis or showed a defective adherence to the therapy (iSp/C). Considering only women with a definite diagnosis, the transmission rate observed in treatment group aSp/C was 7.4%, and the CT incidence observed in treatment group iSp/C was 10.6% ($p = 0.5$). The transmission rate observed in women with a definite/probable diagnosis treated with aSp/C was 3.8%, while the transmission rate observed in the iSp/C group was 7.6% ($p = 0.09$). Considering all diagnoses (definite, probable, possible and improbable), the incidence of CT in the aSp/C group (8/233, 3.4%) compared to the iSp/C group (21/272, 7.7%) revealed a statistically significant difference ($p = 0.039$). Half of the infants born from untreated women were infected, while only 5% of CT was confirmed in women treated with Pyrimethamine–Sulphonamide. Among the 178 infants from 177 mothers (1 twin pregnancy) with complete neonatal follow-up presenting pre-/peri-conceptional infections treated with Spiramycin alone, no congenital toxoplasmosis has been reported (see Supplementary Table 1). In this group, 96 (54.2%) mothers were treated with Spiramycin starting <4 weeks from the diagnosis until delivery, 67 (37.9%) with Spiramycin starting >4 weeks after diagnosis or discontinued for more than a week. A total of 41 reactivations or reinfections have a complete follow-up and no cases of infected newborns have been reported. Logistic regression analyses showed that iSp/C (apart from no treatment) tripled the risk of CT when compared to aSp/C (OR 3.05, 95% CI 0.8–8.7; $p = 0.026$). Among other possible prognostic factors analysed, maternal anti-toxoplasma IgA positivity, amniocentesis diagnostic for intrauterine infection and maternal symptoms were not significantly associated with a higher risk of transmission. A total of 338 amniocentesis were performed in the second trimester, comprising 173 cases of PCR analyses and 160 biological assays, with only 3.8% positivity for toxoplasma intrauterine localisation. Among the 13 cases, 6 (46.1%) received Spiramycin plus Cotrimoxazole (5 aSp/C vs. 1 iSp/C, respectively) and 8 received Pyrimethamine–Sulphonamide (61.6%). The only one case iSp/C presented foetal malformations and neonatal symptoms.

Adverse reactions to Spiramycin plus Cotrimoxazole treatment have been reported by 65/505 women (12.8%). In 27 cases (5.3%) patients required treatment, while in 38 women presented only minor reactions (7.5%). Most common adverse effect reported was gastrointestinal intolerance ($n = 47$), followed by skin reactions ($n = 13$) and neurologic symptoms ($n = 5$). Discontinuation of therapy was necessary in only five women for hepatic disorders (1%) while in six cases, Cotrimoxazole was not administered because of allergy to sulphonamides. Two women who developed a cutaneous allergic reaction to Spiramycin and one to Cotrimoxazole underwent preparatory tolerance-induction therapy. A relationship between Cotrimoxazole administration in pregnancy and neonatal hyperbilirubinemia has been investigated in a subgroup of 337 non-infected infants born from 2003 to 2015. The 22.9% (36/157) of infants born from mothers treated with Cotrimoxazole had neonatal hyperbilirubinemia compared to the 16.5% (28/170) of cases in infants from mothers who did not receive Cotrimoxazole ($p = 0.092$). No cases of kernicterus or jaundice requiring treatment have been reported (Table 3).

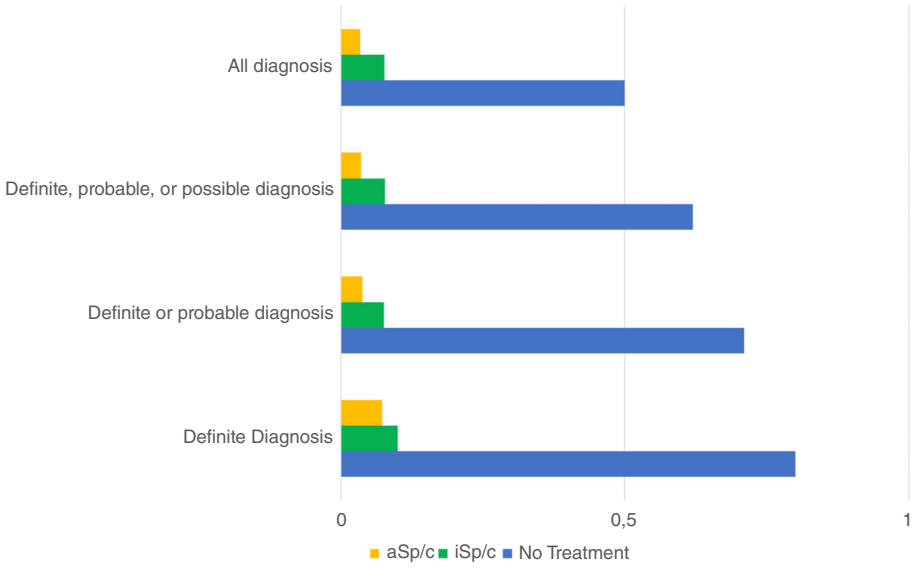
DISCUSSION

Prenatal treatment for the prevention of CT is not supported by randomised controlled trials and recent studies have questioned the efficacy of the traditional Spiramycin-based treatment, with or without Pyrimethamine–Sulphonamide. Any grade of benefit from prenatal treatment cannot be ruled out [29]. A first report [20] and a larger retrospective study [23] have shown promising results of Spiramycin plus Cotrimoxazole treatment in reducing foetal transmission, which has been demonstrated to be superior to Spiramycin alone. The results from our long-term experience in treating toxoplasmosis-positive pregnant women go in the same direction. Furthermore, this report emphasises the importance of appropriate administration of Spiramycin plus Cotrimoxazole in terms of a well-timed start of treatment and correct adherence to the therapy. Women treated correctly using Spiramycin plus Cotrimoxazole presented a lower incidence of CT compared to the expected rate of neonatal infection for each trimester [7] (respectively 1.6% vs. 5% in the first trimester; 7.1% vs. 13% in the second; 22.2% vs. 32% in the third). In the case of inadequate Sp/C treatment, the incidence of CT was constantly higher than aSp/C, with a statistically significant difference when all the diagnoses in all the trimesters were considered ($p = 0.039$) (Figure 2). Very interestingly, among the severely symptomatic newborns, no pregnant women were subjected to aSp/C, while 60% occurred in case of iSp/C. This supports the rationale that using a second-line drug that accumulates in foetal tissues is helpful to avoid CT. In our sample, Spiramycin plus Cotrimoxazole therapy seems to be safe for both mother and foetus, and a very limited number of women present adverse events or medical contraindications. Continuous

TABLE 3 Rates of vertical transmission in relation to treatment, type of infection and timing of diagnosis.

		Treatment groups, cases of vertical transmission (%)				
			Spiramycin + Cotrimoxazole			
Diagnosis of maternal infection	**Trimester of maternal infection**	**No treatment**	**aSp/C**	**iSp/C**	***p* value[a]**	**OR[a]**
Definite diagnosis	All trimesters	4/5 (80%)	5/68 (7.4%)	7/66 (10.6%)	0.501	1.50
	First trimester	0/1 (0%)	1/28 (3.5%)	1/21 (4.7%)	0.833	1.35
	Second trimester	2/2 (100%)	2/33 (6.1%)	4/34 (11.7%)	0.415	2.07
	Third trimester	1/1 (100%)	2/7 (28.6%)	2/9 (22.2%)	0.771	0.71
	Undatable	1/1 (100%)	0/0 (0%)	0/2 (0%)	—	—
Definite or Probable diagnosis	All trimesters	5/7 (71.4%)	8/209 (3.8%)	16/210 (7.6%)	0.09	2.07
	First trimester	0/1 (0%)	3/158 (1.9%)	6/138 (4.3%)	0.221	2.35
	Second trimester	2/2 (100%)	3/42 (7.1%)	6/52 (11.5%)	0.409	1.83
	Third trimester	1/1 (100%)	3/9 (22.2%)	3/12 (25%)	0.677	1.02
	Undatable	2/3 (66.7%)	0/0 (0%)	1/8 (12.5%)	—	—
Definite, probable or possible diagnosis	All trimesters	5/8 (62.5%)	8/218 (3.7%)	18/229 (7.8%)	0.051	2.24
	First trimester	0/1 (0%)	3/167 (1.8%)	7/157 (4.4%)	0.166	2.55
	Second trimester	2/2 (100%)	3/42 (7.1%)	6/46 (11.5%)	0.361	1.95
	Third trimester	1/1 (100%)	2/9 (22.2%)	4/12 (33.3%)	0.408	2.25
	Undatable	2/4 (50%)	0/0 (0%)	1/8 (12,5%)	—	—
All diagnosis	All trimesters	5/10 (50%)	8/233 (3.4%)	21/272 (7.7%)	0.039	2.35
	First trimester	0/2 (0%)	3/182 (1.6%)	9/192 (4.7%)	0.095	2.93
	Second trimester	2/2 (100%)	3/42 (7.1%)	7/59 (11.9%)	0.433	1.75
	Third trimester	1/1 (100%)	2/9 (22.2%)	4/13 (30.8%)	0.658	1.56
	Undatable	2/5 (40%)	0/0 (0%)	1/8 (12,5%)	—	—

[a]Comparisons between aSp/C and iSp/C.



aSp/C: Appropriate maternal treatment with Spiramycin-Cotrimoxazole.
iSp/C: Inappropriate maternal treatment with Spiramycin-Cotrimoxazole.

FIGURE 2 Transmission rates in treatment groups in relation to type of diagnosis of maternal infection.

administration of Cotrimoxazole despite the results of amniotic fluid PCR may prevent late dissemination from placental antibiotic-resistant quiescent foci. The trimester of maternal infection has been confirmed to be a determining factor for vertical transmission. No cases of CT following a pre-/periconceptional infection were present in our cohort, even if other

authors report transmission rates as high as 3.8% in this period [30]. The reduction of the transmission rates and the absence of CT cases in patients treated appropriately according to our protocol in comparison with those who started treatment later or who did not complete a cycle of Cotrimoxazole demonstrates the efficacy of our protocol, confirming the importance of starting promptly [15, 31]. Our experience confirms once again that monthly screening of seronegative women is extremely important, as many authors recommend [32]. Reduction in transmission rate by therapy seems to be less evident in the case of third-trimester infections, possibly because a longer period of drug administration is needed to reach effective concentrations in tissue and exert their antimicrobial activity [33].

The strength of our study is the large population size and its homogeneity, being our protocol unchanged since 1978 and independent from prenatal diagnosis.

The main limitations of our study are its retrospective nature, the absence of a consistent untreated control group and the lack of a comparison with a representative group of women treated with Pyrimethamine–Sulphonamide. Other possible biases could be found in the very long period considered: during the last 40 years thanks to the technological evolution of ultrasound the detection rate of foetal abnormalities has changed. Furthermore, invasive diagnosis of infection, once based on bioassay, now presents an increased performance due to PCR technology on amniotic fluid. The same considerations about the availability of IgA serological tests for toxoplasmosis after 2000s. We firmly deem that those potential biases do not alter the results of the proposed therapy and its efficacy in CT prevention.

In conclusion, the effectiveness of the available treatments in reducing the risk of vertical transmission in the case of toxoplasma infection during pregnancy is still controversial. Ideally, evidence from a large randomised controlled trial is needed to standardise and validate or change current clinical practice on the management of this infection in pregnancy but, to date, there is sufficient evidence to consider randomisation against placebo unethical. Our experience showed that adequate maternal treatment with Spiramycin plus Cotrimoxazole even in the case of negative amniocentesis could significantly reduce the expected transmission rate of neonatal CT with no harm to the mother and the foetus.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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