

1 **Maternal anti-*Toxoplasma* treatment during pregnancy is associated with**
2 **reduced sensitivity of diagnostic tests for congenital infection in the neonate**

3
4 **Running Title:** Anti-toxoplasma treatment impacts neonatal diagnosis

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19
20 **Key-words**

21 Congenital toxoplasmosis, *Toxoplasma gondii*, serology, IgM, IgA, western-blot, maternal treatment,
22 neonatal diagnosis, qPCR

24 **Abstract**

25 Neonatal diagnosis of congenital toxoplasmosis is based on the combination of serological and molecular
26 tests. Maternal screening and treatment differ according to national policies, and may impact on the
27 sensitivity of diagnostic methods in infants at birth.

28 In this multicenter study, 115 neonates born to 61 treated (53%) and 54 (47%) untreated women were
29 retrospectively included in three centers (France, Serbia, USA) to assess the impact of maternal anti-
30 *Toxoplasma* treatment on the performance of neonatal workup at birth (neosynthesized anti-*Toxoplasma*
31 IgM, IgA and IgG, and qPCR), using univariate and multivariate approaches.

32 Independently of the time of maternal seroconversion, the serological techniques were differently
33 impacted by maternal treatment. The detection of IgM by ISAGA and western-blot (WB) dropped from
34 90.7% and 88.2% in untreated neonates to 53.3% and 51.9% in treated neonates ($p<0.05$), whereas IgM
35 ELISA and IgA ISAGA were not significantly affected by maternal treatment. A two-fold reduction in the
36 sensitivity of neosynthesized IgG by WB was also observed in case of treatment during pregnancy (37.7%
37 versus 82.3%). Interestingly, the effect of treatment was shown to be duration-dependent, especially for
38 IgM detection, when treatment course exceeded 8 weeks, whatever the therapy. The sensitivity of
39 *Toxoplasma* PCR in blood was also lowered by maternal treatment from 39.1% to 23.2%.

40 These results highlight that anti-*Toxoplasma* therapy during pregnancy may set back biological evidences
41 of neonatal infection at birth, and underline the need for a careful serological follow-up of infants with
42 normal workup.

43

44 INTRODUCTION

45 Toxoplasmosis is a widespread protozoan foodborne infection (1, 2), affecting about one third of humans,
46 with great differences in prevalence according to geographical areas (3). Hence, public health policies vary
47 among countries, as the disease burden is diversely appreciated (4, 5). Indeed, *Toxoplasma gondii*
48 infection is largely asymptomatic, except in immunocompromised subjects. It can also be responsible for
49 congenital infection when primary infection is acquired during pregnancy. The only way to know if a
50 pregnant woman has acquired toxoplasmosis during pregnancy is to determine her serological status at
51 the beginning of gestation, and repeat serological testing regularly until delivery, if it shows an absence
52 of protective immunity. Such serological screening has been implemented in some countries including
53 France, Austria, Belgium, Italy (6–8), to allow early maternal anti-*Toxoplasma* therapy, and reduce vertical
54 transmission and severe fetal damages (9–15). The usual treatment consists in spiramycin (SPI)
55 administration until delivery (16), but if prenatal diagnosis demonstrates the presence of *Toxoplasma* DNA
56 in amniotic fluid, it is strongly recommended to switch SPI to the association of pyrimethamine and
57 sulfadiazine (PYR-SD), as the latter is more potent to reduce fetal sequelae (17). However, in about 10%
58 of cases, neonates are diagnosed after birth, despite a negative prenatal diagnosis, and in about 30% of
59 cases, prenatal diagnosis is not performed because of late infection during gestation (18). Therefore,
60 postnatal diagnosis is essential to diagnose infected neonates and start treatment, as recommended (19–
61 21).

62 Clinical and biological work-up for postnatal diagnosis of congenital toxoplasmosis relies on a combination
63 of methods including, parasite detection in placenta or amniotic fluid collected during delivery (18), cord
64 blood or newborn blood, by PCR and serological screening (22, 23). The serological workup is now well-
65 established (6, 21, 24) and usually relies on the detection of specific IgA or IgM in the neonate serum. As
66 specific IgG antibodies developed by the mother are transferred to the fetal compartment, the only way

67 to detect specific IgG synthesized by the neonate himself, is to characterize anti-*Toxoplasma* IgG profiles
68 of mother and newborn paired serum samples at birth by western-blotting. This technique has been
69 added to the routine serological workup since many years in most reference labs in France (25, 26), and
70 is widely used across Europe. Sensitivity data of these techniques have been published over years, but
71 show various performances according to studies and countries.

72 A possible impact of maternal treatment has been suggested for parasite detection in placenta or IgM
73 detection in neonates, with a higher rate of positive qPCR or positive IgM, respectively, when mothers
74 had received SPI, compared to PYR-SD (18, 27). However, both studies were conducted in France, where
75 women usually receive specific therapy during pregnancy, thus the comparison of the sensitivity of
76 biological tests in neonates born to treated and untreated mothers was not addressed. Recently, Olariu
77 et al. (28) reported a possible impact of maternal treatment on IgA and IgM detection, but the number of
78 treated cases was small, and the trimester of infection, which was shown to influence IgA and IgM
79 antibody detection in the neonate, was not taken into account.

80 Therefore, the aim of the present study was to assess the impact of maternal anti-*Toxoplasma* treatment
81 during pregnancy on the sensitivity of various tests in neonates at birth, using a multivariate analysis
82 performed on data from three countries with different national policies, i.e. maternal screening and
83 treatment (France), occasional maternal screening (Serbia), and no maternal screening (USA).

84

85 **MATERIALS AND METHODS**

86 **Ethics**

87 All analysis were performed during routine work-up, as implemented in the three participating centers.

88 Data were recorded anonymously. The study design was approved by the local ethics committee of the
89 University Hospital of Rennes (approval number: #20.08).

90

91 **Patients**

92 All congenitally infected infants diagnosed over a 10-year period (2010-2019) in the three labs (Dr. Jack S.
93 Remington Laboratory for Specialty Diagnostics, Palo Alto, CA, USA, (JSRLSD), National Reference
94 Laboratory for toxoplasmosis from Serbia (CNLTS) and Rennes University Hospital Parasitology lab (RUH-
95 PL)) were retrospectively included if a blood sample had been analyzed at birth or during the first month
96 of life. Diagnosis of congenital toxoplasmosis relied on a positive prenatal diagnosis (parasite DNA
97 detection by qPCR on amniotic fluid), and/or detection of specific IgM or IgA in peripheral blood at >7
98 days of life, or positive *Toxoplasma* qPCR in peripheral blood or cerebrospinal fluid, or detection of
99 neosynthesized IgG or IgM by western-blot.

100 Other relevant data were recorded for analysis: age of gestation at the time of maternal infection, type,
101 date of onset and duration of anti-*Toxoplasma* targeted therapy, gender of the newborn, clinical signs at
102 birth or during the first year of life. When the date of maternal infection could not be known with accuracy,
103 due to the absence of early serological screening, the estimation was graduated as “1st , 2nd or 3rd
104 trimester”, according to clinical or imaging findings or serological results during pregnancy or
105 “undetermined” when discovered only after birth.

106

107 **Serological methods**

108 Anti-*Toxoplasma* IgM were detected by ELISA (Platelia Toxo IgM, Biorad, Marnes-la-Coquette, France) and
109 ISAGA (BioMérieux, Marcy-l’Etoile, France) in CNLTS and RUH-PL, and only by ISAGA in JSRLSD. Anti-
110 *Toxoplasma* IgA were detected by ISAGA IgA (BioMérieux) in CNLTS and RUH-PL, and by an in-house ELISA
111 in JSRLSD. Comparison of maternal and neonatal IgG and IgM antibody profiles was performed using the
112 *Toxoplasma* WB IgG/IgM assay (LDBIO, Lyon, France) in CNLTS and RUH-PL.

113 ELISA indexes for IgM and IgA were positive when ≥ 1 and negative when < 0.8 . For performance
114 calculation, ELISA indexes in grey zone were merged with positive results. ISAGA IgM and IgA scores were
115 positive when ≥ 3 , according to the manufacturer's recommendations. When a positive result was
116 obtained on a cord blood sample using a quantitative test, it was taken into account only if it could be
117 confirmed on peripheral blood or if WB could rule out contamination of cord blood by maternal IgM
118 (different profiles).

119 WB profiles of the mother and her baby were compared to determine if additional bands were observed
120 in the neonatal serum (neosynthesis by the infected neonate). When cord blood samples were tested, if
121 the IgM patterns of the mother and baby were similar, then the cord blood was considered contaminated
122 by the maternal serum and was excluded from the study. When maternal and neonatal patterns differed
123 by only one faint band, the WB was considered as doubtful. In both instances, if there were no other
124 arguments in favor of the diagnosis of infection, all tests were repeated on a neonatal blood sample
125 collected several days later.

126

127 ***Toxoplasma* real-time PCR**

128 For parasite DNA detection, 200 μL of total blood or cerebrospinal fluid (CSF) were extracted using Qiamp
129 DNA Mini-kit (Qiagen) and 5 μL of DNA was used for amplification. In CNLTS and RUH-PL, the *Toxoplasma*-
130 specific quantitative real-time PCR targeted the repetitive *rep529* sequence and was carried out as
131 previously described (18, 29, 30). In JSRLSD, the PCR used two target sequences from the B1 gene (22).

132

133 **Statistical analysis**

134 All serological and PCR results were included as dichotomous variables (positive / negative), with
135 borderline test results classified as positive, and were analyzed according to maternal treatment.

136 An univariate analysis was performed on population characteristics according to treatment group. The
137 association of the different test results with qualitative variables (gender, type of sample, trimester of
138 pregnancy, treatment group) was analyzed using Pearson's χ^2 test or Fisher's exact test. For continuous
139 variables (age at time of sampling and duration of treatment) for which the Skewness and Kurtosis tests
140 showed non-Gaussian distribution, we used the nonparametric Mann–Whitney U test, and the
141 distributions were displayed as medians with interquartile ranges [25th;75th percentile].
142 Multivariate analysis was then carried out, and a logistic regression model was set up with each particular
143 test result as an outcome variable and the trimester of infection, the gender, the newborn age at sampling
144 (in days), the center, and the maternal treatment (yes / no) as covariates. The duration of treatment (by
145 categories ≤ 8 / > 8 weeks or in number of weeks) and the type of treatment (SPI or SPI \pm PYR-SD) were
146 further analyzed on the treated group. Only variables that were found to be significant by univariate
147 analysis, with $P < 0.2$, were tested forward by stepwise Wald logistic regression. The Odds ratio ([OR]) and
148 95% confidence intervals (95% CI) were used to describe the associated factors if $P < 0.05$.
149 All statistical tests and procedures were performed using the IBM SPSS 21 statistical package (IBM, NY,
150 USA).

151

152 **RESULTS**

153 **Patient file**

154 A total of 115 mother-neonate pairs were included from the 3 centers; 46 from France, 26 from Serbia
155 and 43 from the USA. Congenital infection was diagnosed antenatally ($n=27$) and/or after birth on the
156 basis of neosynthesized antibody detection or positive PCR on newborn samples (Supplementary Table
157 1). The first positive post-natal test was obtained as soon as birth until one year of age when treatment
158 was stopped (Supplementary Table 1). Neonates were aged from 0 to 35 days (median days 3 [1; 11],

159 when the first blood sample was obtained and included in the study. The time of sampling was earlier in
160 newborns from treated mothers than from untreated ones, as a result of the follow-up protocol
161 implemented in case of maternal *Toxoplasma* infection in Europe (Table 1). Blood samples included 46
162 cord blood and 69 peripheral blood samples. Most samples were taken before 5 days of life (n=70) (Table
163 1). The frequency of detection of specific IgG, IgM, or IgA neosynthesized antibodies did not depend on
164 the sample type in any of the analyzed diagnostic tests ($p>0.05$). Maternal seroconversion occurred during
165 the first (T1), second (T2) and third (T3) trimester in 14 (12.2%), 28 (24.3%) and 36 (31.3%) of cases,
166 respectively, while the date of maternal infection remained unknown in 37 (32.2%), of which 34 were
167 symptomatic cases (Table 1).

168 An anti-*Toxoplasma*-targeted therapy during pregnancy was given to 61 (53.0%) pregnant women. The
169 proportion of treated women did not differ according to the trimester of seroconversion (Table 1). As
170 expected, the median duration of treatment was significantly shorter when infection was diagnosed in T3
171 (median weeks 5 [2; 7]) in comparison with T2 (median weeks 12 [7.25; 14]) and T1 (median weeks 17.5
172 [8; 24], $p<0.001$), respectively. The treatment consisted of SPI alone (n=34), PYR-SD (n=8) or SPI followed
173 by PYR-SD (n=19). Interestingly, the proportion of infections acquired during the first, second and third
174 trimester didn't differ among treated and untreated mothers (Table 1), ruling out a possible interplay of
175 these two parameters in the interpretation of results.

176 The incidence of *Toxoplasma*-related clinical manifestations within the first year of follow-up reached
177 53.1% (60/113) (Table 1). The main disorders included cerebral lesions, i.e intracranial calcifications,
178 ventriculomegaly and meningitis in 47/60 (78.3%), ocular lesions in 26/60 (43.3%), and growth
179 retardation/prematurity in 7/60 (11.7%) neonates. The rate of newborns with clinical signs was
180 significantly lower when treatment was given during pregnancy (31.1% versus 75.9% in treated and
181 untreated groups, respectively) (Table 1).

182

183 **Maternal treatment is associated with lower frequency of detection of anti-*Toxoplasma* IgM and IgG in**
184 **the neonate, but not of IgA**

185 As a first approach to investigate the impact of maternal anti-*Toxoplasma* treatment on the results of
186 diagnostic methods, we calculated the overall sensitivity of each test for prenatally treated and untreated
187 infants. The overall sensitivity of IgM detection by ELISA and/or ISAGA was lower in infants born to treated
188 mothers ($p < 0.0001$, Table 2). However, when analyzing separately each technique, the ELISA-based IgM
189 detection was not affected by treatment, whereas the sensitivity of IgM ISAGA dropped from 90.7% to
190 53.3%, when treatment was administered ($p < 0.0001$, Table 2). WB-based IgM detection was also
191 significantly lower in the treated group, as it was positive in only 55.8%, compared to 88.2% without
192 treatment ($p < 0.05$). This reduction was also observed when gathering doubtful IgM WB profiles (only one
193 additional faint band obtained with the neonate's profile, compared to the mother's IgM profile) with
194 negative results instead of with the positive ones ($p = 0.009$, data not shown). An above two-fold reduction
195 of neosynthesized IgG detection by WB was also observed in neonates from treated mothers, 82.3%
196 versus 37.7% ($p < 0.01$). No significant differences were observed in the detection of specific IgA (ELISA or
197 ISAGA) according to maternal treatment (Table 2).

198 To further analyze the differences observed between ISAGA and ELISA for IgM detection, we evaluated
199 the sensitivity on serum samples analyzed concomitantly with both techniques (Serbian and French
200 neonates). Results showed that poorer ISAGA sensitivity was associated with maternal treatment,
201 whereas ELISA sensitivity was even in both treated and untreated groups (Table 3).

202 As the absence of specific IgM detection in the neonate could be solely due the physiologic disappearance
203 of antibodies before birth when infection occurred in early pregnancy, we then performed a multivariate
204 analysis to confirm the results obtained by univariate analysis. Interestingly, results showed that
205 treatment significantly altered IgM detection by ISAGA and WB ($p < 0.001$ and $p < 0.01$, respectively), as well
206 as neosynthesized IgG detection by WB (Table 4), thus confirming univariate analysis. Multivariate analysis

207 also showed that the trimester of maternal infection influenced the positivity of these serological tests
208 with more frequent positivity for infections acquired during the third trimester, while the age of the
209 newborn at the time of sampling had no effect (Table 4 and Supplementary Table 2). Unexpectedly, the
210 gender was significantly associated with IgM detection, with male and female infants being most likely to
211 have positive ISAGA IgM, and positive WB IgM, respectively (Table 4 and Supplementary Table 2).

212

213 **The duration of anti-*Toxoplasma* therapy during pregnancy is unequally associated with reduced**
214 **sensitivity of serological tests**

215 To further analyze if the duration of maternal anti-*Toxoplasma* therapy influenced the sensitivity of
216 diagnostic methods, we divided the group of 61 infants from treated mothers into two sub-groups,
217 according to the length of maternal treatment, i.e. ≤ 8 weeks or > 8 weeks of any treatment. Interestingly,
218 the duration of treatment interfered heterogeneously with the detection of IgM, IgA or IgG. The detection
219 of specific IgA, regardless of the method, was not altered, but when distinguishing ISAGA and ELISA, it
220 appeared that ISAGA sensitivity was significantly reduced if treatment course was > 8 weeks ($p < 0.05$, Table
221 5). Sensitivity of IgM detection, whatever the technique used (ELISA, ISAGA or WB), was dramatically
222 decreased when treatment exceeded 8 weeks ($p < 0.001$, $p < 0.01$, $p = 0.001$, respectively) (Table 5). Overall,
223 the range of IgM detection decreased from 72-79% to 29-32%, when treatment lasted ≤ 8 weeks and > 8
224 weeks, respectively, depending on the method. By contrast, IgG detection by WB was not significantly
225 modified according to the cut-off of 8 weeks of treatment (Table 5). The duration of treatment seems to
226 have a progressive effect, as illustrated in Fig 1.

227 Multivariate analysis using the same cut-off of treatment duration (≤ 8 weeks or > 8 weeks), confirmed
228 these findings (Table 5). Additionally, when taking into account the precise duration of treatment (number
229 of weeks), a longer duration of treatment was associated with a lower sensitivity of ISAGA IgA, as well as
230 of IgM detection by any technique, but no impact on neosynthesized IgG detection by WB (Table 6).

231 Overall, among the 61 treated neonates, no statistically significant difference ($p > 0.05$) was found
232 between neonates treated with SPI or PIR-SD \pm SPI, in any serological test results (Table 6).

233

234 **Prenatal therapy is associated with decreased sensitivity of the detection of parasite DNA in the**
235 **neonate at birth**

236 Eighty-four neonate specimens consisting of blood (n=66) or CSF samples (n=18) were also collected for
237 *Toxoplasma* detection by qPCR. The sensitivity dropped from 43.6% to 24.4% in neonates from untreated
238 and treated mothers, respectively (Table 2). When the precise duration of treatment was analyzed for
239 each type of treatment, regression analysis showed that there was no effect of SPI alone, whereas there
240 was a significant time-dependent effect of PYR-SD on the DNA detection by PCR (Table 6).

241

242 **DISCUSSION**

243

244 The laboratory diagnosis of congenital toxoplasmosis at birth requires the use of multiple techniques to
245 prove infection of the neonate, through detecting either antibody neosynthesis by the newborn, or
246 circulating parasite DNA. Postnatal screening of neonates is paramount in three situations: i) when
247 evocative clinical signs are present at birth and the mother has not benefited from *Toxoplasma* serological
248 screening during gestation, ii) when the mother acquired toxoplasmosis during gestation and the prenatal
249 diagnosis was negative, and iii) when the mother acquired toxoplasmosis, but prenatal diagnosis was not
250 performed (late infection during pregnancy).

251 As most French women with primary infection during pregnancy are given specific treatment, we included
252 data of infants from Serbia, where maternal screening has been only recently adopted, and from the USA,
253 where women are diagnosed and treated only in case of ultrasound anomalies. We chose to include only

254 early samples, because we wanted to draw conclusions on the effect of maternal on the early diagnosis
255 of congenital toxoplasmosis. To ensure perfect interpretation of IgM or IgA detection in cord blood
256 samples, we considered them as positive only if IgM neosynthesis by the newborn was confirmed by WB,
257 thus ruling out maternal contamination for both IgM and IgA.

258 Using univariate and multivariate analyses, we found strong evidence that maternal treatment was
259 associated with a dramatic decrease in the sensitivity of IgM and neosynthesized IgG in the neonate.
260 Whereas a direct impact of treatment on parasite replication and dissemination was expected, as it is
261 assumed to reduce parasite loads (27), the demonstration of an impact on antibody synthesis by the
262 newborn is a novelty. Indeed, it could be hypothesized that the absence of IgM or IgA detection in
263 newborns was due to a transient synthesis by the fetus, particularly if infection occurred early in gestation.
264 Indeed, we observed that the positivity of tests was associated with the trimester of infection (Table 4),
265 with a higher detection rate for infections acquired during the third trimester (Suppl. Table 2), which also
266 coincides with shorter courses of treatment. However, there was no differences in the time of infection
267 among treated and untreated mothers (Table 1), thus the time of infection cannot bias the conclusions
268 on the effect of treatment. Taken together, multivariate analysis clearly demonstrated an association of
269 treatment with a lower sensitivity of IgM and neosynthesized IgG antibody detection that could be
270 explained by a delayed synthesis. Additionally, this effect was time-dependent, with an apparent cut-off
271 of 8 weeks of any treatment (SPI or PYR-SD) for most serological tests. Besides, the ISAGA technique was
272 more impacted by maternal treatment than the ELISA technique. Since the IgM ISAGA technique uses
273 “global” antigens from entire parasites, this finding would suggest that maternal treatment could more
274 likely affect the ability of the fetus to react against surface antigens, than against cytoplasmic antigens.
275 Why SPI and PYR-SD administered to the mother had roughly the same effect on the fetus or neonate
276 antibody response is difficult to explain, as SPI is known as hardly transferring through the placental
277 barrier. A previous study by Gilbert et al. found no impact of maternal treatment on the sensitivity of IgM

278 detection by multivariate analysis, but the group treated with PYR-SD was compared to a control group
279 including both SPI-treated and untreated mothers (31).

280 In contrast to our results, Naessens et al. investigated IgM detection in peripheral blood samples from 86
281 congenitally infected newborns and found no significant effect of treatment despite an apparent decrease
282 of sensitivity (85% in untreated versus 25% in treated, $p=0.19$) ; they attributed it to the gestational age
283 of maternal infection rather than to prenatal treatment (32). However, the vast majority of mothers (75%)
284 had been treated, and the duration of treatment was not known with accuracy, nor the time of maternal
285 infection, as monthly serological screening was not current practice in most centers. In a Brazilian study
286 primarily aiming at measuring the duration of IgM synthesis by infected infants, Lago et al. observed that
287 in 23/28 neonates whose mother had been diagnosed with seroconversion during pregnancy, IgM were
288 detected during the first month of life. However, the difference in sensitivity between treated (75%) and
289 untreated (88%) groups was not statistically significant ($p=0.4$) (33).

290 While IgA detection appeared not to be affected by treatment using univariate analysis, there was a trend
291 towards a time-dependent effect of treatment in infants from treated mothers, irrespective of the type
292 of treatment. Again, this was significant only with the ISAGA technique, but it must be acknowledged that
293 there was a small number of samples analyzed with ELISA. Interestingly, it has been shown in experimental
294 murine infection that specific IgA synthesis was the least affected of all antibody classes by several anti-
295 *Toxoplasma* chemotherapeutic regimens (34).

296 In contrast, despite a significant effect of treatment on the detection of neosynthesized IgG by WB, it was
297 not related to the duration of maternal treatment. It was not related to the time of sampling, i.e. the age
298 of the newborn, either (Table 4). In infants from untreated mothers, neosynthesized IgG were found in
299 4/5 (80%) in the early days after birth (0-4 days) and in 7/9 (78%) from day 15 to 35 (data not shown). Few
300 reports are available on the detection of neosynthesized IgG using WB. In a cohort of 55 neonates, a
301 French multicenter study reported a sensitivity of IgG WB similar to ours at birth (37.7% in the treated

302 group), increasing to 40% during the first 10 days of life, and to 63.3% between 0.5 and 1.5 months (35).
303 Similar findings were published in Brazil, where the IgG WB was positive in 40% (6/15) of infected
304 newborns born to treated mothers up to 3 months of age (36). In another Brazilian series of newborns
305 aged up to 1 month and born to women who had not been given treatment during pregnancy, the
306 sensitivity of IgG WB reached 73.5 % (130/177), which is not far from the results obtained in our untreated
307 group (82.3%) (37). However, they found a lower sensitivity of IgM WB (54.8 %) compared to that of IgG
308 WB.

309 In a previous study, Olariu et al (2019) analyzed IgM and IgA detection in infants from 25 treated and 164
310 untreated mothers, and similarly found that IgM were less frequently detected in infants of treated
311 mothers (44%) than of untreated mothers (86.6%, $p < 0.001$)(28). The detection of IgA was not modified
312 according to maternal treatment, either ($p = 0.06$). However, they did not take into account the gestational
313 age at the time of maternal infection, which is a major factor impacting the sensitivity of serological tests
314 (27, 32). In addition, samples were obtained within the first six months of life, whereas in the present
315 study, we focused on the results obtained early after birth (mean age of 7 days).

316 It is interesting to note that, in case of maternal anti-*Toxoplasma* treatment, the detection of IgA was the
317 most sensitive approach to detect neonatal infection, outperforming IgM and IgG-based screening (68.4%
318 versus 59% and 38%, respectively), while it was less sensitive in untreated cases.

319 Still, the overall sensitivity of IgA and IgM detection (71.8% and 73.9%, respectively) is quite high
320 compared to previous reports, which showed sensitivity ranges from 47.8 to 72.5% and 44 to 67.5%, for
321 IgA and IgM, respectively, irrespective of the technique (24, 27, 31, 35).

322 Whereas the contribution of PCR testing in AF has been extensively evidenced for prenatal diagnosis, the
323 use of PCR on newborn blood or CSF at birth has been sparsely investigated (22). In our cohort, the
324 sensitivity of blood PCR was low (28.6%) but it is interesting to note that it ranged from 23% to 39% in
325 treated and untreated infants, respectively. Unexpectedly, while univariate analysis was significant,

326 multivariate analysis showed that treatment onset did not impact PCR positivity. However, among treated
327 neonates, the longer the treatment, the lower the sensitivity of PCR, provided that the treatment was
328 PYR-SD, which is in agreement with the expected in utero anti-parasitic effect of this bi-therapy. The
329 trimester of pregnancy had no impact on the positivity of PCR, while it might be expected to find more
330 positive PCR results when infection occurred at the end of pregnancy, due to a shorter course of
331 treatment. These findings may suffer from a bias of center, as the proportion of positive cases was higher
332 in the JSRLSD (38% versus 23% in RUHPL), but 37 cases were excluded from the multivariate analysis
333 because of missing data, thus the power of the analysis may have been impaired.

334 The strength of our study is that it was performed on a well-balanced cohort of prenatally-treated and
335 untreated infants, diagnosed prospectively in three reference centers. A limitation can result from the
336 uncertainty of the time of maternal infection in some US cases, as no serological follow-up was
337 undertaken, which led us to discard 32% of cases for the multivariate analysis.

338 Taken together, we provide evidence that anti-*Toxoplasma* therapy in the mother may contribute to set
339 back serological confirmation in the neonate, and underlines the need for careful serological follow-up of
340 neonates even if the workup at birth is normal. We also recall that prenatal diagnosis can detect at most
341 90% of infected fetus, thus postnatal serological follow-up is essential.

342

343 **ACKNOWLEDGMENTS**

344 This study was supported by the Ministry of Education, Science and Technological Development of Serbia,
345 through project no. III 41019; and by a grant to the Institute for Medical Research (contract no. 451-03-
346 68/2020-14/200015). The authors also received a grant from Campus France (PHC Pavle Savic
347 no.40734WC) (Serbian project number 451-03-01963/2017-09/15).

348

349 **LEGENDS TO FIGURES**

350 **Fig. 1: Sensitivity of diagnostic tests according to the duration of treatment.**

351 Statistical significance was graduated as * ($p < 0.05$) and ** ($p < 0.01$). Data obtained for ELISA IgA are not
352 shown because only 6 infants had been treated in utero.

353

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- 463

464 **Table 1. Population characteristics (N=115) and comparison according to treatment group**
 465

Characteristics	Maternal treatment during pregnancy		Univariate analysis [§]
	No (N = 54)	Yes (N = 61)	p
Sex ratio (M/F)	0.69 (22/32)	1.10 (32/29)	0.209, ns
Type of blood sample, n (%)			<0.0001
Cord blood (n=46)	4 (7.4)	42 (68.8)	
Peripheral blood (n=69)	50 (92.6)	19 (31.1)	
Time of blood sampling, median (interquartile range)	10 (4-17)	1 (0-3)	<0.0001
0-4 days of life (n=70), n (%)	16 (29.6)	54 (88.6)	
5-14 days of life (n=21), n (%)	20 (37.0)	1 (1.6)	
15-35 days of life (n=24), n (%)	18 (33.3)	6 (9.8)	
Trimester of maternal infection, n (%)			0.063, ns
TI (n=14)	4 (7.4)	10 (16.4)	
TII (n=28)	2 (3.7)	26 (42.6)	
TIII (n=36)	11 (20.4)	25 (41.0)	
ND (n=37)	37 (68.5)	0	
Clinical manifestation during the first year of life, n (%)			<0.0001
Yes (n=60)	41 (75.9)	19 (31.1)	
No (n=53)	11 (20.4)	42 (68.9)	
ND (n=2)	2 (3.7)	0	
Type of treatment, n (%)	na		na
SPI alone		34 (55.7)	
SPI then PYR-SD		19 (31.1)	
PYR-SD alone		8 (13.1)	

466 [§] Pearson's χ^2 test or + Fisher's exact test compared distributions in treated and untreated groups
 467 na: not applicable; nd: not determined; ns: not significant; SPI: spiramycin; PYR-SD: pyrimethamine-sulfadiazine
 468

469

470 **Table 2: Sensitivity of serological tests and qPCR in newborns according to treatment group (N=115)**

Diagnostic method	Overall sensitivity according to maternal treatment			Univariate analysis p (Fisher's test)
	% (n/N)			
	All N=115	Untreated N=54	Treated N=61	
IgA [‡] (ELISA or ISAGA)	71.8 (79/110)	75.5 (40/53)	68.4 (39/57)	0.416
IgA ELISA	72.1 (31/43)	73.0 (27/37)	66.7 (4/6)	1
IgA ISAGA	71.6 (48/67)	81.2 (13/16)	68.6 (35/51)	0.526
IgM [‡] (ELISA or ISAGA)	73.9 (85/115)	90.7 (49/54)	59.0 (36/61)	0.0001
IgM ELISA	59.2 (42/71)	68.8 (11/16)	56.4 (31/55)	0.564
IgM ISAGA	71.1 (81/114)	90.7 (49/54)	53.3 (32/60)	<0.0001
IgM WB	63.8 (44/69)	88.2 (15/17)	55.8 (29/52)	0.0198
IgG WB	48.6 (34/70)	82.3 (14/17)	37.7 (20/53)	0.0018
qPCR	33.3 (28/84)	43.6 (17/39)	24.4 (11/45)	0.104
Blood	28.8 (19/66)	39.1 (9/23)	23.2 (10/43)	0.254
CSF	50.0 (9/18)	50.0 (8/16)	50.0 (1/2)	1

471 [‡]IgM and IgA ELISA values in the grey zone were grouped with positive results

472 Na, not applicable

473

474 **Table 3. Compared sensitivity of ISAGA and ELISA assays for IgM detection (N=70) according to**
 475 **treatment group**

	Sensitivity according to maternal treatment		p (Fisher test)
	% (n/N) [£]		
	Treated	Untreated	
IgM ELISA [¤]	55.6 (30/54)	68.8 (11/16)	0.400
IgM ISAGA	51.9 (28/54)	81.2 (13/16)	0.045

482 [£]This analysis included only neonates for whom both IgM ELISA and IgM ISAGA were performed on the same
 483 sample

484 [¤] IgM ELISA values in the grey zone were grouped with positive results

485

486

487 **Table 4.** Multivariate analysis of the effect of treatment, trimester of maternal infection, and age at blood
 488 sampling on the sensitivity of diagnostic tests

489

Outcome Variable	Covariates			
	Treatment	Trimester of maternal infection	Gender male	Newborn age
Diagnostic method	p OR (95% CI)	p OR (95% CI)	p OR (95% CI)	p OR (95% CI)
IgM ISAGA (n=144)	0.000 0.117 (0.041-0.333)	0.001 3.417(1.648-7.085)	0.021 2.680 (1.160-6.192)	0.104
IgM WB (n=69)	0.002 0.049 (0.007-0.322)	0.000 6.623 (2.461-17.874)	0.003 0.174 (0.055-0.547)	0.292
IgG WB (n=70)	0.005 0.115 (0.025-0.518)	0.007 2.931 (1.348-6.372)	ND	0.242
qPCR* (n=80)	0.480 0.292 (0.068 0.990)	ND	ND	0.007 1.091(1.024-1.162)

490 *blood or CSF

491 ND; not determined because the p value in univariate analysis was <0.2 (results not shown)

492

493 **Table 5. Sensitivity of serological tests and qPCR in newborns from treated mothers (N = 61), and**
 494 **comparison according to treatment duration ≤ 8 or > 8 weeks**

495

Diagnostic test	Sensitivity according to the duration of treatment % (n/N)		Univariate analysis [§]	Multivariate analysis [§]	
	≤ 8 wks	> 8 wks	p	p	OR [95% CI]
	N = 33	N = 28			
IgA (ELISA or ISAGA) (N=57)	78.1 (25/32)	56.0 (14/25)	0.091	0.079	na
IgA (ELISA) (N=6)	33.3 (1/3)	100 (3/3)	1	nd	na
IgA (ISAGA) (N=51)	82.8 (24/29)	50.0 (11/22)	0.017	0.009	0.161 [0.041-0.629]
IgM (ELISA or ISAGA) (N=61)	75.6 (25/33)	39.3 (11/28)	0.005	0.04	0.208 [0.070-0.619]
IgM (ELISA) (N=55)	76.7 (23/30)	32.0 (8/25)	0.001	0.048	0.288 [0.083-0.972]
IgM (ISAGA) (N=60)	71.9 (23/32)	32.1 (9/28)	0.004	0.035	0.117 [0.026-0.517]
IgM (WB) (N=52)	78.6 (22/28)	29.2 (7/24)	0.001	0.001	0.112 [0.032-0.396]
IgG (WB) (N=53)	46.4 (13/28)	28.0 (7/25)	0.256	nd	na
qPCR # (N=45)	30.8 (8/26)	15.8 (3/19)	0.309	nd	na

496 na: not applicable

497 nd: not determined because the p value in univariate analysis was < 0.2

498 [§] Fischer's exact test

499 [§] multivariate analysis was done using the treatment duration as qualitative variable (> 8 or ≤ 8 weeks); data for other
 500 covariates are not shown

501

502 **Table 6: Sensitivity of diagnostic tests according to treatment duration (weeks)**

Diagnostic test	Statistical significance of treatment duration in weeks on diagnostic tests*					
	Any treatment (N=61)		SPI (N = 34)		PYR-SD ± SPI (N =27)	
	p-value [§] (Mann–Whitney test)	Regression Analysis [#] P OR [95% CI]	p-value [§] (Mann–Whitney test)	Regression analysis p OR [95% CI]	p-value [§] (Mann–Whitney test)	Regression analysis p OR [95% CI]
IgA (ISAGA)	0.052	0.047 0.929 [0.864-0.999]	0.069	0.056	0.375	ND
IgM (ELISA)	0.000	0.001 0.518 [0.581-0.962]	0.052	0.052	0.346	ND
IgM (ISAGA)	0.001	0.003 0.815 [0.440-0.956]	0.030	0.040 0.859 [0.7430.993]	0.002	0.004 0.864 [0.778-0.953]
IgM (WB)	0.000	0.001 0.857 [0.761-0.965]	0.000	0.027 0.872 [0.758 -0.901]	0.000	0.023 0.845 [0.730-0.977]
IgG (WB)	0.149	0.155	0.435	ND	0.755	ND
qPCR	0.124	0.193	0.615	ND	0.065	0.042 0.102 [0.011-0.902]

503 *data for other covariates are not shown

504 [§] comparison of the mean durations of treatment in patients with negative and positive tests results

505 [#] calculated on N= 51, 55, 60, 52, 53 and 45, respectively for the various tests

506 ND: not determined because the p value in univariate analysis was <0.2

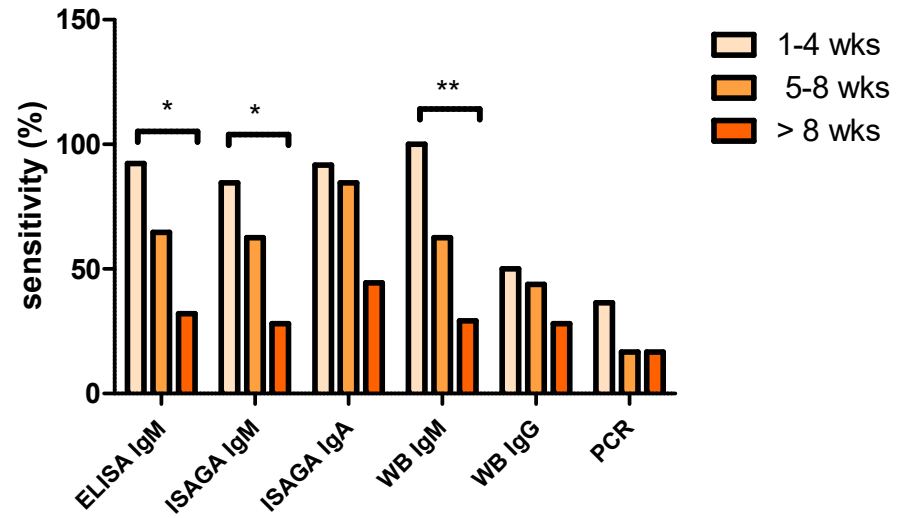
507 SPI: spiramycin; PYR-SD: pyrimethamine-sulfadiazine

508

509

510 **Figure 1**

511



512

513