

The Comparison of Polymerase Chain Reaction Directed to the 529 bp Gene and the B1 Gene in the Detection of Experimental Mouse Toxoplasmosis

Deneyisel Fare Toksoplazmozunu Tanısında 529 bç Gen Bölgesi ve B1 Geninin Polimeraz Zincir Reaksiyonu ile Belirlenmesi

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ABSTRACT Objective: This study was performed to evaluate and compare the sensitivity of Polymerase Chain Reaction (PCR) targeting the 529 base pair (bp) gene and B1 gene to detect *Toxoplasma gondii* DNA. **Material and Methods:** A total of 49 mice (*Mus domesticus domesticus*) were divided into 4 groups and they were infected with tachyzoites of *T. gondii* RH strain. Brain, liver, spleen and blood samples were obtained from infected mice at 24, 48, 72 and 96 hours following the onset of the infection. The extracted DNAs were amplified by using primers designed for the 529 bp gene and the B1 gene. **Results:** Both PCR assays succeeded to detect *T. gondii* DNA in most mice after 24 hours of infection. For each sample type, 529 bp gene PCR was more sensitive than B1 gene PCR in detecting *T. gondii* DNA (McNemar $p < 0.05$). The smallest number of tachyzoites, that Toxo DNA could be detected by B1 and 529 bp repeat gene PCR assays was 1×10^4 tachyzoites/mL and 1×10^2 tachyzoites/mL respectively, by using DNAs from serial tachyzoite dilutions. **Conclusion:** For the diagnosis of toxoplasmosis in mice, 529 bp PCR was more sensitive than B1 PCR to detect *T. gondii* DNA.

Key Words: Toxoplasma; polymerase chain reaction

ÖZET Amaç: Bu çalışmada *Toxoplasma gondii* DNA'sının saptanmasında 529 baz çifti (bç) gen bölgesine ve B1 gen bölgesine yönelik Polimeraz Zincir Reaksiyonu (PZR) uygulamalarının duyarlılıkları karşılaştırılmıştır. **Gereç ve Yöntemler:** Dört grup fare (*Mus domesticus domesticus*) *T. gondii* RH suşunun takizoitleri ile enfekte edildi. Enfeksiyonun 24, 48, 72 ve 96. saatlerinde farelerin beyin, karaciğer, dalak ve kan örnekleri alındı. İzole edilen DNA, 529 bç tekrarlayan gen bölgesi ve B1 genine spesifik primerleri kullanılarak PZR ile çoğaltıldı. **Bulgular:** Enfeksiyonun 24. saatinden itibaren farelerin tüm dokularında hem 529 bç hem de B1 genine ait PZR ürünleri saptandı. Her örnek tipi için, *T. gondii* DNA'sının belirlenmesinde, 529 bç PZR'nin B1 geni PZR'den daha duyarlı olduğu belirlendi (McNemar $p < 0.05$). Takizoitlerin seri dilüsyonundan elde edilen DNA kullanılarak 529 bç PZR ile sınır değeri 1×10^2 takizoit/mL olarak belirlenirken, B1 geni için bu değer 1×10^4 takizoit/mL olarak saptanmıştır. **Sonuç:** Fare toksoplazmozunda, 529 bç geninin, B1 genine göre *T. gondii* DNA'sının belirlenmesinde daha duyarlı olduğu kanaatine varılmıştır.

Anahtar Kelimeler: *Toxoplasma gondii*; polimeraz zincir reaksiyonu

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Nicolle and Manceaux first identified *Toxoplasma gondii* in 1908 from *Ctenodactylus gundi*, a rodent living in North Africa. The apicomplexan *T. gondii* is the sole member of genus *Toxoplasma* that is pathogenic to mammals and avian species. The life cycle of the parasite consists of asexual reproduction in mammalian intermediate hosts and sexual and asexual reproduction in the intestinal mucosa of feline definitive

host.^{1,2} This zoonotic nature of *T. gondii* accounts for its worldwide distribution. It is estimated that one third of the world population is infected by this parasite.² *T. gondii* has been detected in 30% of adults in USA. In Southern Europe 54% seroprevalence rate was reported.^{1,3} In Turkey, seroprevalence of anti-*Toxoplasma* IgG antibody titer varies between 23.1-48.6% according to various studies.⁴⁻⁸

Primary toxoplasmosis is mainly asymptomatic; only 10% of the individuals display a self limiting mild fever and lymphadenopathy.^{1,2,9} However, reactivation of the latent infection in immunocompromised hosts can lead to encephalitis or ocular toxoplasmosis.¹⁰⁻¹³ Maternal toxoplasmosis creates a risk for the development of a number of fetal abnormalities, such as chorioretinitis, cerebral calcifications and hydrocephalus.^{9,14-16}

The laboratory diagnosis of toxoplasmosis is generally based on the demonstration of the parasite in clinical samples and serology. Sabin Feldman dye test which relies on live parasites, indirect fluorescent antibody (IFA), indirect hemagglutination, latex agglutination and enzyme linked immunosorbent assay (ELISA) are the most commonly used diagnostic tests.^{9,17,18} The major drawback of these serological methods is that, the results can be difficult to interpret in the diagnosis of toxoplasmosis in immunocompromised hosts and in pregnant women. Molecular diagnostic methods offer a promising alternative in the diagnosis of toxoplasmosis, regardless of the immunological status of the patient.^{9,19,20} However, currently only a few potential DNA targets are available, due to the gap in the basic molecular biology of the organism. The first and currently the most widely used target is the 35-fold repetitive gene B1.²¹ Another popular target is the single copy gene p30 which encodes major surface antigen P30.²² Primers directed to 110-fold repetitive small subunit ribosomal ribonucleic acid (rRNA) gene, single copy alpha tubulin, beta tubulin genes, apparently non-coding repetitive TGR1_E, have also been described and used by several groups.²³⁻²⁸ Homan et al. described 200-300 fold repetitive 529 bp tandem repeat region, which they

reported to be more sensitive in the diagnosis of toxoplasmosis by PCR.²⁹

In this study, primers directed to B1 gene and 529 bp repeat gene region were used to detect *T. gondii* DNA in liver, spleen, brain and blood samples of mice infected by *T. gondii* RH strain and the sensitivity of both PCR methods were analysed.

MATERIAL AND METHODS

Animal Toxoplasmosis Model

Male mice *Mus domesticus domesticus* 4-6 weeks of age and weighing between 18-21 g were selected for this study. The animals were maintained at Gazi University under conventional conditions. All experimental procedures on mice were consistent with the International Guiding Principle for Biomedical Research Involving Animals and our research plan was approved by the Local Ethics Committee For Experiments on Animals in Gazi University. A total of 49 mice were distributed in 4 groups in addition to a control group. Group 1, 2, 3 and 4 included 10, 10, 13 and 10 mice respectively. Six animals were assigned in the control group. The animals in the control group were inoculated intraperitoneally (IP) with *T. gondii* RH strain at a dose of 50,000 tachyzoites in 0.1 mL. From each study group one mouse was sacrificed at 24, 48, 72 and 96 hours following the inoculation consecutively. Blood, brain, liver and spleen samples were collected from each animal. Giemsa stained thin blood films were prepared. Tissue samples were stained with hematoxyline eosin for histopathological examination.

Extraction of *T. gondii* DNA

The detection of *T. gondii* DNA in blood and tissues was performed on mice infected IP with virulent *T. gondii* RH strain. Blood samples were collected in Vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ, USA). DNA extraction from blood samples was performed by using cell lysis buffer (10 mM Tris-Cl pH 8.0, 1 mM EDTA pH 8.0, 0.1% SDS), potassium acetate solution (5 M potassium acetate), red blood cell lysis buffer (20 mM Tris-Cl pH 7.6) and pro-

teinase K (400 µg/mL).³⁰ DNA extraction from tissue samples was made by SNET lysis buffer (20 mM Tris-Cl pH 8.0, 5mM EDTA pH 8.0, 400 mM NaCl, 1%SDS) including 400 µg/mL proteinase K in each mL of SNET. DNA was dissolved in 200 µL 10 mM Tris-Cl, 1mM EDTA, pH 8.0.³¹ In order to measure the sensitivities of 529 bp repeat gene PCR and B1 gene PCR, serial tachyzoite dilutions of RH strain ranging from 1×10^0 to 1×10^7 were prepared. Following DNA extraction, each dilution was amplified by using primers directed to 529 bp and B1 genes.

529 bp and B1 PCR:

The 529 bp PCR assay was performed as described by Homan et al Forward primer TOXO4 (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and reverse primer TOXO5 (5'-CGCTGCAGACA-CAGTGCATCTGGATT-3') (TibMolBiol, Germany) were used.²⁹ Each reaction tube contained 50 µL of a mixture containing 0.5 µM of each primer, 100 µM dNTP, 10 µM Tris-HCl (pH 8.8), 50 µM KCl, 0.08% NP-40, 2 µM MgCl₂, 1U Taq DNA polymerase (Fermentas, USA). Amplification protocol consisted 3 minutes of initial denaturation at 94°C, followed by 35 cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, extension for 1 minute at 72°C and a final extension for 10 minutes incubation at 72°C. The B1 nested-PCR assay was performed as described by Burg et al with buffer conditions as described for the 529 bp PCR at both runs. For B1 PCR outer forward (5'-TGTTCTGTCCTATCGCA-ACG-3'), B2 outer reverse (5'-ACGGATG-CAGTTCCTTTCTG-3'), B3 inner forward (5'-TCTTCCCAGACGTGGATTTTC-3'), B4 inner re-

verse (5'CTCGACAATACGCTGCTTGA3') (TibMolBiol, Germany) were used as described by Grigg et al (Table 1).^{21,32} Both runs of amplification consisted of 3 minutes of initial denaturation at 94°C, followed by 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C, extension for 30 seconds at 72°C and a final 5 minutes incubation at 72°C. Finally the amplification products were visualized on an ethidium-stained 2% agarose gel.

Statistical Analysis

The McNemar and Wilcoxon Signed Ranks tests were used for statistical analysis in SPSS (13.0 version) software program, and the 95% confidence interval (CI) was calculated. A P value less than 0.05 was considered statistically significant.

RESULTS

At 96 hours of infection, hemorrhage foci in brain samples and in organomegalia, hemorrhage and necrosis foci in spleen samples were observed macroscopically. Histopathological evaluation revealed granulomatous formations in the spleen and livers but no cystic formation. *T. gondii* tachyzoites were observed in blood smears stained by Giemsa under microscopy.

In order to determine the sensitivity of the PCR assays in detecting *T. gondii* DNA, extracts from 10-fold dilutions of tachyzoites were tested by using TOXO-4 and TOXO-5 primer pairs directed to the 529 bp repeat gene, and B1-B2, B3-B4 nested primer pairs directed to the B1 gene. While 529 bp PCR detected down to 1×10^2 tachyzoites/mL, B1 nested-PCR was not able to detect less than 1×10^4 tachyzoites/mL (Figure 1).

TABLE 1: Oligonucleotide primers, target regions and amplicon sizes were used in this study.

Primer	Primer sequence	Target region	Amplicon size
B1	5'-TGTTCTGTCCTATCGCAACG-3'	128..147 ^a	579
B2	5'-ACGGATGTCAGTTCCTTTCTG-3'	688..707 ^a	
B3	5'-TCTTCCCAGACGTGGATTTTC-3'	152..171 ^a	530
B4	5'-CTCGACAATACGCTGCTTGA-3'	663..682 ^a	
TOXO4	5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3'	-2..24 ^b	533
TOXO5	5'-CGCTGCAGACACAGTGCATCTGGATT-3'	504..529 + 2 ^b	

^a: B1 gene, GenBank accession no. AF179871.

^b: 529 bp repeat gene region, GenBank accession no. AF146527.

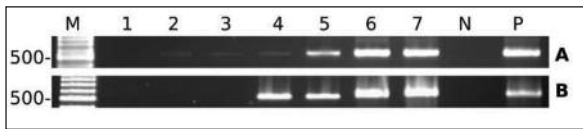


FIGURE 1: Sensitivity determinations of 529bp (A) and B1 nested-PCR (B). DNA was extracted from dilutions of tachyzoites as described in material and methods. M: 100 bp molecular weight marker (Gibco, BRL), lanes 1-7: of tachyzoites dilutions (1 x 10⁻¹ x 10⁻⁷), N: Negative control, P: Positive control.

Combining the assay results of all four tissue/blood samples of individual mice, failure to diagnose toxoplasmosis occurred in one mouse with 529 bp PCR and in four mice with B1 PCR. Overall sensitivities of the PCR assays were 97.67% and 88.37% respectively (McNemar p= 0.125) (Figure 2). Although the sensitivities of the two assays with combined samples were similar, 529 bp PCR was positive in 2, 3, 3 and 4 out of four sample types at 24, 48, 72 and 96 hours of infection respectively. However, B1 gene PCR was positive in 1, 2, 2 and 3 out of four sample types at the respective time points (Consecutive Wilcoxon Signed Ranks Test p values of 0.059, 0.024, 0.031 and 0.004) (Table 2). Either PCR assay failed to amplify toxoplasma DNA in one animal at 24 hours group. Results revealed that 529 bp PCR had a higher sensitivity compared to B1 PCR (Table 3). The success of DNA extraction was confirmed by using mouse IL-1 gene specific PCR primers (data not shown).

DISCUSSION

Detection of IgG and IgM class antibodies against *T. gondii* offers a sensitive, specific and cost-effective tool for the diagnosis of toxoplasmosis. Recently, IgA, IgE levels and IgG avidity have gained importance in accurate diagnosis of toxoplasmosis.^{9,18} However, the serodiagnosis of toxoplasmosis can be quite compli-

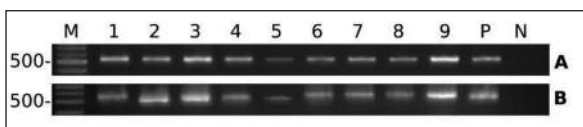


FIGURE 2: Amplification products of various tissue and blood samples. (A) 533 bp products were amplified by using TOXO4-TOXO5 primer pairs. (B) 530 bp products were amplified by using B1-B2 and B3-B4 primer pairs. M: 100 bp molecular weight marker (Gibco, BRL), lanes 1-9: Mouse tissue and blood samples N: Negative control, P: Positive control.

cated since IgM antibodies may persist for up to 2 years after acute infection and the presence of rheumatoid factor in serum samples and elevated antinuclear antibodies may cause false positive results. In such cases, seroconversion of IgA and IgE antibodies may be helpful in the diagnosis of acute infection. Another pitfall in the serodiagnosis of toxoplasmosis is the window period of several weeks between parasitemia and detectable levels of antibodies. This delay in the diagnosis of acute infection may create a risk for the fetus. Avidity of IgG antibodies to discriminate acute and chronic infection may be difficult to interpret because of the delayed IgG avidity responses.^{9,18,19,33} In tandem use of specific serological tests and PCR method has been suggested particularly in pregnant women with toxoplasmosis. However, because the diagnosis of infection is based on increased antibody titration, seropositivity alone is not sufficient to suggest infection.^{18,34}

The difficulties in serodiagnosis of toxoplasmosis such as insufficient antibody response in immunocompromised patients, prolonged antibody response in congenital toxoplasmosis cases, and inability to differentiate acute and chronic infections have increased the importance of well-designed molecular diagnostic methods.²⁰

Compared to conventional methods (serological tests, cell culture and animal inoculation) PCR provides a much more sensitive and earlier diagnosis in congenital toxoplasmosis starting from the 18th week of gestation.^{9,19} Patients with toxoplasmic encephalitis - revealed by brain biopsy - and immunocompromised patients with ocular toxoplasmosis, have been successfully diagnosed by PCR.^{11,19,35-37} The sensitivity of PCR methods were reported as 65%-80% in the amniotic fluid, 60% in placenta samples, 17%-100% in cerebrospinal fluid (CSF), 15%-53% in aqueous humor and in bronchoalveolar lavage fluid.¹⁹

Lack of a standard molecular method hinders comparisons of the sensitivity and specificity of molecular tests by conventional methods.^{19,20} On the other hand, the results of PCR methods used in determining *T. gondii* were reported to be consistent with the results of animal inoculation experiments.^{14,17,38}

TABLE 2: 529 bp repeat gene PCR and B1 gene PCR results of blood, brain, liver, spleen and all four samples types combined.

Group	Blood		Brain		Liver		Spleen		Combined	
	529bp	B1	529bp	B1	529bp	B1	529bp	B1	529bp	B1
24 hours	-	-	-	-	+	-	-	-	+	-
	-	-	+	+	+	+	-	-	+	+
	-	-	-	-	-	-	-	-	-	-
	+	-	+	+	+	-	-	-	+	+
	-	-	-	-	-	-	+	+	+	+
	+	+	+	-	+	+	+	+	+	+
	-	-	-	-	+	+	-	-	+	+
	+	+	-	-	+	+	+	+	+	+
48 hours	-	-	-	-	+	-	-	-	+	-
	+	+	+	-	+	+	+	-	+	+
	+	+	+	+	+	+	+	+	+	+
	+	-	+	+	-	-	+	-	+	+
	+	-	+	+	+	+	-	-	+	+
	+	+	-	-	+	+	+	+	+	+
	+	-	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
72 hours	+	+	-	-	-	-	+	+	+	+
	-	-	-	-	+	+	+	-	+	+
	+	+	+	+	+	+	+	+	+	+
	+	-	+	+	+	-	+	+	+	+
	+	+	+	-	+	-	+	+	+	+
	+	-	+	+	+	+	+	+	+	+
	-	-	+	-	+	-	+	+	+	+
	+	+	-	-	+	-	+	+	+	+
	+	+	+	-	+	-	+	+	+	+
	+	+	+	+	-	-	+	+	+	+
	+	-	-	-	-	-	-	-	+	-
96 hours	+	+	-	-	+	+	-	-	+	-
	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	-	+	+	+	+
	+	+	-	-	+	-	+	+	+	+
	+	+	+	-	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	-	+	+
	+	-	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
									42/43	38/43
Sensitivity (%)									97.67	88.37
*McNemar p									0.125	

529bp: 529 bp PCR; PCR, B1: B1 gene PCR, *CI: 95%.

TABLE 3: The sensitivity comparison of B1 and 529bp repeat gene PCR assays.

Sample type	B1 PCR	Blood		Brain		Liver		Spleen	
		Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
529 bp PCR	Negative	8	0	16	0	7	0	10	0
	Positive	11	24	9	18	12	24	7	26
	Total	19	24	25	18	19	24	17	26
Sensitivity* (%)	B1 gene	55.81		41.86		55.81		60.47	
	529bp gene	81.40		62.79		83.72		76.74	
**McNemar p	0.001	0.004		0.000		0.016			

Neg.: Negative, Pos.: Positive, *Sensitivities were calculated as (the number of PCR positives for a given sample type/total number of animals) x 100.⁴³ **CI: 95%.

In studies by various study groups, different primers were drafted for different gene regions and therefore uncomparable results were obtained.^{19,20} Real-time PCR using 529 bp repeat gene region was reported to be ten times more sensitive than PCR directed to amplify B1 gene, which has a copy number of 200 per genome, compared to 20 of the former.³⁹

In animal models, histological diagnosis was negative particularly in mice infected with few parasites. To observe the cysts in mice brains, a 40-day period is required. However, tachyzoites can be identified 3-4 days after IP inoculation of the parasite.^{17,40} In our study, the pathological lesions in various organs of the mice were observed macroscopically at 96 hours of infection. Hemorrhagic foci were observed in brain specimens; however, parasitic proliferation along with hemorrhagic and necrotic foci was present in spleen samples of mice. The histopathological evaluation also revealed tachyzoites in the liver and granulomatous formations in the spleen at 96 hours of infection. In all groups, tachyzoites were observed in Giemsa stained blood smears.

Variability of the sensitivity of B1 gene specific PCR depending on sample type was previously reported. In blood samples of dogs and cats, a parasite load of at least 100 tachyzoites/mL was required for successful amplification, which is quite insensitive compared to 10 tachyzoites/mL in aqueous humor, CSF and serum samples. In the placental tissues of aborting sheep, PCR was as sensitive as to detect 10 parasites/mL, and results were confir-

med by mouse inoculation studies.¹⁷ Our results showed that 529 bp PCR could detect down to 1 x 10² tachyzoites, while B1 specific nested-PCR detected a minimum of 1 x 10⁴ tachyzoites/mL.

PCR with B1 gene was shown to be a fast and sensitive method. However, for reliable and reproducible results, a sufficient amount of sample - at least 2 mL of the fluid and 10 mg of the tissue - is required.⁴¹ In determining *T. gondii* in aqueous humor samples by PCR, B1 (nested-PCR) was 20 times more sensitive than P30 (nested-PCR), and 18S rDNA regions.⁴²

Mice were infected IP with 5 x 10³ tachyzoites and in the serum samples of the animals, no trace of infection was noted at 3-15 hours by all three methods. PCR detected the infective agent in three mice out of five, at 18 hours and in all animals on 1-7 days of infection, while using other methods the infective agent was determined only 24 hours after the infection.⁴³ Paugam et al used B1 and TGR 1_E genes in mice infected orally and in blood samples, *Toxoplasma* DNA was determined on 2-21 days of infection. No growth was detected in the cell cultures, at this period.⁴⁴ Weiss et al, in their acute infection model of mice, demonstrated parasite DNA in the tissue samples of the animals on the second day of infection and from the blood samples on the 5th day using P30 gene specific PCR. Thus, they reported that P30 PCR could determine as few as 10 parasites.⁴⁵ In our study, using both the B1 gene and the 529 bp PCR, *T. gondii* DNA was detected in the blood and tissue samples starting from the 1st day of infection.

Wastling et al infected sheep by injecting *T. gondii* S48 strain subcutaneously and used primers directed to B1 and P30 gene regions in blood and lymph fluid samples. They found that, similar to the mouse toxoplasmosis model, the B1 gene was more sensitive than the P30 gene. Moreover, the false positivity of P30 PCR was twice higher than that of B1 PCR. The lack of false positivity in the mouse model was attributed to inability to detect non-living tachyzoites in mouse tissues.⁴⁶

T. gondii DNA was detected in urine and blood samples but not in brain samples of mice infected with the RH strain between 24 and 96 hours of infection using P30 specific PCR. These results were in concordance with the cell culture studies with MRC5 fibroblasts, indicating that, the PCR method was also similarly sensitive.⁴⁷

Garcia et al used a pig model of *T. gondii* with the VEG strain. On the 60th day of infection, brain, heart, tongue, and diaphragm tissues of the animals were evaluated with the 529 bp repeat gene region PCR in comparison to mouse inoculation and histopathological evaluation. Histopathologically, all the tissues were negative, while mouse inoculation was more sensitive than PCR. This was attributed to the heterogenous distribution of tachyzoites in the tissues.⁴⁸

In a study by Filisetti et al, on umbilical cord blood and amniotic fluid samples of the newborn with suspected congenital toxoplasmosis, 18SrDNA, B1, and 529 bp PCRs and mouse inoculation method were compared. Notably, 529 bp repeat gene region PCR was in complete concordance with mouse inoculation. The sensitivity and specificity of 529 bp gene were comparable to mouse inoculation and higher than that of the B1 gene.¹⁴ Comparisons of Real-Time PCR based B1 and 529 bp PCRs in the blood samples of 30 organ donors with reactive toxoplasmosis showed that the 529 bp repeat gene region was able to identify down to one genomic copy of the parasite (80 fg DNA), while B1 gene identified ten parasite genomes (800 fg DNA).⁴⁹ Similarly, Cassaing et al used Real-Time PCR method and compared the sensitivity of B1 and 529 bp PCR in determining the infection in amniotic fluid, placenta, aqu-

eous humor, blood, CSF and bronchoalveolar fluid samples. They found that 529 bp PCR was more sensitive than the other methods in all samples, particularly in the amniotic samples.⁵⁰ Colombo et al determined a sensitivity of 80% and specificity of 98% for PCR applied on the blood samples of patients with AIDS.¹¹ In studies on CSF samples, the sensitivity of the PCR method was 11.5%-100%, and the specificity was 96%-100%.^{35,51,52} Studying with CSF samples, however, was considered disadvantageous because of the invasive nature of the process and comparable results obtained from blood samples.¹¹

In our study, 529 bp PCR was 100 times more sensitive than B1 PCR. A minimum of 1×10^4 tachyzoites/mL was detected by B1 PCR, compared to 1×10^2 tachyzoites/mL detected by 529 bp PCR. Combining all four sample types, *T. gondii* DNA was determined as early as the 1st day of infection by using 529 bp PCR in all but one mice. In contrast, B1 PCR failed to diagnose toxoplasmosis in three mice within the 1st 24 hours of infection. Nevertheless, both 529 bp PCR and B1 PCR perform equally well in a molecular diagnostic protocol based on concurrent blood, brain, liver and spleen sampling. However, in routine, it is not realistic and feasible to obtain such a variety of samples from the vast majority of the patients. Practically, it is more desirable to obtain only one sample per patient, and preferably the least invasive method is the choice. In this aspect, 529 bp and B1 PCR were performed for each sample type.

In the diagnosis of toxoplasmosis, molecular tests are more advantageous than serological, histopathological tests and cell culture/animal inoculation because they provide results in relatively shorter time, have higher sensitivity, can be evaluated independently from the immune system, and aid conventional methods.

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