

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Detection of *Toxoplasma gondii* from Sera and Urine of Experimentally Infected Mice by PCR

Saeedeh Shojaee, Sasan Rezaie and Hossein Keshavarz

Department of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, P.O. Box 6446/14155, Tehran University of Medical Sciences, Tehran, Iran

**Abstract:** *Toxoplasma gondii* was detected in sera and urine of acutely infected mice. Animals were inoculated intraperitoneally with tachyzoites of *T. gondii*, RH strain. Ten animals were killed every day from day 1 up to day 7 post infection. Urine and sera of animals were collected and stored at -20°C until use. PCR performed by B<sub>1</sub> gene amplification. *Toxoplasma* was detected in sera from 3 days post infection and in urine from 5 days post infection. No parasite was detected in control group.

**Key words:** *Toxoplasma gondii*, PCR, sera, urine

### INTRODUCTION

Toxoplasmosis is a world-wide endemic disease, caused by *Toxoplasma gondii*. Infected immunocompetent individuals present only with mild symptoms or may even be completely asymptomatic. In congenitally infected children and immunocompromised persons infection causes high rates of morbidity and mortality (Fuentes *et al.*, 1996).

The classical diagnosis of toxoplasmosis is based on detection of specific antibodies that have poor efficiency, especially in neonates and in immunocompromised patients. There have been many studies to detect parasite or its components in experimental infection (Raizman and Neva, 1975; Hafid *et al.*, 1991) or in human toxoplasmosis (Van Knapen and Panggabean, 1977; Hafid *et al.*, 1995). Recently PCR assays for the detection of *T. gondii* DNA with primers specific to different regions of genome of the parasite have been developed (Burg *et al.*, 1989). Several studies were performed for the diagnosis of different forms of toxoplasmosis with different kinds of samples by PCR (Grover *et al.*, 1990; Johnson *et al.*, 1993). Huskinson *et al.* (1989) had suggested the detection of *T. gondii* in urine of experimentally infected mice by immunoblotting. Nguyen *et al.* (1996) detected *T. gondii* in blood, urine and brain of infected mice by PCR-DEIA and Hafid *et al.* (2000) have compared PCR, capture ELISA and immunoblotting for detection of *T. gondii* in sera of experimentally infected mice. In this study PCR was evaluated for detection of *T. gondii* in serum and

urine of experimentally infected mice with tachyzoites of RH strain, with the aim of improvement of diagnosis in human toxoplasmosis.

### MATERIALS AND METHODS

The this study was performed at the Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Science from Nov. 2005 to April 2006. One hundred male Balb/c mice were infected by intraperitoneal route with  $5 \times 10^4$  tachyzoites of *T. gondii* RH strain. Urine samples were collected from animals then they were killed and whole blood were removed from them and centrifuged for 10 min at 200 g. This procedure was done from day one up to day seven post infection. The collected sera and urine were kept frozen at -20°C until use. Sera and urine of ten uninfected mice were collected as negative controls.

The DNA extraction was performed on 200 µL of serum and urine from infected and non-infected mice and tachyzoites of *T. gondii* with PCR kit (QIA Gene amp DNA mini kit, Germany) in accordance with the manufacturer's instructions.

Amplification of the B<sub>1</sub> gene was performed according to the method of Burg *et al.* (1989) with two primers, 5'ATTGCCCGTCCAAACTGCAACAACACTG and 5'TGGGTCTACGTCGATGGCATGACAAC.

Briefly 10 µL of DNA product extraction were added to 10 µL of 10 × PCR buffer (10 mM. Roche, Germany), 5 µL of 25 mM MgCl<sub>2</sub> (Roche, Germany), 2 µL of each

10 mM deoxynucleoside triphosphate (dATP, dCTP, dGTP and dTTP; Roche, Germany), 20 pmol of each primer (Roche, Germany), 10 IU of Taq-DNA Polymerase and 18  $\mu$ L of distilled water. The reaction performed in thermocycler (Techne, Uk).

After an initial denaturation step at 94°C for 4 min, 35 cycles were run, consisting of denaturation (94°C for 1 min), annealing (58°C for 45 sec), extension (72°C for 1 min) and a final extension at 72°C for 10 min.

PCR Products and DNA mol. wt marker (100 bp ladder, Biolab, England) were analyzed simultaneously by electrophoresis in an agarose 1% w/v gel (Merck, Germany) in 1 $\times$ TAE buffer. Amplified fragments of 570 bp were visualized under UV illumination after staining with ethidium bromide (Sigma, USA). A positive control (DNA extracted from tachyzoites) and negative control (DNA extracted from non-infected mice) were tested in each experiment.

## RESULTS

The PCR assay gave positive results from serum and urine samples. The parasite was detected from day 3 post infection in serum of all experimentally infected mice. The results were positive in days 3, 4, 5 6 and 7 post infection in serum samples. The amplified fragments related to sera of 6th day were more intensive than fragment related to other days of infection. All control groups had negative results (Fig. 1a).

The parasite was detected in urine of all mice from 5 days post infection. The PCR gave positive results in days, 5, 6 and 7 post infections in urine samples. The results for urine of control groups were negative (Fig. 1b).

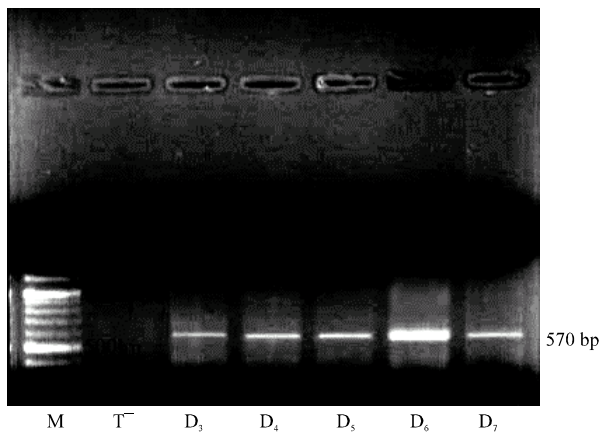


Fig. 1a: Detection of *T. gondii* RH strain in mice sera by PCR. M, mol. wt marker (100 bp ladder); T<sup>-</sup>, negative control, D, day post infection

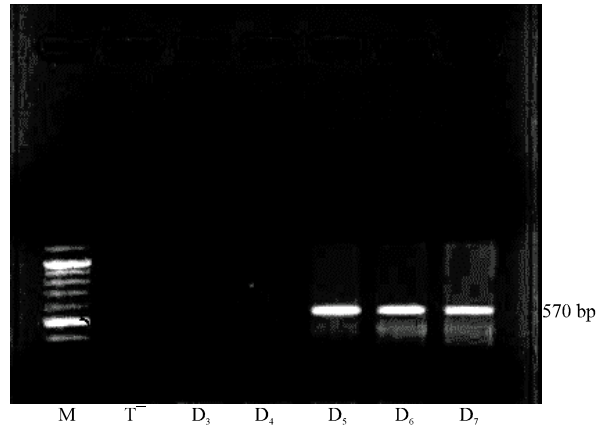


Fig. 1b: Detection of *T. gondii* RH strain in mice urine by PCR. M, mol. wt marker (100 bp ladder); T<sup>-</sup>, negative control, D, day post infection

The amplified fragments were more intensive in sera than urine.

## DISCUSSION

The molecular diagnosis of toxoplasmosis essentially relies on the PCR assays. Numerous PCR assays have been developed, using different sets of primers for different DNA targets and each has been tested, usually, with small numbers of biological samples from different body sites (Weiss, 1995). The most widely used targets are B<sub>1</sub> and P<sub>30</sub> genes. In this study primers from the 35 fold repetitive B<sub>1</sub> gene were designed. Serum and urine of infected mice were compared. DNA was detected in sera from 3 days and in urine from 5 days post infection.

Although 2 initial studies could not detect any parasitemia, either in infected mice (Savva *et al.*, 1990) or human (Ho-Yen *et al.*, 1992), all subsequent studies did so. In experimental model, with virulent strain, parasitemia was detected (Angel *et al.*, 1997; Wiess *et al.*, 1991; Joss *et al.*, 1993; Hafid *et al.*, 2000), between 1 and 5 days post infection. There are few studies performed for *Toxoplasma* detection from urine. In one study four infants suspected of having *Toxoplasma gondii* infection were studied with different methods including PCR. *T. gondii* was detected by PCR in the urine of all of them (Fuentes *et al.*, 1996). In other study, *Toxoplasma gondii* antigens were detected in urine of acutely infected mice by enzyme-linked immunosorbent assay (Huskinson *et al.*, 1989).

In present study, *T. gondii* was detected in both serum and urine of infected mice by B<sub>1</sub> gene amplification. The results suggest that isolation of parasite is possible both in sera and urine of experimentally infected mice.

Taken together, parasitemia could be detected in infection with *T. gondii* by PCR. So it would be a useful tool for early detection of toxoplasmosis in human infection especially in neonates and immunocompromised patients but the time period at which the parasite is present in human urine is unknown so, more studies are necessary in human toxoplasmosis especially in acute period of the disease.

#### REFERENCES

- Angel, S.O., M. Matrajt, J. Margarit, M. Nigro, E. Illescas, V. Pszenny, M.R.R. Amendoeira, E. Guarnera and J.C. Garberi, 1997. Screening for active toxoplasmosis in patients by DNA hybridization with the ABGTg7 probe in blood samples. *J. Clin. Microbiol.*, 35: 591-595.
- Burg, J.L., C.M. Grover, P. Pouletty and J.C. Boothroyd, 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J. Clin. Microbiol.*, 27: 1787-1792.
- Fuentes, I., M. Rodriguez, C.J. Domingo, F. Castillo, T. Juncosa and J. Alvar, 1996. Urine sample used for congenital toxoplasmosis diagnosis by PCR. *J. Clin. Microbiol.*, 34: 2368-2371.
- Grover, C.M., P. Thulliez, J.S. Remington and J.C. Boothroyd, 1990. Rapid prenatal diagnosis of congenital *Toxoplasma* infection by using polymerase chain reaction and amniotic fluid. *J. Clin. Microbiol.*, 28: 2297-2301.
- Hafid, J., R. Tran Manh Sung and B. Posseto, 1991. Kinetic of circulating antigens by capture ELISA and immunoblotting during toxoplasmosis. *Eur. J. Parasitol.*, 27: 40-45.
- Hafid, J., R. Tran Manh Sung, H. Raberin, Z.Y. Akono, B. Pozzetto and M. Jana, 1995. Detection of circulating antigens of *Toxoplasma gondii* in human infection. *Am. J. Trop. Med. Hyg.*, 52: 336-339.
- Hafid, J., D. Guichard, P. Flori, T. Bourlet, H. Raberin, C. Genin and R.T. Sung, 2000. Detection of *Toxoplasma gondii* by polymerase chain reaction in sera of acutely infected mice. *J. Parasitol.*, 86: 857-859.
- Ho-Yen, D.O., A.W. Joss, A.H. Balfour, E.T.M. Smyth, D. Baird and J.M.W. Chatterton, 1992. Use of polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. *J. Clin. Pathol.*, 45: 910-913.
- Huskinson, J., P. Stepick-Biek and J.S. Remington, 1989. Detection of antigens in urine during acute toxoplasmosis. *J. Clin. Microbiol.*, 27: 1099-1101.
- Johnson, J.D., P.D. Butcher, D. Savva and R.E. Holliman, 1993. Application of the polymerase chain reaction to the diagnosis of human toxoplasmosis. *J. Infect. Dis.*, 26: 147-158.
- Joss, A.W.L., J.M.W. Chatterton, R. Evans and D.O. Ho-Yen, 1993. *Toxoplasma* polymerase chain reaction of experimental blood samples. *J. Med. Microbiol.*, 38: 38-43.
- Nguyen, T.D., M. DE Kesel, G. Bigaigenon, P. Hoet, G. Pazzaglia, M. Lammens and M. Delmee, 1996. Detection of *Toxoplasma gondii* tachyzoites and bradyzoites in blood, urine and brains of infected mice. *Clin. Diag. Lab. Immunol.*, 3: 635-639.
- Raizman, R.E. and F.A. Neva, 1975. Detection of circulating antigen in acute experimental infections with *Toxoplasma gondii*. *J. Infect. Dis.*, 132: 44-48.
- Savva, D., J.C. Morris, J.D. Johnson and R.E. Holliman, 1990. Polymerase chain reaction for detection of *Toxoplasma gondii*. *J. Med. Microbiol.*, 32: 25-31.
- Van Knapen, F. and S.O. Panggabean, 1977. Detection of circulating antigen during acute infection with *Toxoplasma gondii* by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.*, 6: 546-547.
- Wiess, L.M., S.A. Udem, M. Salgo, H.B. Tanowitz and M. Wittner, 1991. Sensitive and specific detection of *Toxoplasma* DNA in an experimental murine model: Use of *Toxoplasma gondii* specific cDNA and the polymerase chain reaction. *J. Infect. Dis.*, 163: 180-186.
- Weiss, J.B., 1995. DNA probes and PCR for diagnosis of parasitic infections. *Clin. Microbiol. Rev.*, 8: 113-130.