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Original Article

Rapid Detection of *Toxoplasma gondii* Antigen in Experimentally Infected Mice by Dot- ELISA

S Jafar pour Azami, H Keshavarz, M Rezaian, M Mohebali,*S Shojaee

Dept of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Toxoplasmosis is a worldwide endemic disease. In congenitally infected infants and AIDS patients, toxoplasmosis causes high rates of morbidity and mortality. In these cases antibody detection is difficult; so detection of parasite or its components could be useful tool for early detection and following treatment of the infection.
Methods: Sixty-three BALB/c mice were injected intra-peritoneal with 5×10^3 tachyzoites of *Toxoplasma gondii* RH strain, nine mice were sacrificed daily for 7 days. Fourteen mice were injected with phosphate buffer saline as control group. Dot–ELISA was performed for detection of *T.gondii* antigen in mice sera and capture – ELISA was done as golden standard assay too.
Results : *Toxoplasma gondii* antigen was detected from day 2 in mice sera ; 22% of mice sera on day 2, 33% on day 3,77% on day 4 and 100% on day 5 till their death on day 7 had shown antigenemia by dot – ELISA, no positive result was detected in control mice by dot- ELISA.
Conclusion: Dot-ELISA is a sensitive method for diagnosis of *T. gondii* infection in the animal model; also, this technique is more rapid and easy to perform method in comparison with capture-ELISA.

Keywords: Toxoplasma gondii*, dot-ELISA, capture-ELISA, antigenemia*

***Corresponding author**: **Email**: s_shojaee@tums.ac.ir

Introduction

Toxoplasmosis is an endemic worldwide disease. It is caused by the parasitic protozoan *Toxoplasma gondii* (1). In healthy individuals the course of toxoplasmosis is harmless and frequently without symptoms.
However in some individuals, such as congenitally infected infants (2, 3) and immunocompromised patients (AIDS patients) (4, 5) and transplant recipients (6-8), toxoplasmosis can be life – threatening. The diagnosis is routinely based on serological tests with detection of specific antibodies. "In this category of patients, however, serology is inadequate because antibody production either fails or is significantly delayed" (4, 7, 9, 10). Moreover, the demonstration of antibodies in neonates is hampered by the presence of maternal immunoglobulin (IgG). "The extent of damage can be reduced by early treatment, for which a rapid diagnosis is mandatory" (10). "Therefore, detection of parasite or its component could improve the diagnosis of acute toxoplasmosis" (11).

Among the diagnosis methods, dot – ELISA is a sensitive and easy to perform technique. In this point of view, the present study was performed to establish dot – ELISA for detection of *T. gondii* antigens in sera of experimentally infected mice.

Material and Methods

Antigen

Toxoplasma gondii antigen was prepared from peritoneal exudates of BALB/c mice infected 3 days earlier with tachyzoites of *T. gondii* RH strain (12).
The peritoneal exudates of mice were centrifuged at 2000 g for 20 min, washed 3 times with phosphate buffer saline (PBS), sonicated for twelve 5-10 periods, centrifuged at 12000g for 1 hour and the supernatant collected as soluble antigen. Protein content was determined by Bradford method and the soluble antigen stored at -20˚ C until use (12, 13).

Antibody

Antibodies to *T. gondii* were obtained by immunization of white rabbits. Initial immunization was performed with antigen and complete Freund´s adjuvant. Second and third immunizations were done with antigen and incomplete Freund´s adjuvant. Immunized rabbits were bled and sera separated by blood centrifugation in 2000 g for 10 min (12).
Polyclonal antibody was isolated from rabbit sera with ammonium sulfate precipitation and Ion- exchange column chromatography. SDS- PAGE and immuonoblotting were done to confirm purification and specification of isolated polyclonal antibody.
For capture- ELISA assay, half of the isolated polyclonal antibody were conjugated with horse radish peroxidase enzyme (HRP) (Sigma, USA) by means of periodate method in a three day procedure according to the Kawaoi and Nakane(14).

Infection of mice

Sixty three male BALB/c mice weighting 20-25 gr were injected intra- peritoneal with 5×10^3 tachyzoites of *T. gondii* RH strain, nine mice were anesthetized by ether and then sacrificed daily for 7 days, control group including fourteen mice were injected with PBS and two of them were sacrificed daily (12). Whole blood was removed from each animal by cardiac puncture. Sera were separated and kept at- 20˚C until use.

Dot-ELISA technique

Nitrocellulose paper 0.45µm (MN, Germany) was cut in to strips and put in the glass Petri dish. Serum samples (5µl) were

placed on the nitrocellulose paper and allowed to air dry for 1 h at room temperature. Each time, *T. gondii* antigen was used as positive and normal mice sera as negative controls. The paper was then blocked with 2.5% skimmed milk in PBS and incubated for 1 h at room temperature.

Solution of rabbit anti – *T. gondii* antibody in 2.5 % skimmed milk was added in Petri dish and incubated at 37˚C for 1 h, then washed three times for 10 min with PBST (PBS, Tween 20). The anti – rabbit antibody conjugated with horseradish peroxidase (HRP) (Dako, Denmark) was added and incubated for 1h in 37˚C. Petri dish was washed with PBST as described above, then diamino benzidine substrate (DAB) (Sigma. USA) was added and incubated for 10 min, rinsed with distilled water, and blotted dry (12).

Capture- ELISA

Capture- ELISA was done as golden standard. Multilevel plates (Nunc) were absorbed with polyclonal rabbit antiserum to *T. gondii* concentration of 30 µgr/ ml in coating buffer (PBS, pH 7.2). After over night incubation at 4˚ C and washing, the samples (sera) were added to each well and held for 1 hour at 37˚ C. The plates were washed three times with PBST.

Rabbit anti *T. gondii* IgG conjugated with HRP were diluted 1:10, in PBST, added to each well, and held for 1h at 37˚ C. After washing, the chromogenic substrate orthophenylen-diamidine (Sigma –Aldrich, USA) was added to each well. After incubating for 20 min the enzymatic activity was revealed. The reaction was stopped by addition of sulfuric acid 20 %. Absorbance was recorded at 492 nm as detected with an automated ELISA reader (13).

Results

Results were compared visually with the positive and negative control dots (Fig.1: a,b). Antigenemia was detected in mice sera experimentally infected with *T. gondii* RH strain by dot – ELISA from day 2 of infection ; 22% (two out of nine) of mice sera had detectable antigen by day 2 of infection, 33% (three out of nine) by day 3 and 77% (seven out of nine) by day 4. Antigenemia was confirmed in 100% of mice sera from day 5 up to their death on day 7.

Antigenemia was detected from day 3 in infected mice sera by capture- ELISA; 40% of mice sera had detectable antigen by day 3 and 100% by day 4 up to day 7. The results for normal sera of control group were negative by these two methods (Table 1).

Table 1: Results of dot- ELISA and capture – ELISA for *T. gondii* antigen detection in experimentally infected mice

Day after Infection	Dot-ELISA		Capture- ELISA		Sensitivity (%)		Specificity (%)		Concordance (%)
	No of Positive sera	No of Negative sera	No of Positive sera	No of Negative sera	Dot-ELISA	Capture-ELISA	Dot-ELISA	Capture-ELISA	
1	-	-	-	-	-	-	-	-	-
2	2	7	0	9	22	0	100	100	77
3	3	6	4	5	33	44	100	100	88
4	7	2	9	0	77	100	100	100	77
5	9	0	9	0	100	100	100	100	100
6	9	0	9	0	100	100	100	100	100
7	9	0	9	0	100	100	100	100	100

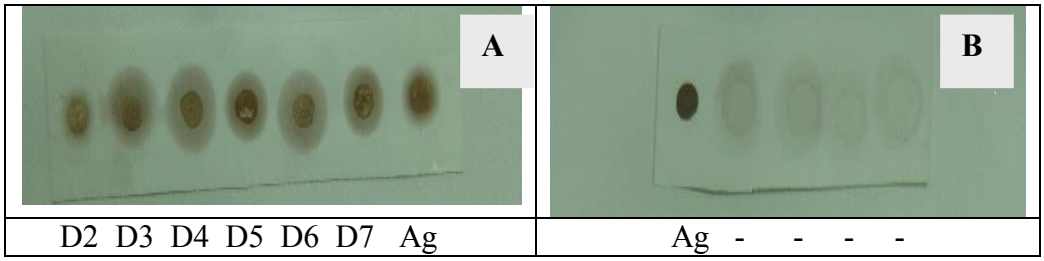

Fig.1: Detection of *T. gondii* antigen in mice sera by dot- ELISA
(a) : case group
D2, D3, D4, D5, D6, D7: days post infection
Ag: *T. gondii* antigen
(b): Control group
Ag: *T. gondii* antigen

Discussion

The diagnosis of toxoplasmosis is most commonly made by serological means with detection of specific antibodies. Rapid diagnosis of *T. gondii* infection cannot be made by measuring antibody levels alone. In infants congenitally infected with *T. gondii* IgM antibodies cannot be detectable, also in AIDS patients IgM antibody may be absent (15, 16). Thus, it would be helpful to use method that could detect whole parasite or its antigen in body fluids. Raizman and Neva were the first who used counter – current electrophoresis and agar gel diffusion to show presence of circulating antigen in mice sera on day 2-4 of infection (17). Shojaee et al. detected antigenemia from day 4 after infection in mice sera by immunoblotting (18). In this study, antigenemia was detected from day 2 in mice sera.

Foroghiparvar et al. used capture- ELISA for detection of *T. gondii* in mice sera from day 3 (13). In our study, antigenemia was detected from day 3 by capture – ELISA. Hafid et al. compared PCR, capture – ELISA and immunoblotting for detection of *T. gondii* in infected mice and showed PCR as the most sensitive assay among them (11). Bitkowska et al. detected *T. gondii* antigens in the sera of mice and rabbits by dot – ELISA and mentioned that this method was sensitive, reproducible, and easy to perform (19).

The present study confirms the sensitivity of dot – ELISA for detection of *T. gondii* antigen in experimentally model; also, the assay is comparable with the sensitive method, capture – ELISA. Robert et al. detected antigenemia by dot-immunoblotting technique on day 2 of infection (12).

In this study, the mice were injected with 5 $\times 10^{3}$ tachyzoites, and antigenemia was detected in their sera from day 2: as though 22% of infected mice had shown antigenemia on day 2, 33% on day 3, 77% on day 4 and 100% on days 5, 6 and 7 by dot – ELISA. Furthermore, some researchers had used dot –ELISA to detect IgM, IgG and IgA in men and animal sera (20, 21).

Regarding to the results obtained here, dot-ELISA is a fast, sensitive, and easy to perform method for detection of acute toxoplas-

mosis in animal model. Studies for detection of human acute toxoplasmosis by this method are suggested.

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References

1. Fuentes I, Rodriguez M, Domingo CJ, Castillo F, Juncosa T, Alvar J, Urine sample used for congenital toxopl-asmosis diagnosis by PCR. J Clin Microbiol. 1996;34 (10): 2368-2371.
2. Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of 378 pregnancies. N Engl J Med. 1974; 290:1110-1116.
3. van de Ven E , Melchers W, Galama J, Camps W, Meuwissen J. Identification of *Toxoplasma gondii* infection by Bl gene amplification. J Clin Microbiol . 1991; 29(10):2120-2124.
4. Luft BJ, Hafner R. Toxoplasmic encephalitis. AIDS. 1990;4:593-595.
5. Luft BJ, Remington JS. Toxoplasmic encephalitis. AIDS Commentary. J Infect Dis. 1988;157:1-6.
6. Derouin F, Gluckman E, Beauvasis B, Devergie A, Melo R, Monny M, Lariviere M. *Toxoplasma* infection after human allogeneic bone marrow transplantation :Clinical and serological study of 80 patients. Bone Marrow Transplant. 1986;1:67-73.
7. Heurkens AHM, Koelma IA, Planque MM; Polderman AM, Vander Meer JWM. Failure to diagnose Fatal disseminated toxoplasmosis in a bone marrow transplant recipient. J Infect. 1980;18:283-288.
8. Pendry K, Tait RC, Mclay A, Hoyen D, Baird D, Burnett AK. Toxoplasmosis after BMT for CML. Bone Marrow Transplant. 1990;5:65-67.
9. Desmonts G, Forestier F, Thulliez P, Daffos F, Capella Pavlovsky M, Chartier M. Prenatal diagnosis of congenital toxoplasmosis. Lancet. 1985;1:500-504.
10. Holliman RE. The diagnosis of toxoplasmosis. Serodiagn Immunother Infect Dis. 1990;4:83-93.
11. Hafid J, Raberin H, Tran Manh Sung R. Comprison of capture – ELISA and immunoblotting for detection of *Toxoplasma gondii* in infected mice. J Med Microbiol. 2001;50:1100-1104.
12. Brooks RG, Sharma SD, Remington JS. Detection of *Toxoplasma gondii* antigen by a dot-immunobiding technique. J Clin Microbiol.1985; 21(1):113-116.
13. Foroghiparvar F, Keshavarz H, Shojaee S. Detection of *Toxoplasma gondii* antigens in sera and urine of experimentally infected mice by capture – ELISA. Iranian J Parasitol. 2008;3(1):1-5.
14. Nakan PK, Kawaoi A. Peroxidase – labeled antibody: a new method of conjugation. The J of Histo & Cytochemistry. 1974;22(12):1084-1091.
15. Naot Y, Desmonts G, Remington JS. IgM enzyme – linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. J Pediatr. 1981; 93:32-36.
16. Wong B, Gold JWM, Brown AE, Lange M, Fried R, Grieco M, Mildvand D, Giron J, Tapper ML, Lerner CW, Armstrong D. Central – nervous system toxoplasmosis in homosexual men and parenteral drug abusers. Ann Intern Med. 1984;100:36-42.
17. Raizman RE, Neva FA. Detection of circulating antigen in acute experimental infection with *T. gondii*. J Infect Dis. 1975;132(1):44-48.
18. Shojaee S, Keshvarz H, Mohebali M. Detection of *Toxoplasma gondii* antigens in sera from experimentally infected mice. Pak J Med Sci. 2007;23(1):100-102.

19. Bitkowska E, Dzbenski TH. Detection of circulating *Toxoplasma gondii* antigens by the dot – ELISA method. Wiad Parasitol.1989;35(4)283-288.
20. Youssef MM, el- Ganayni GA, el-Shazly AM. The efficacy of IHAT, IFAT and dot ELISA in sero–diagnosis of toxoplasmosis in complicated pregnancies. J Egypt Soc Parasitol. 1992;22(2):343-347.
21. Pappas MG, Lunde MN, Hajkowski R, Mc Mahon J. Detection of IgM and IgG anti- bodies to *Toxoplasma* using the IFA test, ELISA, and dot – ELISA procedures. Vet Parasitol. 1986;20(1-3):31-42.