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Detection of *Toxoplasma gondii* Infection in Butchers and Buffaloes by Polymerase Chain Reaction and Latex Agglutination Test

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Abstract. For detection of toxoplasmosis, latex agglutination test (LAT) and polymerase chain reaction (PCR) based on the amplification of repetitive B 1 gene of *Toxoplasma gondii* was used in this study. The study was based on total of 100 samples from 50 butchers and 50 buffaloes. Through PCR the incidence of toxoplasma infection was found 20% in butchers and 22% in buffaloes. All the samples which were positive to PCR for toxoplasmosis were seropositive at 1:64 dilution, both in butchers and buffaloes, indicating good correlation amongst the two tests.

Key words: Toxoplasmosis, zoonosis.

INTRODUCTION

Toxoplasma gondii is an important intracellular protozoan (Smith, 1995), and is the causative agent of toxoplasmosis in humans and animals. It is a zoonotic, endemic worldwide and, depending on the geographic location, 15 to 85% of the human population is asymptomatically infected (Reisch *et al*., 2003). It is normally caused by eating undercooked infected meat or by ingestion of oocysts excreted by its definitive host, the cat (Dubey, 1998). The parasite has got affinity for epithelial, reticulo-endothelial and blood cells (Sadun, 1974).

T. gondii causes abortion and congenital defects in humans and is an important cause of abortion in domestic livestock. Infection in farm animals poses a risk to public health, as well as economic losses to the farming industry. *T. gondii* infection is established by rapid multiplication of the tachyzoite stage of the parasite. Although generally effective, the immune response does not completely eliminate the parasite; instead the tachyzoites differentiate into bradyzoites that form quiescent cysts in the brain and other tissues causing chronic infection that persists for the lifetime of the host (Sibley *et al.,* 1999). Infection with *T. gondii* is extremely common, 20.80% prevalence in Europe and North America, but the majority of people show no overt clinical symptoms. Immunosuppression, however, rapidly leads to the breakdown of the tissue cysts, recrudescence of infection, and development of toxoplasmosis.

Rapid and accurate diagnosis of toxoplasmosis is essential for the management of the human infections and is also an important research tool for understanding the role of animal reservoirs in the spread of infection. Indirect diagnosis by detection of antibodies to *T. gondii* is feasible, but is less useful in determining the timing of infection. Various serological tests used are indirect haemagglutination (Nieto and Melendez, 1998); indirect immunofluorescence (Van der Puije *et al*., 2000), enzyme linked immunosorbent assay (Hashemi-Fesharki, 1996), and Latex Agglutination Test (Ahmad, 1999).

The ability to detect low numbers of parasites rapidly, in a range of tissues, is an important consideration for the management of congenital and potentially acquired toxoplasmosis. Direct detection of *T. gondii* parasites by amplification of DNA using polymerase chain reaction (PCR) has, therefore, become a valuable asset (Ellis, 1998). The most widely used gene target consists of the 35-fold repetitive B1 locus (Burg *et al.*, 1989). It is an important research tool for the study of the parasite in humans and animals. Independent studies have shown that the B 1 locus is the best target identified so far for the routine and sensitive detection of *T. gondii* in human and animal tissues (Jones *et al.*,

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2000; Pelloux *et al.*, 1998).

Considering the close interaction between livestock and human being in Pakistani society, the issue of toxoplasmosis is of special significance and hence warrants a thorough investigation.

MATERIALS AND METHODS

A total of 100 blood samples (butchers n = 50 and buffaloes n = 50) were collected at random from Lahore abattoir. The serum samples were analyzed for toxoplasma antibodies using Latex Agglutination Test LAT. For this purpose, a commercial LAT kit (LXTX 0100 manufactured by Fortress Diagnostics Limited, BT 161 QT, United Kingdom) was used. PCR was performed to amplify DNA by using: PI (5 -GGA ACT GCA TCC GTT CAT GAG- 3), and P4 (5 TCT TTA AAG CGT TCG TGG TC-3) as primers (E-oligos, NY).

The results for LAT were interpreted as: agglutination at <1:16 dilution was considered indicative of no immunity or toxoplasmosis. A titer of 1:16 was considered indicative of residual immunity or non-specific immunity. Titers = or > 1:164 was considered due to acquired toxoplasmosis or evolving immunity (Zaki, 1995).

PCR protocol was followed as described by Jonathan and Mattson (2003) with some modifications. Detection of the *T. gondii* was carried out by amplification of repetitive-B1 locus with specific primers mentioned above.

RESULTS AND DISCUSSION

Fifty serum samples from butchers were analyzed for *T. gondii* antibodies by latex agglutination test. Out of 50 samples, 14 samples showed agglutination at 1:16 dilution. Out of them 10 were seropositive on 1:64 dilutions. It shows 20% prevalence of the toxoplasmosis in butchers, while 40 samples (80%) were negative in this test (Table I). Out of 50 blood samples from buffaloes, 16 samples showed agglutination at 1:16 dilution, out of them 11 samples also showed agglutination at 1:64 dilutions, which was the indication of acquired toxoplasmosis or evolving immunity against *T. gondii.* Five samples showed agglutination at 1:16 and indicated residual or non-specific immunity, while 39 sera samples (78%) showed no agglutination and were considered as negative (Table I). These findings are in agreement with Mazumder *et al*. (1988) who used LAT test for the detection of antibodies against *T. gondii* in butchers and found that 24% suspected cases were positive for *T. gondii* infection. Similar results were reported by Zaki (1995) who studied the sero-prevalence of *T. gondii* in domestic animals in Pakistan using commercial LAT Kit.

Samad *et al*. (1997) randomly selected 49 professional blood donors, 617 pregnant women, 14 butchers, 528 slaughtered goats and 24 buffaloes and found the presence of *T. gondii* antibodies using LAT. Overall 12.4% blood donors, 11.18% pregnant women, 50% butchers, 12.88% slaughtered goats and 33.33% buffaloes had diagnostically significant antibody titers (> or = 1:64) to *T. gondii.*

PCR was used as a confirmatory test for the detection of *T. gondii* genomic DNA from blood of butchers and buffaloes. For PCR same specimens of blood were used, as used for LAT. Ten samples of butchers were found positive by PCR, which were positive at 1:64 dilution in LAT. Amplified DNA was detected by running PCR product on 2.5% Agarose gel that produced 193 bp bands (Fig. 1). Out of remaining 40 samples, 4 positive and 36 negative at 1:16 dilution in LAT, were also negative by PCR. It represents 20% prevalence of toxoplasmosis in butchers.

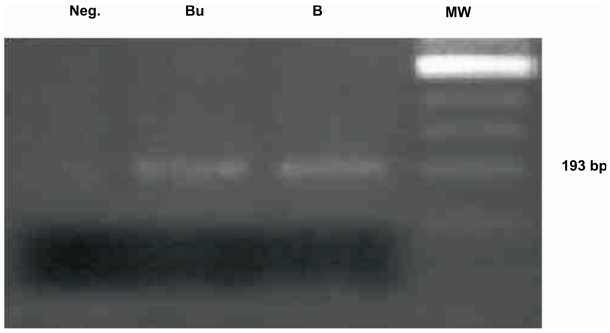

Fig. 1. Amplified DNA by PCR in butcher and buffalo. B, buffaloes; Bu, butcher; MW, molecular weight marker; Neg, negative.

Eleven blood samples which were seropositive at 1:64 dilutions in LAT were found to be positive by PCR which indicated 22% prevalence

Table I.- Latex agglutination test and polymerase chain reaction for detection of *Toxoplama gondii* infection in butchers and buffaloes.

Total No. of Samples	Latex agglutination test (LAT)				Polymerase chain reaction (PCR)				
	Sero-positivity on 1:16 dilution	Sero-positivity on 1:64 dilution	Sero-negative on 1:16 dilution	Pre-valence (%)	Total No. of samples		Positive (+)	Negative (–)	Pre-valence (%)
1. Butchers									
50	14	10	36	20	50	A	10	Nil	20
						B	Nil	4	Nil
						C	Nil	36	Nil
2. Buffaloes									
50	16	11	34	22	50	A	11	Nil	22
						B	Nil	5	Nil
						C	Nil	34	Nil

of the disease. Out of the remaining 39 samples (78%), 5 samples showing agglutination at 1:16 and 34 seronegative were also negative to PCR (Table I, Fig. 1).

Zhang and Wei (2000) evaluated the diagnostic value of LAT which was used for the detection of antibodies to *T. gondii.* A total of 288 human serum samples were assayed using LAT. The results showed 8.9% seropositivity with positive titers (> or = 1:32). These findings were in partial support with the results of this report.

In the present study, 35-fold-repetitive Bl gene was used as a target for amplification *T. gondii* DNA in blood. Burg *et al.* (1989) showed that a single *T. gondii* parasite could be directly detected from cell-lysate material using the 35-fold-repetitive B1 gene as a target from amplification. The same target was used successfully with diagnostic purpose by Bretagne *et al.* (1995), Dupon *et al.* (1995), and Johnson *et al.* (1993).

Guy and Johnson (1995) tested blood samples from 54 patients suffering from acute lymphadenopathy for the presence of *T. gondii* using PCR. Test results were positive in 19 (35%) of the 54 patients. Findings indicated that PCR was helpful indicator for the detection of toxoplasmosis. Figueroa *et al.* (2000) found that in reactivated ocular toxoplasmosis blood samples from 3 patients were positive for *T. gondii* DNA by out of 7 patients. The studies regarding the use of PCR in detection of toxoplasma infection in animals are limited, hence data for comparison are not available.

It is concluded for this study that there is a good correlation between detection efficiency of the LAT and PCR, which could be used successfully for diagnosis of toxoplasmosis. The study has confirmed the zoonotic importance of the disease because the incidence was 20% in the butchers, who are in contact with the animals. Further studies are recommended to include the persons in the study who are not in contact with animals and the ones who are in contact with the other domestic and pet animals.

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