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Toxoplasmosis; seroprevalence, comparative analysis of diagnostic techniques and identification of risk factors in humans in Malakand Agency, Khyber Pakhtunkhwa, Pakistan

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Abstract

Toxoplasmosis is caused by *Toxoplasma gondii* (*T. gondii*) and is a wide spread parasitic zoonoses affecting a broad range of mammals and birds. Members of family Felidae are specific hosts for *T. gondii*. Specific IgA, IgM, and then IgG antibodies appearance usually indicate the onset of an acute *T. gondii* infection. The aim of the study was to check the seroprevalence of *T. gondii* and related risk factors in Tehsil Batkhela, Malakand Agency, Khyber Pakhtunkhwa, Pakistan. A total of 420 females were screened during four months study. Sera were collected and examined by Immune Chromatographic Technique (ICT), Enzyme Linked Immunosorbent Assay (ELISA) and Latex Agglutination Test (LAT) for detection of antibodies against *T. gondii*. The results were evaluated for occurrence rate; age wise occurrence, comparison of occurrence of *T. gondii* in pregnant and non-pregnant females, relation between animal contact and toxoplasmosis, *T. gondii* and abortion, comparison of ICT, ELISA and LAT. The overall occurrence rate was 65.71%, which was quite alarming. Middle age group (21-30 years) females were highly affected (41.31%). Pregnant women had high infection level than non-pregnant. No any positive relation was found between the occurrence of toxoplasmosis and cats contact. Higher occurrence rate was found in aborted women. ELISA IgM method was found to be the most sensitive, reliable and accurate for the detection of *T. gondii* infection as compared to other methods. It may be recommended to study the same work on large population to identify further risk factors and to develop a molecular diagnostic assay for the accurate diagnosis of the infection.

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Introduction

Toxoplasmosis is one of the most widespread parasitic zoonoses affecting a wide range of mammals and birds (Parmeswaran, 2008). About 30% of human population is infected worldwide with *T. gondii* (Jittapalapong *et al.,* 2010). The causative agent is *Toxoplasma gondii* (Parmeswaran, 2008), which is an obligate, intracellular, parasitic protozoan (Sukthana *et al.,* 2003). The members of family Felidae are specific hosts for *T. gondii* that are infected by oocysts from the environment or by tachyzoites and bradyzoites from intermediary hosts, such as all kinds of vertebrates including human. In the life cycle of *T. gondii*, there are three stages; oocysts, tachyzoites and bradyzoites. The oocysts are found in the environment while the tachyzoites and bradyzoites are found in host tissue (Parmeswaran, 2008). The infection usually does not cause a considerable problem for immunocompetant people, but can be life threatening for congenitally infected and immunosuppressed individuals (Jittapalapong *et al.,* 2010). Specific IgA, IgM, and then IgG antibodies appearance usually indicate the onset of an acute *T. gondii* infection. After few months, anti-toxoplasma specific IgA and IgM disappear, and specific IgG remains detectable throughout life and indicate chronic stage of infection. After acute infection, in some patients, IgA and/or IgM anti- toxoplasma antibodies may persist not just for some months but even years (Horvath *et al.,* 2005). Pregnant women and immune compromised peoples have to adopt hygienic principles not only after contact with soil, cats, before eating, but also after contact with dogs (Luptakova *et al.,* 2009). The present study was aimed to study *T. gondii* in human, identification of major risk factors and to find out an accurate and cost effective diagnostic technique for the screening of *T. gondii* in Tehsil Batkhela, Malakand agency. Objectives were to identify risk factors for *T. gondii*, its prevalence with age, relation with pregnancy, abortion, its occurrence, transmission and diagnosis. Also information about the most accurate diagnostic test ought to be given to clinical laboratories.

Materials and methods

A total of 420 serum samples were collected from human (females) at Bahadur Khan, Rozi Khan Memorial Hospital, Batkhela, Malakand Agency, Khyber Pakhtunkhwa, Pakistan. Prior permission was taken from the hospital administration and also a written consent was signed from all the patients. History was recorded on printed questioneers. All serum samples were analyzed for presence of IgG and IgM antibodies against *T. gondii* using Immuno-chromatographic technique (ICT), Latex Agglutination Test (LAT) and Enzyme Linked Immuno-sorbant Asssay (ELISA).

Immuno-chromatographic tests (ICT)

Sera were first screened for antibodies against *T. gondii* with the help of immuno-chromatographic technique using strips (CTK, USA). Positive and negative samples were further subjected to ELISA.

Latex agglutination test

All samples were screened for antibodies against *T. gondii* using Latex Agglutination Technique (LAT, UK). Both qualitative and quantitative analysis was done. Positive and negative samples were subjected to ELISA.

Enzyme linked immune-sorbent assay

Screening of positive samples was done with the help of ELISA technique for both IgM and IgG antibodies by using EIA Test Kit (BioCheck, Inc, USA). Test kit was used according to the manufacturer's instructions. After incubation of antigen-coated microplates with test sera diluted upto 1:20, *T. gondii*-specific antibodies were detected by binding the antigen/antibody complex with a peroxidase labeled anti- human IgM or IgG monoclonal antibody conjugate for 90 minutes. The optical density (OD) of the reaction was read on a strip reader (DOS Instruments Inc, China) at a wavelength of 450 nm. The results were calculated according to the control serum readings; i.e. the percent ratio between the ODs for the sample and the positive control corrected for the OD of the negative control, and interpreted as recommended by the manufacturer, as follows: <0.90% negative, 0.91-0.99% ambiguous, 1.00 and

>1.00% positive (Lashari and Tasawar, 2010).

Results

A total of 420 patients having different ages, referred by different Gynecologists were diagnosed for their pregnancy and for *T. gondii* infection through Immuno Chromatographic Technique, Latex Agglutination Test and ELISA for anti-*toxoplasma* antibodies. On the prescribed form, each patient history was recorded. Five age groups of the patients were made. Two groups were made on the basis of pregnancy. The patients were also divided into two groups on the basis of their contact with animals. Sera were collected from these patients and screened for anti-*Toxoplasma* antibodies.

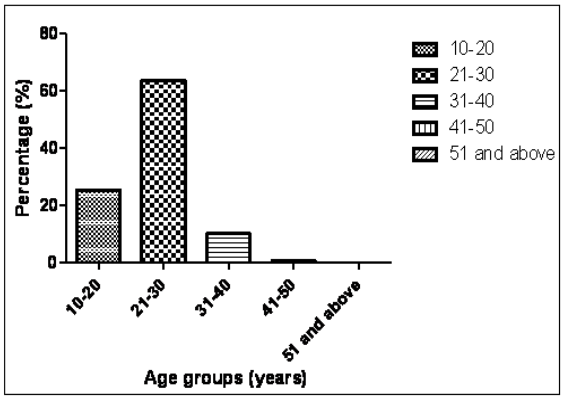

Fig. 1. Age wise distribution of Toxoplasmosis in positive female patients Cats contact and Toxoplasmosis.

Age wise distribution of Toxoplasmosis

In total 276 infected female patients, 70 (25.4%) females were in the age group of 10-20 years, 176 (63.77%) were in age group of 21-30 years, 28 (10.14%) were in age group of 31-40 years, and 02 (0.72%) females were positive in age group of 41-50 years and no patients were above 50 years (Figure. 1). Out of 276 positive females, 142 (51.44%) had contact with cats and 134 (48.55%) females had no any contact. The results showed that there was no significant difference between the females that had contact with cats and those who had no contact. It has been suggested that contacts with cats may have no any importance in transmission of toxoplasmosis.

Toxoplasmosis in pregnant and non-pregnant females

Among 276 positive females, 164 (59.42%) were pregnant, while 112 (40.58%) were non-pregnant (Figure 2).

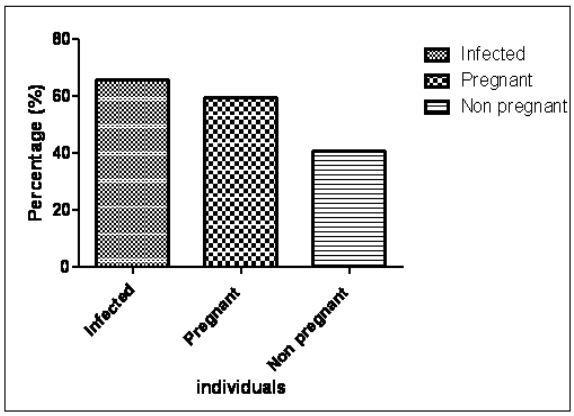

Fig. 2. Toxoplasmosis in pregnant and non-pregnant females.

Toxoplasmosis and abortion

There were 420 female samples that were tested for toxoplasmosis. Out of which 276 were positive for toxoplasmosis. Among 276 positive patients, 218 (78.98%) aborted the fetuses, 52 (18.84%) had no abortion. 144 females had no Toxoplasmosis; but 54 (37.5%) aborted the fetuses spontaneously and 90 (62.5%) had no any abortion history (Figure 3).

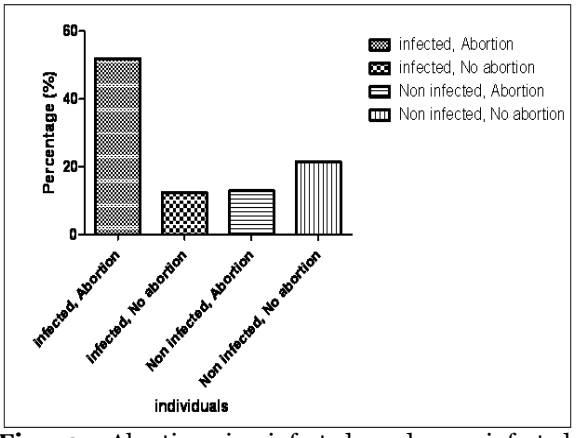

Fig. 3. Abortion in infected and non-infected females.

Comparison of different diagnostic tests

Different diagnostic tests i.e. ICT, LAT and ELISA are available for detection of Toxoplasmosis. These tests were considered in the present study as these are normally used in local laboratories of the region. Through ICT IgG, 13.15% female were positive for Toxoplasmosis, while 14.1% females were positive

through LAT. Among the tests, ELISA (IgM) was found the most sensitive and all the tests were positive. Total samples were 420; among which, 276 samples were positive through ELISA (IgM). Only 6.1% female were positive though ELISA (IgG) (Figure 4).

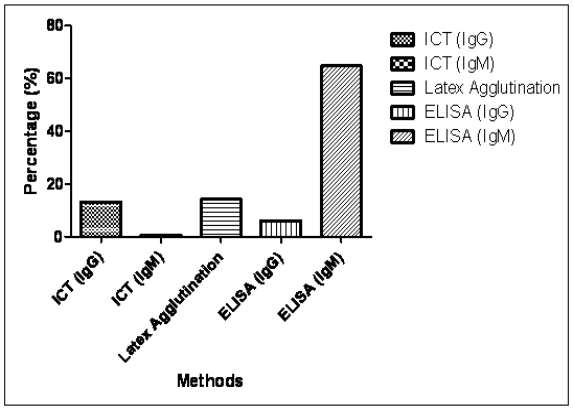

Fig. 4. Comparison of different diagnostic tests for detection of *Toxoplasma gondii*. Among the tests, ELISA (IgM) was the most sensitive and all tests were positive. Total samples were 420; among which, 276 (65.7%) samples were positive through ELISA (IgM). Latex Agglutination was at second number; 14.3% samples were positive. Through ICT, 13.3% samples were positive. Through ELISA (IgG), 6.2% samples were positive.

Discussion and conclusion

Toxoplasmosis is a parasitic zoonosis affecting a broad range of mammals and birds. Specific IgA, IgM and IgG antibodies usually indicate the onset of *T. gondii* infection (Horvath *et al.,* 2005). It is estimated that about 30% of human population is infected worldwide with *T. gondii* (Jittapalapong *et al.,* 2010). A total of 420 blood samples were collected from females that were referred by Gynecologist for the diagnosis of pregnancy and *T. gondii* infection in Bahadur Khan, Rozi Khan Memorial Hospital Batkhela, Malakand Agency, Khyber Pakhtunkhwa, Pakistan. Serum was isolated and subjected to ICT, LAT and ELISA.

The study revealed that middle age group; 21-30 years was highly affected (41.31%) with *T. gondii*. The presence of infection in this age group may be due to pregnancy, because *T. gondii* is active during pregnancy and remain dormant in other life stages. The results are in agreement with studies of Jittapalapong *et al*., (2010**)**, Kun Wu *et al*., (Wu *et al.,* 2009) and Ramzan *et al*., (2009). Lashari *et al*., (2010) observed high prevalence of Toxoplasmosis at age of 16-28 months sheep that are not correlated with the present study. It may be due to the difference between the experimental subjects. In the present study, both types of patients were present; those who had contact with cats and those having no contact. There was no significant difference for this parameter. Same results have also been found by Ayi *et al*., (2009). Toxoplasmosis may be caused due to contact with cat feces, only which may seem the major risk factor. And the locality where the study has been performed is such that no one keeps such pets at their homes. In present study, toxoplasmosis was more in pregnant females as compared to non-pregnant. The same results have also achieved by Ayi *et al.* (2009), Kayman and Tuba (2010). The present results showed that in pregnancy chances of getting infection is high. In present study, the relationship between *T. gondii* infection and abortion was also studied. The results showed that there was high abortion rate due to *T. gondii* in pregnant females, as previous history was taken from them. The results are in correlation with Lashari *et al*., (2010) and Sunsnta *et al*., (2009). The comparison of different methods through which *T. gondii* infection was diagnosed was also made. ELISA for IgM antibodies technique gave the highest sensitivity, followed by Latex Agglutination Test and Immuno Chromatographic Technique for IgG antibodies. The lowest results were through ELISA for IgG antibodies detection. Ahmed *et al*., (1989) used only direct agglutination test for the detection of *T. gondii* and obtained good results. The present results are not in agreement with Bari *et al*., (1990). ELISA IgG method gave the highest sensitivity than ELISA IgM method; ELISA IgG (46%) and ELISA IgM (27.7%). Results of EI-Moghazay *et al*., (2011) are also not in agreement with results of the present study. Modified agglutination test (MAT) gave the highest sensitivity than ELISA method; MAT (56.6%) and ELISA (52.2%). The results by Sunsnta *et al*., (2009) are also not in correlation with the present results.

Latex agglutination test gave the highest sensitivity than ELISA. This may be due to use of different technique used e.g. MAT and also different kits provided by different companies. Jadoon *et al*., (2009) found similar results with the present study. The 46.88% prevalence was observed in dogs with the Latex agglutination test. The results of Kook *et al*., (1999) are also in agreement with the present study. 7.7% children were found positive with Indirect Latex agglutination test. In the present study ELISA IgM was the most sensitive diagnostic test.

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