Seroprevalence and Risk Factors of Toxoplasmosis among Women in District Chitral, Khyber Pakhtunkhwa, Pakistan

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Abstract: Toxoplasmosis; a common zoonosis caused by protozoan parasite Toxoplasma gondii, could be serious medical condition in women by causing abortion and still birth and congenital malformations in infants. The current study is one of the prime investigations carried to evaluate the seroprevalence and possible risk factors of toxoplasmosis among women in Chitral; the northern most District of Pakistan. This study was conducted from July-December 2013. A total of 300 blood samples from women, aged 15-40 years was tested for Toxoplasma IgG/IgM antibodies using lateral flow chromatographic immunoassay test kit®. Chi-square test was employed for statistical evaluation and odd ratios were used to quantify association between toxoplasmosis and risk factors. Antibodies were detected in 74 samples showing 24.7% prevalence. Association of toxoplasmosis was significant (P=0.05) with age, living place and rearing of animals in homes. Infection was higher (28.6%) in women aged 25 years. Similarly, higher infection (33.1%) was detected in women from rural areas and in those who raised cattle, goats or sheep in homes (27.2%). This study confirmed the presence of toxoplasmosis among women in Chitral and showed that women in this area are vulnerable to infection, therefore, public awareness and suitable control measures are needed.

Key words: Chitral - Risk Factors - Seroprevalence - Toxoplasmosis

INTRODUCTION

Toxoplasmosis is a common zoonotic disease caused by Toxoplasma gondii (T. gondii), an obligate Apicomplexan protozoan [1]. Cats serve as definitive hosts for T. gondii while birds and mammals including humans act as intermediate hosts [2]. In humans, toxoplasmosis may be acquired or congenital [3]. Acquired toxoplasmosis occurs by ingesting the resistant stage of the parasite called oocysts in food and water or through the encysted stage of parasite called tissue cyst infecting about one third of human population [7]. In infected meat [4]. Oocysts are excreted in infected cat faeces while tissue cysts are present in muscles and brain of mammals and birds. Acquired toxoplasmosis is usually mild; however, in immunocompromised person can cause lesions especially in brain [2]. Congenital toxoplasmosis results when the parasite is vertically transmitted from mother to fetus through placenta [5]. The classical congenital toxoplasmosis is characterized by chorioretinitis, hydrocephalus or microcephalus, intracranial calcification and convulsions. More than 90% of newborns with congenital infection do not show clinical signs at the time of birth. If not treated promptly, these children are at major risk for developing toxoplasmosis with severe consequences in later life. Up to 85% of the infected children may develop chorioretinal disease. Acute infection of mother during pregnancy is also a major cause of death inside the uterus [6].

Toxoplasma gondii is ubiquitous in distribution infecting about one third of human population [7]. Epidemiological surveys are useful tools to assess the different sources of toxoplasmosis in humans [8]. In Pakistan, some studies about epidemiology of T. gondii have been conducted [9-13] but search of the available literature shows that data regarding prevalence of toxoplasmosis in humans in many parts of Pakistan is still questionable to date. The present study was therefore, planned to find out the seroprevalence and risk factors of
toxoplasmosis among women in district Chitral, Khyber Pakhtunkhwa Pakistan. This study would not only provide fundamental data for prevention and control of this important zoonosis but would also give base-line information on potential risk factors associated with public health.

MATERIALS AND METHODS

Study Area: Chitral is a mountainous area in the extreme north of Pakistan, lying in the Hindukush range at 4,900 feet height from the sea level, between 36.15N° and 72.15E°. Covering an area of 14,850 km², Chitral is the largest district of Khyber Pakhtunkhwa Province in terms of territory. According to the development statistics of Khyber Pakhtunthwa-2014, the estimated current population of the district is 0.475 millions. About 76% of the land is occupied by mountains and glaciers. Climate is dry, Mediterranean. Winter is severe with heavy snow fall and temperature may drop to -10°C at night time. Summer is pleasant and temperature ranges between 25°C- 40°C [14].

Study Population: Blood samples were collected from women who visited Gynecology Department, THQ Hospital Chitral and other private maternity clinics in Chitral Town and Drosh. All the women in study were advised test for toxoplasmosis by the gynecologist. A total of 300 blood samples was collected during July-December 2013. A printed proforma was filled from each woman at the time of sample collection. The proforma included information about living place, age, education level and pregnancy status of participant, presence of animals and cats in homes and date of sample collection. Permission from concerned hospital was obtained before data collection.

Serum Separation: Serum was obtained by centrifuging the blood at 4000 rpm for 10 minutes and was tested immediately for detection of antibodies against *Toxoplasma gondii*.

Serological Test: Serum was tested using commercially available Onsite Toxo IgG/IgM rapid test cassette® (CTK Biotech, Inc, San Diego, USA) which is a lateral flow chromatographic immunoassay used for the simultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma gondii* in human serum. The test was performed according to manufacturer’s instructions.

Statistical Analysis: Data were analyzed through Chi-square test using Statistical Package for Social Sciences version-16 (SPSS Inc., Chicago, IL, USA) software. Odd ratios were used to quantify association between risk factors and toxoplasmosis. *P*-value was considered significant when *p* = 0.05.

RESULTS

A total of 300 women were evaluated during the study period. Most of them were: below 26 years of age (60.7%), from urban areas, i.e. Lower Chitral (58.7%), literate (74.7%) and non-pregnant (52.7%). All of the participants had never heard about toxoplasmosis and were unable to identify any of the associated risk factors. Out of tested samples (n=300), 74 were found infected with *T. gondii* showing an overall 24.7% prevalence (Figure 1).

Analysis of data collected from questionnaire revealed significant association of toxoplasmosis with age, place of living and presence of animals in homes. Age of all studied individuals ranged from 15-40 years.

| Table 1: Association of risk factors with prevalence of toxoplasmosis in women (n=300) in District Chitral, Khyber Pakhtunkhwa Pakistan |
|-----------------|----------------|----------------|-----------------|-----------------|-----------------|
| Factor          | Category       | No. examined | No. positive | Prevalence (%)  | Odds Ratio (95% C.I) | *p*- value |
| Age             | 25 years       | 182          | 52            | 28.6            | 1.74 (0.99-3.06)   | 0.034        |
|                 | 26 years       | 118          | 22            | 18.6            | 1.00 (0.63-1.63)   | 0.998        |
| Area            | Upper Chitral (Rural areas) | 124          | 41            | 33.1            | 2.14 (1.25-3.64)   | 0.004        |
|                 | Lower Chitral (Urban areas) | 176          | 33            | 18.8            | 0.95 (0.56-1.62)   | 0.863        |
| Education       | Illiterate     | 76           | 23            | 30.3            | 1.47 (0.82-2.63)   | 0.125        |
|                 | Literate       | 224          | 51            | 22.8            | 1.00 (0.63-1.63)   | 0.998        |
| Pregnancy       | Non-pregnant   | 158          | 42            | 26.6            | 1.24 (0.73-2.11)   | 0.249        |
|                 | Pregnant       | 142          | 32            | 22.5            | 1.00 (0.63-1.63)   | 0.998        |
| Animals* in homes | Present       | 246          | 67            | 27.2            | 2.51 (1.08-5.83)   | 0.018        |
|                 | Absent         | 54           | 07            | 13.0            | 1.00 (0.63-1.63)   | 0.998        |
| Cats in homes   | Present        | 168          | 45            | 26.8            | 1.29 (0.76-2.21)   | 0.205        |
|                 | Absent         | 132          | 29            | 22.0            | 1.00 (0.63-1.63)   | 0.998        |

*Cattle, Goats or Sheep.*
Based on age, all the participants were divided into two groups; group one comprised of women aged up to 25 years and group two above 25 years. Infection rate was higher in women aged 25 years. Similarly, infection rate was almost double (33.1%) in women from Upper Chitral, i.e. rural areas than from Lower Chitral, i.e. urban areas (18.8%) and in women rearing animals in their homes (27.2%) compared to those having no animals in homes (13.0%). Although infection rate was lower in literate women, statistically, there was no significant association between education level and toxoplasmosis. Similarly, association of toxoplasmosis with pregnancy status of women and presence of cats in homes was also non-significant at $P=0.05$ (Table 1).

**DISCUSSION**

Toxoplasmosis is found throughout the globe and its prevalence differs in different countries and in different areas within a country [15]. Factors which affect *T. gondii* prevalence include; geographic location and age of host [6], difference in weather conditions, life style and customs of people [16], differences in sensitivity of tests used for diagnosis and number of cats in the area [17]. Seroprevalence of *T. gondii* reported in women of child bearing age was 58% in Central Europe, 51-72% in Latin America, 54-77% in West Africa, 4-39% in Southwest Asia [16]. Overall seroprevalence of toxoplasmosis in women recorded in this study was 24.7% which is comparable to results previously reported in Southern Punjab Pakistan 25.9% [11] and in District Swabi Pakistan 19.29% [9]. However, the current prevalence rate is much higher than the 14.4% prevalence reported by Khan *et al.* [10] in pregnant women in Kohat Pakistan using ELISA. This difference may be due to difference in diagnostic techniques used. The current prevalence is lower than the 34 and 62% reported from Southern Punjab and Lahore Pakistan respectively [12, 18]. This may be due to change in climatic conditions in these areas. Climate of Punjab is much warm compared to Chitral which is a hilly area in the
northern parts of Pakistan. Toxoplasmosis is more prevalent in humid warm climates as hotter weather favor sporulation of oocysts [15]. Other authors have also reported lower prevalence of toxoplasmosis in colder areas from USA [19] and Iran [20].

In the present study, younger women were found more infected (28%) than aged (18%) with significant difference between the two groups. Higher infection rate in younger individuals has also been reported by Tasawar et al. [11] in Southern Punjab Pakistan, Sharif et al. [20] in Iran and Studenicova et al. [21] in Slovakia. High prevalence during younger age suggests that exposure to soil, which is common during this age may be the principal mechanism for transmission of infection [3]. Other authors also reported significant association between toxoplasmosis and age of the host [22, 23].

Prevalence of toxoplasmosis varies with locality [19]. Significantly higher prevalence was recorded in Upper Chitral (rural areas) compared to Lower Chitral (urban areas) in the present study. This may be attributed to differences in life styles of people in two parts of the district. Being a rural area, most of the females in Upper Chitral help their men in livelihood by farming and rearing animals in homes. This increased their chances of becoming in contact with oocysts of T. gondii. This hypothesis is further strengthened by the fact that significant higher rate of prevalence in the present study was recorded in women having cattle, goats or sheep in their homes. Almost every village in Chitral has its own pasture and people used to send animals there for grazing. As a result, these animals become more prone to T. gondii oocysts and can then transmit the infection to humans either through direct contact or through consumption of their meat or milk. Live tachyzoites of T. gondii have been detected in milk of sheep, goats and cattle [24] and also isolated from vaginal mucosa, saliva, nasal secretions and urine of experimentally infected goats [7].

Cats are necessary for completion of T. gondii life cycle by acting as definitive hosts [15]. Many reports have associated presence of cats with increased seroprevalence of toxoplasmosis [4, 25-31]. However, presence of cats was not found a significant risk factor for toxoplasmosis in the current study. It can be explained by the fact that stray cats from neighboring areas might have accessed the places where cats were reported absent and thus contaminated the environment. Stray cats are more prone to infection compared to pet cats [32]. Other authors also reported non-significant association between presence of cats and toxoplasmosis [33-39]. Moreover, direct contact with cats is not a main source for spread of toxoplasmosis [40] and oocysts of T. gondii are not found on cat’s fur [41]. Infected cats contaminate the environment with oocysts which are then transmitted to humans and other animals through contaminated food and water [42-44].

CONCLUSION

It was concluded that toxoplasmosis exist among women in Chitral and young females who rear animals in homes in rural areas are particularly prone to infection. Therefore, every woman visiting the antenatal care center should be tested for toxoplasmosis and also educated about the spreading routes and preventive measures of infection. Moreover, domestic animals in the study area need to be screened for T. gondii in order to lower the risk of zoonotic transfer of the infection.

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REFERENCES


