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Research Article

Seroprevalence of Toxoplasmosis in Different Areas of Pakistan

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Abstract

Background: Parasites are organisms that feed and shelter on other species. The majority of individuals carry a variety of parasites, including helminths and protozoans. Toxoplasmosis, caused by *Toxoplasma gondii* is a major protozoan infection in tropical and sub-tropical climates, as well as in Pakistan. This parasite infects humans and most other warm-blooded animals, but the felid family is its main host. It is anticipated to infect one-third of the world's population.

Material and Methodology: Seroprevalence of Toxoplasmosis was investigated in local populations of Lahore, Faisalabad and Mardan, Pakistan. The interviews were used for the questioner survey, while serological investigation was done using ELISA technique. About 360 samples were selected randomly for the detection of *toxoplasma gondii* antibodies.

Results: Immunoglobulin IgG antibodies were used to detect past parasite infection by using a commercial enzyme immunoassay kit (ELISA). Gender, age, socio- demographic and associated factors were all used to characterize these samples. The samples for seroprevalence analysis were chosen randomly. A total of 169 people were found seropositive for this infection having the prevalence of 46.9%. Males were found with a high percentage prevalence of (20%) followed by females (17%) and children (9.7%).

Conclusion: This study has revealed that males were exposed to toxoplasmosis at a higher rate than females and children.

Recommendation: Contact with the soil and dirt should be avoided to reduce the risk of toxoplasmosis from the environment. The report suggests that more research be conducted nationwide utilizing various hematological and biochemical parameters.

Keywords: *Toxoplasma gondii;* ELIZA; Toxoplasmosis

Introduction

Toxoplasmosis is a common and serious disease caused by the apicomplexan parasite *Toxoplasma gondii*. Toxoplasmosis usually causes asymptomatic infection in warm-blooded mammals and birds, but it may cause serious infection in majority of subjects. In livestock, it may be apparent as a congenital disease of newborns. In these animals, it can cause premature birth or death of infant. *Toxoplasma gondii* can cause life-threatening infections in humans [1,3].

The parasite has a Heteroxenous lifecycle and reproduces sexually as well as asexually. The sexual cycle is exclusive to Felidae

members, while mammals and birds complete the asexual cycle. The parasite is transmitted to humans through eating inadequately cooked meat from infected animals or through unintentional consumption of food contaminated with cat faeces. Contaminated water and food is a key source of toxoplasmosis transmission in numerous animal species. Fever, convulsions and ataxia are signs of the condition. The condition can also cause abortions and congenital childbirth abnormalities in newborns [2]. Except Antarctica, *T. gondii has* been recovered from different worldwide locations. Among adults in many countries, Seroprevalence has been reported up to 90%. Primary maternal infection that takes place during pregnancies has been reported from 1 to 310 per 10,000 pregnancies in different regions of the world such as America, Asia, Europe and Australia [14].

In developed countries, the prevalence rate is not the same, such as in France, 88% of the total population is the carrier of the parasite, perhaps due to high usage of lightly cooked and raw meat. The prevalence rate in Brazil, Germany and the Netherlands is 67%, 68% and 80% respectively [15]. Britain has a 22% prevalence rate and South Korea has lower prevalence rate, which is 4% [16]. *T. gondii* causes a serious infection among pregnant females. The possibility for transmission of the parasite from mother to fetus rises if the infections occur in the 2nd or 3rd trimester of pregnancy, but the severity of the disease decrease and causes blindness, mental retardation retinochoroiditis, encephalomyelitis, hydrocephalus and microcephaly in infected babies [20].

The transmission rate of congenital infection and the level of harshness of *Toxoplasma* infection in newborns depend on the stage of gestation period at the time of infection. The transmission risk is lower in the first trimester and higher during the last trimester of pregnancy [21]. The objective of present study was to investigate the prevalence and risk factors related to Toxoplasma infection in individuals from different areas of Pakistan.

Methodology

Sample collection

The present study was conducted to find out the seroprevalence of Toxoplasmosis in local population of Lahore, Faisalabad and Mardan, Pakistan. Blood samples (n=360) were collected from males, females, pregnant females and children in above mentioned areas of Pakistan. For blood sampling from different hospitals permission was taken from hospital's authority. Written consents were also signed by patients. Preliminary information about gender, age, occupation, education level, socioeconomic status, hygienic conditions, contact with cats and other animals, drinking water from different sources, consumption of meat, vegetables and fruits. Blood was centrifuged in clinical laboratories, and then transferred to Research Laboratory of LCWU, Lahore. All serum samples were analyzed for detection of specific anti-*toxoplasma gondii* IgG antibodies by using Enzyme Linked Immuno-Sorbant Assay (ELISA).

Enzyme linked immuno-sorbant assay

All serum samples were screened with the help of the ELISA technique for the detection of IgG antibodies by using an EIA test kit (Bio check, Inc, USA). The test kit was used in accordance with the manufacturer's instructions. Antigen-coated microplates were incubated with test sera dilution 1:40. In each well, 100ul of diluent sera, calibrators, and controls were inserted and dispensed 100ul sample diluents in 1A well location for the reagent bank. It was incubated for 30 minutes at 37°C. The liquid was removed from all wells at the end of the incubation period. The microtiter well was rinsed and flicked 5 times with diluted wash buffer (1x). Each well received 100ul of enzyme conjugate, gently mixed for 10 seconds before being incubated at 37°C. After this, Enzyme conjugate was removed from all wells and rinsed, and the microtiter wells were flicked 5 times with diluted wash buffer (1x). Each well received 100ul of TMB reagent. After gently mixing for 10 seconds, the mixtures were incubated at 37°C for 15 minutes. To stop the reaction, 100ul of stop solution (1N HC1) was added. It was gently blended for 30 seconds. The binding combination of antigen/antibody detects *T. gondii-specific* antibodies. A micro well reader was used to read the O.D at 450 nm in 15 minutes.

Results

A total of 360 samples, including males (n=130), females (n=180) and children (n=50), were collected from Lahore, Faisalabad and Mardan. They were diagnosed for the presence of anti- *Toxoplasma gondii* antibodies by using the ELISA technique. History of each patient, including gender, age, occupation, education level, socioeconomic status, hygienic conditions, contact with cats and other animals, drinking water from different sources, consumption of meat, vegetables and fruits, and medical information

about abortion and pregnancy was identified with the help of specifically designed questionnaire. Patients were divided into four groups according to their age.

Based on contact with cats, they were also divided into two groups. Serum samples were analyzed for IgG antibodies against *Toxoplasma gondii*.

Gender wise omcarison

A total 169 people were found seropositive for this infection having the prevalence of 46.9%. Males were found with a high percentage prevalence of (20%) followed by females (17%) and children (9.7%).

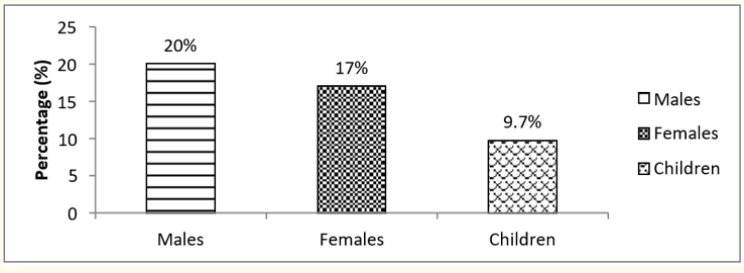

Figure 1: Graph showing percentage (%) of the examined and infected people.

Age wise comparison

A total of 169 people were infected, with 26 (15.3%) being between the ages of 10 and 25, 35 (20.71%) being between the ages of 26 and 40, 47 (27.81%) being between the ages of 41 and 55 and 61 (36.09%) being over 55.

Hygienic conditions

Of 169 infected people, 45 (26.6%) had satisfied hygienic conditions, while 124 (73.3%) lived in unsatisfied sanitary conditions. 150 (88.7%) had contact with animals and more with cats, while 19 (11.2%) had no contact with animals or cats. People consumed water from different sources, such as 113 (66.8%) drinking taps, bores, and hole water. 43 (25.4%) people used boiling water, and 13 (0.07%) used filtered water.

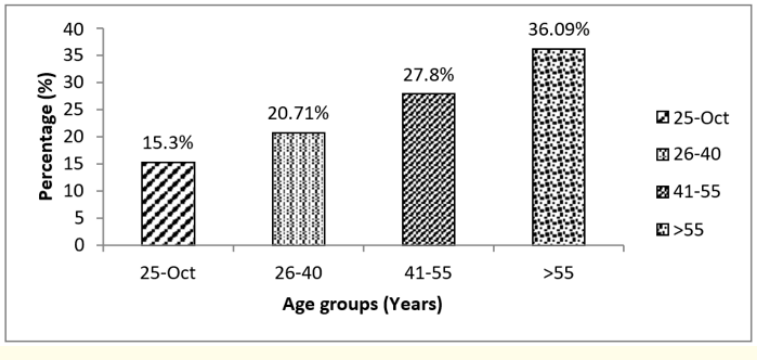

Figure 2: Graph showing percentage (%) of the examined and infected people in relation to age.

Comparison between pregnant and non-pregnant

Of 169 infected people, 62 females were infected, including 35(56.2%) pregnant and 27 (43.4%) non-pregnant. Pregnant females were in different trimesters. 7 were in 1st trimester and 5 were in 2nd trimester while 9 were in 3rd trimester.

Discussion

The present study was conducted to determine the seroprevalence of toxoplasmosis in different areas of Pakistan, such as Lahore, Faisalabad and Mardan. Preliminary information was collected from people. ELISA technique was used to check the seroprevalence of Toxoplasmosis. A total of 169 people were found seropositive for this infection, having a prevalence of 46.9%. Males were found with a high percentage prevalence of (20%) followed by females (17%) and children (9.7%). Another important study was conducted by [10] in Muzaffargarh, and they found a similar result. He also found the highest percentage prevalence of infection in males compared to females. The prevalence of toxoplasmosis was slightly higher in males as compared to females (44% versus 40%) in that study.

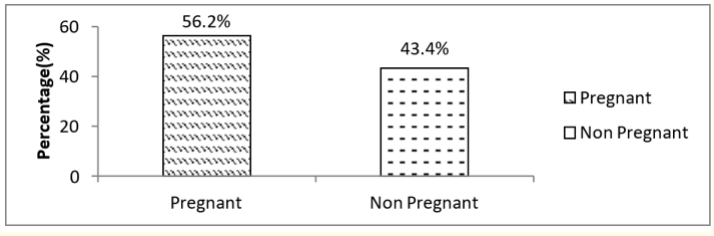

Figure 3: Graph showing percentage (%) of the infected people pregnant and non-pregnant females.

The overall prevalence rate was high in the present study as compared to many other countries. The high prevalence rate in the present study was found due to humidity, which increases the infection rate. The studies conducted in Saudi Arabia show a 30% disease prevalence in the region. Dry and hot weather conditions most probably restrict the transmission of disease. On the other hand, a higher prevalence of disease is found in Ethiopia (90%), Brazil (47%), and Egypt (60%) [5,18,19]. In India, Pakistan and Mexico, a high prevalence was found, while in some regions where hygienic conditions prevail have low percentage,as in Vietnam [6,7].

[17] Researchers conducted a study in India, and they also reported that the infection rate varied from area to area and in the rural populations, the prevalence rate was high as compared to urban. In rural areas, people have to rear farm and domestic animals and get the infection from animals. Of 169 infected people, 62 females were infected, including 35(56.2%) pregnant and 27 (43.4%) non- pregnant. Pregnant females were in different trimesters. 7 were in 1st trimester and 5 were in 2nd trimester while 9 were in 3rd trimester.

The results of present study in non-pregnant women were also in close agreement with the findings of [9] found the same prevalence of toxoplasmosis in pregnant women in Tanzania. According to his study, the prevalence rate of *T. gondii* in pregnant women was 31%. [10] Investigators also worked on toxoplasmosis to check out the seroprevalence of toxoplasmosis in Muzaffargarh Kallarwali village. Who reported a 33% prevalence in non-pregnant females. However, [11] found acute *Toxoplasma gondii* in early pregnancy in Kuwait. He tested pregnant women for IgG toxoplasma antibodies and demonstrated that 53% of women were seropositive for Toxoplasma IgG antibodies.

Similarly, according to other researchers [12], 74% of pregnant women in Brazil were seropositive for *Toxoplasma gondii*. [13] showed that 54% of pregnant women were positive for specific *T. gondii* antibodies. Our findings are also consistent with the results of [4] who systematically reviewed different research articles published from year 2000 to 2020. And found that total 10,924 people were involving in this research out of which 2611 were seropositive. Prevalence rate was higher in those who had direct contact with cats and consumed raw vegetables and under cooked meat, had poor education and lived in rural areas. Above 35y age group prevalence of toxoplasmosis was higher. According to their results infection was more prevalent in Khyber Pakhtunkhwa province than in Punjab.

Conclusion and Recommendations

This study has revealed that males were exposed to toxoplasmosis at a higher rate than females and children. *T. gondii* infection increases in persons as increasing age. Primary care providers should conduct routine serological tests and health education for toxoplasmosis in high risk groups in the endemic zone. Furthermore, health authorities must establish a policy to monitor pregnant women's clinical and laboratory parameters for toxoplasmosis. We cannot afford to lose the struggle against toxoplasmosis. To stop the toxoplasmosis epidemic from spreading too quickly, awareness campaigns about the diseases epidemiology must be launched immediately.

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