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Full Length Article

Comparative Study of *Toxoplasma gondii* Infection in Urban and Rural Areas of District Sheikhupura, Pakistan

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Abstract

Toxoplasmosis is an infectious disease caused by *Toxoplasma gondii* parasite. The infection triggers a large variety of clinical syndromes. Toxoplasmosis results in blindness in children it causes intellectual retardation with congenital infections. The goal of the current investigation is to compare the incidence of *T. gondii* infections in the urban and rural areas of District Sheikhupura, Pakistan. In the course of investigation, blood samples were taken from 300 randomly chosen individuals, including 150 from rural and 150 from urban areas. A commercially available kit, "Latex Agglutination Kit" (Antec Diagnostic Product, UK) was used to analyze the particular immunoglobulin (IgG) antibodies of the parasite. During the analysis it was observed that out of 300 hosts 105 hosts had *T. gondii* infection. Overall *T. gondii* prevalence was 35%. Seroprevalence was higher in males (45.33%) than in females (24.66%). The parasite was most prevalent (58.82%) in the age group of 1 to 10 years and least prevalent (33.33%) in the age group of 61 to 70 years, according to the relationship between age and *T. gondii.* In both urban and rural areas of District Sheikhupura, the overall seroprevalence rate of toxoplasmosis was 35%. Punjab's urban and rural areas require the development of comprehensive, sound, science-based policies at the federal and provincial levels.

Key Words: Toxoplasmosis; *Toxoplasma gondii;* Parasite; Prevalence; Serological

Introduction

Toxoplasma gondii is the most common zoonotic apicomplexan parasite present worldwide. *T. gondii* can create an infection called Toxoplasmosis. *T. gondii* was first described by Nicolle and Manceaux in 1908 from the liver of African rodent, *Ctenodactylus gundi*. *T. gondii* may infect humans and many warm-blooded animals (Silva and Langoni 2005). Domestic and wild cats are the definitive host and natural reservoir of the infective oocysts. The resistant oocysts are excreted in environment *via* feces. The birds and mammals are intermediate hosts that may develop tissue cysts (Pavlovic and Ivanovic 2005). According to Ashburn (1992), serological surveys were carried out in various regions of the world and the results showed that more than 1/3rd human population has antibodies to *T. gondii*.

Age, geographic region, eating habits, and sanitary standards all affect how often *T. gondii* infection is in humans (Tenter *et al.* 2000). The prevalence of *T. gondii* has been reported to be the maximum in warm and humid environments (Coelho *et al.* 2003). The virulence varies by strain and susceptibility according to one's genetic characteristics (Ngo *et al.* 2017). Most infections in people are either asymptomatic or cause very minor clinical illnesses. Acute or chronic illness caused by active infection at any age (Boyer *et al.* 2011; Delair *et al.* 2011). A primary infection during pregnancy may result in a stillbirth or spontaneous abortion (Pappas *et al.* 2009). While toxoplasmosis is asymptomatic in immune-competent people, immune-compromised people with cancer, HIV positivity, and transplant recipients may experience severe illness. These individuals are susceptible to sequelae with a poor prognosis, such as encephalitis, brain abscess, myocarditis, and chorioretinitis, brought on by acute infection or by the reactivation of latent toxoplasmosis. Acute or recurrent infections have the potential to be fatal (Ahmadpour *et al.* 2014; Sutterland *et al.* 2015). Additionally, retinochoroiditis of the ocular disease normally develops in up to 2% of *T. gondii*-infected healthy persons (Holland 2003). Pakistan has been the subject of several research on the incidence of *T. gondii* (Dubey and Jones 2008; Bahia-Oliveira *et al.* 2003), and (Sadaruddin *et al.* 1991; Tasawar *et al.* 2011). Epidemiological studies on *T. gondii* seroprevalence assist impact health policies in many nations due to its long-term effects and ubiquity. The goal of the current investigation is to ascertain the general prevalence of toxoplasmosis in humans and its associations with sex, age, and gender.

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Materials and Methods

In the course of current study, blood samples were taken from 300 randomly chosen people, including 150 from rural and 150 from urban areas. The humans' ages and sexual orientations were noted. With the help of medical technician about 3–5 mL blood was taken using disposable syringe. Collected blood was carefully transferred into a sterile screw-capped tube in order to prevent hemolysis. After allowing it to coagulate, collected blood was centrifuged at 3000 rpm for ten min. Serum was collected in eppendorf tubes using micropipette and was stored at -20°C until it was processed for further analysis. Latex Agglutination Kit was used in the antibody analysis.

Procedure

Analysis was done in accordance with the manufacturer's recommended method. Before starting, the material (serum & reagents) was maintained at room temperature. Using LAT buffer, all samples were diluted at a rate of 1:20 and thoroughly mixed. Each test slide's well was filled with a drop of each diluted serum (40 μL), followed by a drop of the latex reagent, which was then thoroughly mixed. Within a time, frame of no more than four min, agglutination was shown both present and absent. Positive sera showed a clear agglutination while negative sera showed no agglutination. Chi Square test was applied to analyze the data for comparison between the groups and the results were represented as percentages (%) (Chaudhary and Kamal 2000).

Results

The protozoal parasite *T. gondii* is incredibly prevalent and infects nearly all mammalian species, including human (Schluter *et al.* 2014). The prevalence of toxoplasmosis in people from all over the world has been confirmed by numerous reports. To investigate the prevalence of *T. gondii* in the rural and urban areas of District Sheikhupura, 300 sera were tested. The outcomes demonstrated that *T. gondii* overall seroprevalence was 35% (Table 1).

Toxoplasmosis prevalence is significantly influenced by the sex of the hosts; male hosts had a prevalence of 45.33%, which was higher than that of female hosts, who had a prevalence of 24.66% (Table 2).

Different age groups and *T. gondii* infections were linked, and in each age group's prevalence of the parasite were confirmed. In the current study, children aged 1 to 10 years had maximum prevalence of *T. gondii* which was (58.82%) while children aged 11 to 20 years had the lowest prevalence (25.80%). For other age categories, prevalence was 28.20% in the age group of 21–30 years, 35% in the age group of 31–40 years, 45.97% in the age group of 41–50 years, 36% in the age group of 51–60 years, and 33.33% in the 61 to 70-year-old range (Table 3).

Comparative study of toxoplasmosis in population of district Sheikhupura rural and urban areas showed no significant differences (Table 4). In comparison to rural areas, the data showed that the percentage of infection in urban areas was slightly lower (32.66%) as compared to rural areas (37.33%).

There are numerous ways to become infected with toxoplasmosis. The prevalence of human toxoplasmosis was also examined in relation to several risk factors, including work position, pet history, drinking water source, style of housing, and dietary habits. Results showed incidence among individuals who had owned pets in the past (49.01%), drunk tap water (51.26%), lived in mud-brick homes (53.27%), and consumed undercooked meat (47.05%) (Table 5).

Discussion

Toxoplasmosis is a worldwide parasitic disease and is a risk to unborn infants and persons with weak immune systems. The prevalence of toxoplasmosis in many countries has been explored. Similar results (30%) were reported in the eastern region of Saudi Arabia (Qurashi *et al.* 2001) while higher values 47.4, 59.6 and 90% were calculated in Brazil, Egypt and Ethiopia, respectively (Garcia *et al.* 2004; Elsheikha *et al.* 2009; Shimelis *et al.* 2009).

Studies on the prevalence of toxoplasmosis have shown that prevalence of parasite depends on many factors like nutritional habits, socio-economic status, immunity hygienic conditions and consumption of raw or under cooked meat and contaminated soil. The prevalence of toxoplasmosis in the current study was higher in people living in rural area and the reason for high prevalence may be lack of education and environmental conditions.

Gender of the hosts plays a major role in the prevalence of toxoplasmosis. The prevalence of Toxoplasmosis during present survey was found to be 45.33% in males and 24.66% in females. Relatively higher prevalence of *T. gondii* in male may be due to low resistance of male hosts as compared to female hosts. Levels of immunoglobin, including IgG, IgM, and IgA are greater in females than in males. The literature generally shows that the females are more resistance to parasitic infections than males because of the gender associated differences in exposure and testosterone immune-suppressive properties.

The results of current study coupled with those from previous research of a similar nature (Sadaruddin *et al.* 1991; Shafi 2004; Tasawar *et al.* 2011; Ahmad *et al.* 2020) provide strong evidence that *T. gondii* infection is highly prevalent in different regions of Pakistan.

In another study in Muzaffargarh, it was found that males were more likely to have parasites than females (Raza *et al.* 2012). In Southern Punjab, Tasawar *et al.* (2011) found that a larger percentage of men than women had toxoplasmosis. Similar results were reported by Meisheri *et*

Table 1: Overall prevalence of human toxoplasmosis in district Sheikhupura, Pakistan

Name of parasite	No. of hosts examined	No. of hosts infected	Prevalence (%)	*P*- value
T. gondii	300	105	35%	0.5828^{NS}

Highly significant $P < 0.01$, * Significant $P < 0.05$, NS Non-significant P-value > 0.05

Table 2: Relationship between sex and human toxoplasmosis in district Sheikhupura, Pakistan

Gender	Hosts examined	Hosts infected	Prevalence (%)	*P*-value
Male Hosts	150	68	45.33%	0.0000**
Female Hosts	150	37	24.66%	

Highly significant $P < 0.01$, * Significant $P < 0.05$, NS Non-significant $P > 0.05$

Table 3: Relationship between age and human toxoplasmosis in District Sheikhupura, Pakistan

Age groups (years)							*P*-value
1-10	11-20	21-30	31-40	41-50	51-60	61-70	0.0000**
n = 17	n = 31	n = 78	n = 100	n = 37	n = 25	n = 12	
10	8	22	35	17	9	4	
(58.82%)	(25.80%)	(28.20 %)	(35%)	(45.97 %)	(36 %)	(33.33%)	

Highly significant $P < 0.01$, * Significant $P < 0.05$, NS Non-significant $P > 0.05$

Table 4: Area wise prevalence of human toxoplasmosis in district Sheikhupura, Pakistan

Name Of place	Hosts examined	Hosts infected	Prevalence (%)	*P*-value
Urban Area	150	49	32.66%	0.4680^{NS}
Rural Area	150	56	37.33%	
Total	300	105	35%	

Highly significant $P < 0.01$, * Significant $P < 0.05$, NS Non-significant $P > 0.05$

Table 5: Risk factors associated with prevalence of human toxoplasmosis in district Sheikhupura, Pakistan

Risk Factors		No. of hosts examined	No. of hosts infected	Prevalence (%)	*P*-value
Employment status	Employment	203	37	18.22 %	0.0000**
	Unemployment	97	41	42.26 %	
Pet History	Pets	253	124	49.01 %	0.0000**
	No. of pets	47	8	17.02 %	
Source of drinking water	Tap water	119	61	51.26%	0.0000**
	Hand pump water	38	15	39.47%	
	Boiled water	12	3	25%	
	Commercial bottled water	131	21	16.03%	
Type of Housing	Mud paved houses	122	65	53.27 %	0.0000**
	Cemented paved houses	178	41	23.03%	
Eating Habits	Raw vegetables and fully cooked meat	76	26	34.12 %	0.0000**
	Raw vegetables and undercooked meat	27	19	70.37 %	
	Cooked vegetables and undercooked meat	34	16	47.05 %	
	Cooked vegetables and fully cooked meat	163	26	15.95 %	

Highly significant $P < 0.01$, * Significant $P < 0.05$, NS Non-significant $P > 0.05$

al. (2003) from India, who found that men's seroprevalence was 34%, slightly higher than women's seroprevalence of 26.2%. Toxoplasmosis prevalence is significantly influenced by the gender of the hosts.

In the current study, children aged 1 to 10 years had maximum prevalence of *T. gondii* positivity (58.82%) while children aged 11 to 20 years had the lowest prevalence (25.80%). For other age categories, prevalence was 28.20% in the age group of 21–30 years, 35% in the age group of 31–40 years, 45.97% in the age group of 41–50 years, 36% in the age group of 51–60 years, and 33.33% in the 61 to 70-year-old range (Table 3). Arene (1986) found a high prevalence rate of (66%) among the children of ages between 1–5 years, which is consistent with current study.

Children may have a higher incidence because they are exposed to more soil, which increases their risk of infection (Deeb *et al.* 2012; Ahmad *et al.* 2019). In the same way, the age group of 11 to 20 years was found to have the lowest infection rate by Hayat *et al.* (2014). Studies on the provenance of toxoplasmosis in various age groups have shown inconsistent findings (Sadaruddin *et al.* 1991). The majority of the research indicates that as host age increases, toxoplasmosis prevalence rises (Sharif *et al.* 2007; Zahida *et al.* 2011; Tilahun *et al.* 2016; Ahmad *et al.* 2019). The highest seroprevalence was reported in people over the age of forty. It has been observed that seroprevalence grew with age.

Older people had much higher prevalence of IgG antibodies, suggesting that they may be susceptible to infection for the rest of their lives. The constant exposure to

risk factors in older people may be a contributing cause to the higher prevalence, as an infection once contracted lasts a lifetime. IgM positive cases were also prevalently found in older age groups. It may be due to a reason that that younger people do not commonly eat undercooked meat, a high IgM prevalence in older people may be caused by this feeding habit. Similar results from investigations carried out in India and USA were recoded (Pearce *et al.* 2008; Ahmad *et al.* 2019).

Comparative study of toxoplasmosis in population of district Sheikhupura rural and urban areas showed no significant differences. In comparison to rural areas, the data showed that the percentage of infection in urban areas was slightly lower (32.66%) as compared to rural areas (37.33). Infection rates might fluctuate from one country to another and even within the same nation in different regions (Dubey and Jones 2008). Poor hygienic conditions and more exposure to animals in rural regions than in urban ones may be to blame for the increased occurrence there (Ahmad *et al.* 2020).

The prevalence of human toxoplasmosis was also examined in relation to a number of risk factors, including work position, pet history, drinking water source, style of housing, and dietary habits. Current study results showed incidence among individuals who had owned pets in the past (49.01%), drank tap water (51.26%), lived in mud-brick homes (53.27%), and consumed undercooked meat (47.05%). Studies by Tilahun *et al.* (2016), Ahmad *et al.* (2019) have also revealed a positive link between the risk factors and toxoplasmosis (2020). Therefore, there is a pressing need to inform and educate the public regarding the occurrence and risk factors of the infection in animals and humans. The more appropriate ways to educate the people include general health awareness and use of social, print and electronic media.

Conclusion

It has been concluded that the human population in district Sheikhupura had a very high seroprevalence of toxoplasmosis especially in rural areas. The age of the patients, their neighborhood, water source and the pets in home like cats were discovered to be the prominent risk factors for contracting *T. gondii* infection in population.

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Author Contributions

Asif Ilyas: Data curation, Writing Original draft preparation. Sikandar Hayat & Majid Hussain: Supervision, Writing Conceptualization, Methodology, Analysis.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on the request to the corresponding author.

Ethics Approval

Departmental bioethical committee approved the research topic.

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