



Original Article

Seroprevalence of Human Toxoplasmosis in Gujranwala Punjab, Pakistan

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ABSTRACT

Toxoplasmosis, transmitted by *Toxoplasma gondii*, is a disease caused by water pollution. Consuming undercooked food or drinking polluted water can lead to severe infections, including seizures, cerebral necrosis, and hearing loss. **Objective:** To find out the prevalence of human toxoplasmosis in Gujranwala Punjab Pakistan. **Methods:** Human blood samples were obtained at random from various locations in Gujranwala. About 5ml blood was draw by puncturing the vein using the sterilized syringe and collected in EDTA vials. Serum was separated from collected blood by centrifugation at 3000rpm for 15 minutes and was collected in serum cup. The Latex Agglutination Test Kit was used to find the presence or absence of *T. gondii* in the collected serum. **Results:** The overall seroprevalence of human toxoplasmosis was 34.25%, with the highest prevalence found in the age group of 15-25 years (41.26%) and the lowest in 65-70 years (28.57%). The prevalence was found to be 36.5% in males and 32% in females, with the highest prevalence found in the age group of 15-25 years (41.26%). The prevalence was also higher in the age group of 65-70 years (28.57%). Factors contributing to the prevalence of toxoplasmosis include employment, direct contact with pets, drinking from tap water/supply water (51.38%), mud houses (60.46%), and eating raw vegetable and undercooked meat (81.81%) as compared to cooked fully cooked meat and vegetables (13.25%). **Conclusions:** The study highlights the complicated prevalence of toxicity in the Gujranwala community, affecting factors like age, education, employment, water supply, housing, and nutrition, requiring targeted interventions.

INTRODUCTION

Toxoplasmosis, a global foodborne disease caused by *Toxoplasma gondii*, is a common protozoan parasite that belongs to the phylum Apicomplexa [1]. The WHO states that one-third of the world's population is affected by this parasite, with warm and humid areas having a high prevalence [2]. The parasite has a wide range of hosts, including humans and warm-blooded species; however, its primary host is the felid family. The parasite completes its sexual cycle and delivers oocysts only in the intestine of felids, making them the most important animal species in the parasite's life cycle [3]. The infection typically produces a mild flu-like illness with symptoms usually disappearing within the first few weeks [4]. However, people with a weak immune system, such as those infected with HIV, may

experience severe illness and even death. *T. gondii* has three contagious phases in its life cycle: Tachyzoites, Bradyzoites, and Sporozoites [5]. The word "tachyzoite" was used to characterize the rapidly growing stage of the definitive host's non-intestinal epithelial cell and any intermediate host cell [6]. Tachyzoites do not have locomotory organs like pseudopodia, cilia, or flagella, but they can move by flexing, gliding, undulating, and spinning their limbs [7, 8]. Frenkel's term "bradyzoites" refers to organisms that multiply slowly inside tissue cysts, unlike tachyzoites [5, 9, 10]. Bradyzoites have a nucleus located at the posterior end of the cell, whereas tachyzoites have a central nucleus. Tissue cysts form and stay within cells, with intramuscular cysts elongated and spheroidal.

Bradyzoites are slenderer than tachyzoites owing to their amylopectin granules. Before gametogony, *T. gondii* forms five distinct groups of intestinal epithelial cells that undergo schizogony. Merozoite development begins with a rounded merozoite anlagen emerging near the schizont's periphery, where the schizont plasma lemma invaginates each merozoite and forms a plasma lemma. The origin of gametes is unknown; however, merozoites produced by schizonts are believed to be the source of gamete formation [11]. Oocysts are subspherical round, with diameter of 10-12 μ m. Only cats develop oocysts, which they release from their feces after eating tachyzoites, bradyzoites, or sporozoites. Oocysts are the most environmentally sensitive *T. gondii* has many stages and can live in the soil for years. When a moderate temperature, aeration, and humidity are active, oocysts sporulate outside the body of the cat 1-5 days after defecation, and they become infectious [12]. The sporozoites are 2 micrometers wide and 7 to 9 micrometers long [7, 10, 13]. *T. gondii* life cycle involves both intra-intestinal and extraintestinal stages in cats, with the extraintestinal stage being active in intermediate hosts. Tachyzoites form sporozoites that invade host cells and grow rapidly during acute infections [14]. These parasites are less susceptible to stomach secretions and are fewer effective sources of infection than other stages. Bradyzoites, or tissue cysts, are a population of bradyzoites that accumulate in large amounts within host cells [15]. *T. gondii* forms five distinct asexual phases until the start of sexual reproduction, in epithelial cells of the small intestine [16, 17]. After ingesting tachyzoites, bradyzoites, or oocysts, 100% of cats develop oocysts while swallowing the tissue cysts. Oocysts emerge in cat feces three-five days before swallowing tissue cysts, with a peak effect exerted from days to 5-8. Oocysts require oxygen, moisture, and moderate temperature for sporulation [12, 18, 19]. In Gujranwala, food must be cooked at a sufficient temperature to prevent toxoplasmosis. Preparation of beef, lamb, and veal roasts should be at least 145 degrees Fahrenheit, while poultry should be cooked to 190 °F in the thigh. Peeled or thoroughly washed fruits and vegetables are recommended. Pregnant women should wear gloves and avoid replacing cat litter that with of pregnant mothers. This study aimed to identify the overall prevalence of toxoplasmosis in the human population of Gujranwala and to investigate its seroprevalence in different age groups.

METHODS

The current research was conducted to know the comparative analysis of *Toxoplasma gondii* infections occurring in the area of District Gujranwala, Punjab Pakistan. During the study 400 blood samples were

collected randomly from female and male having different ages and data was recorded in the pre-designed Performa. The current research was conducted in the university of Lahore and DHQ hospital Gujranwala Province Punjab, Pakistan. Total 400 blood samples were collected including 100 blood sample from Chanda Qila, 100 from Wapda Town, 100 from Jalil Town and 100 from those persons who visited the DHQ Hospital Gujranwala. The people at this hospital mainly come from four Tehsil's of Gujranwala and near located areas. A questionnaire-guided interview was conducted in accordance with the collection of blood samples. Throughout the study phase, the participants' confidentiality and anonymity is preserved. Age, educational degree, marital status, profession, location, and family size were among the demographic data collected. Such predicted risk factors such as interaction with felines, eating raw meat or unwashed vegetables, consuming unpasteurized milk, drinking water supplies, rabbit rearing, and a history of spontaneous abortion were also taken into account. Different material like Latex reagent, negative and positive control, Glycine buffer diluents (20X) plastic, stick, test slide, micropipette, centrifugation. During the investigation total 400 blood samples of male and female with respect to their age and residence were collected in current study. A total of 5ml of venous blood was taken from participants who agreed to take part in the study. Blood was obtained by puncturing a vein with a sterilized syringe, and the blood was cautiously and deliberately poured into sterile screw-capped EDTA tubes for storage to prevent haemolysis. Then the samples were brought to the CRIMM laboratory of The University of Lahore for further analysis. Collected blood allow for clotting, blood samples were kept at room temperature overnight and centrifuged for 15 minutes at 3000 rpm. Serum was separated and transferred into sterile serum cups with the help of micropipette and store at -20 °C until processed for further analysis. Preserved serum was analyzed using commercial "Latex Agglutination Kit" for specific antibodies of *T. gondii*. Each well of the test slide was filled with a drop of each serum, followed by a drop of latex reagent and blended well. Slide was shaken tenderly and gradually for 5 minutes. The presence and absence of agglutination was observed to unaided eye. There was clear agglutination in the positive sera, but none in the negative sera. The findings were compiled and entered into a data knowledge book.

RESULTS

The total seroprevalence of human toxoplasmosis was assessed in Gujranwala, and the findings show that it was 34.25% in people. 200 males and 200 females were tested for human toxoplasmosis, and 73 males and 64 females

tested positive for the disease. It was discovered that 32% of women and 36.5% of men had toxoplasmosis. The age group of 15 to 25 years had the highest frequency (41.26%), while the age group of 65 to 70 years had the lowest prevalence (28.57%). In contrast to educated people's 27.67%, high frequency was identified in persons who were primarily illiterate (38.58%). The prevalence of toxoplasmosis in humans was induced by a number of circumstances, including low employment (21.72%) and high unemployment (59.39% prevalence). Highest prevalence 51.38% is found in those who drink from tap water/supply water, and lowest prevalence 13.15% was in those who drink commercially available filtered water. Mud houses had the highest prevalence of infection (60.46%), compared to paved cemented homes, which had a prevalence of 21.77%. Consuming raw or undercooked meat and vegetables was more common (81.81%) than thoroughly cooked meat and vegetables (13.25%). The results are presented in Table 1-6.

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

Parasite	Total examined	No. of infected (Overall prevalence)
<i>Toxoplasma gondii</i>	400	137 (34.25)

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

Parasite	Male hosts			Female hosts		
	Total examined	Host Infected	Seroprevalence	Total examined	Host Infected	Seroprevalence
<i>T. gondii</i>	200	73	36.5%	200	64	32%

Table 3: Relationship between age and human toxoplasmosis in district Gujranwala, Punjab, Pakistan

Name of parasite	Total No of hosts examined	Age of host examined (Years)					
		15-25	26-35	36-45	46-55	56-65	65-70
<i>T. gondii</i>	400	N=63	N=123	N=64	N=84	N=45	N=21
		26 (41.26%)	44 (35.77%)	21 (32.81%)	23 (27.38%)	17 (37.77%)	6 (28.57%)

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

Locality	Negative		Positive		Prevalence N (%)
	Male	Female	Male	Female	
Chanda Qila	24	28	26	22	100(48%)
Wapda Town	38	37	12	13	100(25%)
Jalil Town	34	36	16	14	100(30%)
DHQ hospital	31	35	19	15	100(34%)

Table 5: Prevalence of toxoplasmosis between educated and uneducated human population in district Gujranwala, Punjab, Pakistan

Education	Negative		Positive		Prevalence
	Male	Female	Male	Female	
Educated	50	65	24	20	159(27.67%)
Uneducated	77	71	49	44	241(38.58%)

Table 6: Risk factors associated with prevalence of human toxoplasmosis in district Gujranwala, Punjab, Pakistan

Risk factors	No. of host examined & Prevalence	Sero-positive (n)	Sero-negative (n)
Working place			
Employment	267(21.72)	58	209
Un employment	133(59.39)	79	54
Infected host with history			
Pets	221(46.60)	103	118
No pets	179(18.99)	34	145
Source of drinking water			
Tape water	144(51.38)	74	70
Hand pump water	84(46.42)	39	45
Boiled water	20(20)	04	16
Commercially available filtered water	152(13.15)	20	132
Type of housing			
Mud houses	129(60.46)	78	51
Paved cemented houses	271(21.77)	59	212
Eating habits			
raw vegetables and fully cooked meat	84(23.80)	20	64
raw vegetables and undercooked meat	77(81.81)	63	14
cooked vegetables and undercooked meat	73(43.83)	32	41
cooked vegetables and fully cooked meat	166(13.25)	22	154

DISCUSSION

The main aim of this research was to look at the general seropositivity of *T. gondii* in the human population, as well as the association between their sex and the age of the host with *T. gondii*. The sample was collected from different area of Gujranwala, and checked the area wise seroprevalence of toxoplasmosis between educated and uneducated human population, and count other factors that are linked with the prevalence of human toxoplasmosis in Gujranwala. During the current study, 400 blood samples were taken at random. A total of 137 hosts were found to be infected with the toxoplasma parasite out of 400 people. According to the latex agglutination measure, the overall prevalence of human toxoplasmosis was 34.25% (LAT). The parasite's prevalence has been studied in various parts of the world, including Pakistan. Conduct research to investigate the seroprevalence of *T. gondii* between 150 human individuals. According to their results 42% individual were serological positive for *T.gondii* [20]. A research reports the overall seroprevalence of immunoglobulin G antibodies to *T.gondii* was 22.5% in sera from participants in the third national nutrition and health [21]. Shafi et al., studied and investigate to examine the seroprevalence of human toxoplasmosis in Multan, Pakistan. According to her result overall prevalence of *T. gondii* was 32%. Because of differences in eating habits, geography, level of exposure, and educational level, the prevalence of *Toxoplasmosis gondii* varies around the

world. It has been reported that prevalence of toxoplasmosis may be higher in farmers and employees [22]. Another study stated that the seroprevalence of human Toxoplasmosis in 200 male and 200 females in which 73 male and 64 females were seropositive by latex agglutination test (LAT) [23]. According to this survey Toxoplasma was found to be 36.5% in male and 32% in female. The same aspect of present study is also reported by different research workers. Tasawar et al., investigate the seroprevalence of human toxoplasmosis among 150 human individuals, according to them the prevalence of toxoplasma was found to be 44% in males and 40% in females [20]. Ali Raza studied to investigate the seroprevalence of human toxoplasmosis in Muzaffargarh Pakistan. According to his findings, the parasite was found to be more widespread in males (40%) than females (34%) [26]. Moschen et al., studied in Italy, males account for 18.2% of the population, while females account for 17.5% [24]. *T. gondii* was found to be prevalent in 35.6 % of males and 1.9 % of females in Saudi Arabia, according to Yaneza and Kumari [25]. According to Sharif et al., Males had a higher seroprevalence (80%) than females (77.6%) among the residents of recovery centers in Northern Iran [26]. During the current investigation the highest seroprevalence 41.26% was observed in age group of 15–25 years and lowest seroprevalence 28.57% was noted in age group of 70 years. Various researchers have examined the association between age and *Toxoplasma gondii*. Ally and Idris, examined one hundred patients of different age at the ophthalmology units of Jinnah postgraduate Medical Center Karachi. Seroprevalence of anti-Toxoplasma antibodies was highest 60% in age groups 21 to 40 years [27]. Kaisi and Abu Ghadir, researched at Queen Alia Hospital in Jordan to assess the prevalence of Toxoplasma antibody. According to their findings, 41.5 % of the participants were *T.gondii* seropositive [23]. The age range aged 21 to 35 years old has the greatest prevalence of seropositivity. Similar findings have been documented by Sharif et al., who found that the prevalence of *T.gondii* increases with age (due to a decline in host immunity) [26]. Many researchers examined the *Toxoplasma gondii* and investigate the prevalence of this parasite in various locations of the globe including Pakistan. Tasawar et al., investigate the prevalence of toxoplasmosis in different areas of the southern Punjab. They found that seroprevalence of human toxoplasmosis was high 51.0% in Bahawalnagar as compared to 44.33% in Rajanpur and 16.33% in Multan [20]. Kistiah et al., According to their results as compared to other regions in Saudi Arabia and neighboring Gulf countries, the seroprevalence of *T. gondii* in Jazan province is 24.1 %, while the prevalence of *T. gondii* varies between 25 and 36 % [28]. Low prevalence rates of

10% have been recognized by the United Kingdom [29], Korea, and Norway, and as high as 77.5% in Brazil [30, 31]. Higher prevalence rates were also reported in some neighboring Arab countries, such as Jordan (66.9%) and Kuwait (53.1%) [32]. The variation in prevalence rates between countries can be explained by differences in geographical and climatic conditions, as oocyst sporulation is more effective in hotter and wetter regions [33]. According to this survey and study different human blood sample were collected and investigate the seroprevalence of human toxoplasmosis between educated and uneducated human population. High prevalence was found 38.58% in mostly uneducated people as compared to educated 27.67%. The present study investigates the high seroprevalence was recorded in the illiterate people as compared to literate because of their less awareness about contaminated and unhygienic conditions and other factors including their working condition and directly contact to pets and contaminated things with *Toxoplasma gondii* spores. Present study investigates the risk factors that are associated with the seroprevalence of human toxoplasmosis. Many factors caused the prevalence of toxoplasmosis in human including working condition employment has low prevalence 21.72% as compared to high prevalence in unemployment 59.39%. Infected host history taken and those who directly contact with pets has high prevalence 46.60% as compared to others that were not contact to pets has low prevalence 18.99%. Source of drinking water also affect the prevalence of toxoplasmosis highest prevalence 51.38% was found that drink from tap water/supply water and lowest prevalence 13.15% was in that drink commercially available filtered water hand pump water 46.42% and boiled water has found 13.15% of human toxoplasmosis. Mud houses have highest 60.46% prevalence as compared to paved cemented houses 21.77% prevalence. And eating habits include eating raw vegetable and undercooked meat has highest prevalence 81.81%, raw vegetables and fully cooked meat 23.80%, cooked vegetables and undercooked meat 43.83%, cooked vegetables and fully cooked meat has lowest 13.25% prevalence of toxoplasmosis. Many researchers investigate the risk factors that are associated with the prevalence of toxoplasmosis. Nazir et al., investigate the different risk factor that are associated with the prevalence of toxoplasmosis in human. High prevalence 20.2% occurred in those who directly contact to pets as compared to those who did not directly contact to pets 5.6% [34]. Similarly, high prevalence 24.4% identified in those who drink tap water or supply water as compared to commercially available filter water 7.7%. High prevalence occurred in those who live in mud houses 25.3% as

compared to others living in paved cemented houses 12.9%. Eating raw vegetables and undercooked meat have high prevalence of toxoplasmosis 31.3% as compared to other who eats cooked vegetables and fully cooked meat 5.6%[35]

CONCLUSIONS

The study's findings demonstrate the seroprevalence with age, sex, education, employment, water supply, housing, and nutrition all playing a role in the observed alterations in the Gujranwala community. This thorough understanding can aid in the creation of focused interventions and public health plans to lower the incidence of toxoplasmosis in the area.

Authors Contribution

Conceptualization: AM, SH

Methodology: AMB, MAAT

Formal Analysis: AMB, MAAT

Writing-review and editing: AM, SH, AMB, MAAT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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