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RESEARCH ARTICLE
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FIRST STUDY ON SEROPREVALENCE OF TOXOPLASMA GONDII AND HEMATOLOGICAL PROFILING IN LARGE FELIDS AND ASSOCIATED HUMAN SUBJECTS IN PUBLIC AND PRIVATE ZOOLOGICAL GARDENS OF PUNJAB, PAKISTAN

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Abstract:
This study aimed to determine the prevalence of *Toxoplasma gondii* infection in lions, tigers, leopards, and pumas kept in public and private zoological gardens along with human subjects associated with these gardens. One hundred and seventy-five samples collected from large felids were examined using the latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) while one hundred and fifty samples from human subjects were tested with LAT. Besides, hematological analysis of positive and negative samples from lions and humans was also done. The study estimated the overall prevalence of *T. gondii* as 21.1% and 29.7% in large felids using LAT and ELISA respectively. Whereas, the prevalence in human subjects was 31.3% using LAT. The overall prevalence was also different in public (10.2%) and private (14.4%) zoological gardens. The highest prevalence was lions followed by pumas and tigers, and no positive case was found in leopards. In human subjects, 32.1% of cases were found positive. The highest prevalence was found in supervisors (60%), followed by vets (40%), zoo keepers (32.8%), and meat vendors (20%). Moreover, the hematological analysis indicated that White Blood Cells (WBCs) count (22.2 ± 1.17), neutrophils (14.2 ± 0.75), and monocytes (3.25 ± 0.21) were significantly increased ($p < 0.05$) in T. gondii positive lions as compared to those found negative for T. gondii infection. The same trend was found in human subjects in which WBCs (16.7 ± 1.7), lymphocytes (61 ± 2.4), and eosinophils (7.40 ± 0.98) were found significantly ($p < 0.05$) increased in positive cases. The study investigated the association of the ABO blood group system with the occurrence of *T. gondii* infection and established significant ($p < 0.05$) association of B (43.6%), AB (42.8%), and A (34.2%) blood groups. These results highlight the necessity of comprehensive surveillance, preventive interventions, and additional studies to comprehend the epidemiology and dynamics of T. gondii transmission in populations of captive large cats and associated human populations in the zoological gardens of Punjab.

Key Words: *Toxoplasma gondii*, large felids, Zoological Gardens, Humans, Hematology, Punjab.

Introduction

Toxoplasma gondii (T. gondii) is a common parasite belonging to the phylum protozoa and is thought to affect one-third of all people on the globe. It is an important zoonotic pathogen that can infect a wide variety of warm-blooded animal species (Traviezo-Valles 2022). Its life cycle is complex and involves two hosts: a definitive host and an intermediate host. The definitive host, typically a member of the Felidae family, undergoes sexual reproduction, leading to the production of millions of oocysts that are shed in feces, contaminating the environment. These oocytes can survive for 16-18 months in the environment. The intermediate host can be any warm-blooded animal, including humans. Once inside the host, the parasite enters the bloodstream and forms tissue cysts in various organs, where it can remain dormant for long periods. Hence, the disease is mainly transmitted through oocytes, and the most common felids to spread these oocytes are cats. The cats can secrete thousands of oocytes in the environment (Saadatmand et al. 2021). However, large felids also belong to the Felidae family and can be an important source of this pathogen.

This pathogen is also not good for the health of large felids. Clinical toxoplasmosis can manifest itself in many ways in wild felids. It is common for wild felids to carry the parasite asymptomatically and show no outward signs of sickness. Nevertheless, clinical symptoms might occasionally show up and result in major health issues. Anorexia, lethargy, neurological issues, and even mortality are just a few of the signs that can result from clinical toxoplasmosis in wild felids (Hatam-Nahavandi et al. 2021). One of the primary concerns regarding the illness in wild felids is the function that clinical toxoplasmosis plays in the parasite's transmission to other intermediate hosts like humans (Denk et al. 2022). Wild felids regularly stray in ecologically varied locations, which can serve as a bridge for *T. gondii* transmission between human populations and other animal species (Singh 2016).

Generally, humans are infected by these oocytes when they consume raw, undercooked, or under-processed meat (Alvarado-Esquivel et al. 2006). Besides, transplacental transmission from mother to fetus via tachyzoites by the bloodstream can be another source of transmission to humans (Ajioka and Morrissette 2009). Toxoplasmosis is specifically very dangerous during first phase of pregnancy and can cause very serious developmental disorders (Thiébaut et al. 2007). It can trigger abortion and serious complications like vision loss, intellectual weakness, brain cells damage, etc. (Herrmann et al. 2014). The Centers for Disease Control and Prevention (CDC) ranked toxoplasmosis at the top of food-borne diseases that led to either hospitalization or death (Jones et al. 2014; Montazeri et al. 2020). Owing to these reasons, its cases in the human population need to be monitored frequently.

In Pakistan, extensive work on toxoplasmosis has been conducted in poultry (Khan et al. 2020), human population (Shah 2020), caprine (Mumtaz et al. 2022; Rafique et al. 2022), and pets and stray cats (Majid et al. 2021; Hafeez et al. 2022). However, there is currently no study available on important members of the Felidae family, i.e., lions, tigers, pumas, and leopards. There are many public and private zoological gardens in Punjab and these gardens have many of these large felids. Moreover, there is also a gap in prevalence studies of toxoplasmosis in human subjects like veterinarians, zoo keepers, managers, supervisors, meat vendors, and purchasers associated with these zoological gardens in Punjab. Keeping in view, the current study fulfills this gap in research and reports the prevalence of T. gondii in large felids and associated human workforce. The study also reported the hematological profile of these animals and associated humans and highlights important areas of future research in this area.

MATERIALS AND METHODS

Ethical statement:

The sample collection methodology was approved by the Institutional Review Committee for Biomedical Research, University of Veterinary and Animal Sciences, Lahore, Pakistan letter number DR/496/1 dated 19-11-2021.

Study area and design:

The present study was conducted to determine the prevalence of *T. gondii* infection in large felids; including lions, tigers, leopards, and puma at the zoological gardens of Punjab, Pakistan. Punjab is home to over half of the population of Pakistan and is located at 31.1704° N, 72.7097° E. In Punjab, there are seven (n=07) public and five (n=05) private zoological gardens which have a total of 109 and 76 large felids, respectively. These zoological gardens were included in the study with the prior consent of the management to participate in a large-scale study and provided access to the data required. Geographic locations of zoological gardens along with the type of animals are shown in figure 1.

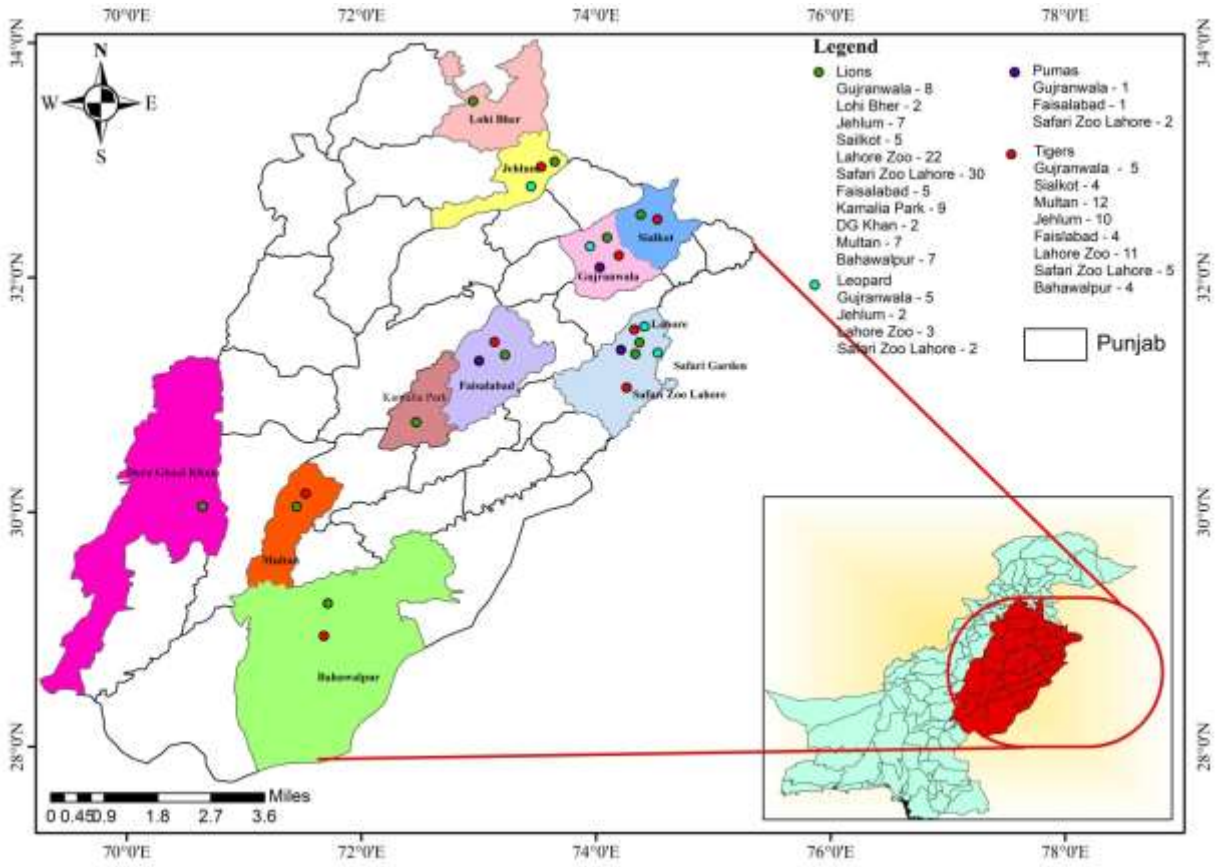

Figure 1: Geographic locations of zoological gardens

The study design is completely randomized. Representative sample size from large felids was calculated by using the following formula (Daniel and Cross 2018):

$n = Z^2 P(1-P)/d^2$

where n is the sample size, Z is calculated with a 99% confidence level, P is expected prevalence or proportion which we kept at 50% because there was no previous prevalence study on *T. gondii* in large felids in the study area, and d is the level of precision which was selected at 99%. The representative sample size was 167 by this formula; however, we collected (n = 175) samples from the study population to minimize the margin of error. Conversely, hundred percent of samples (n = 150) were collected from zoo keepers because these were the total zoo keepers that were in contact with large felids in any capacity.

Sample Collection:

Blood and serum samples were collected by standardized method and preserved for transportation to the Animal Health Research Laboratory, Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore. Briefly, a three ml blood sample was collected from the tail vein of each animal included in the study using a hypodermic needle attached to disposable

syringes under aseptic conditions. The blood samples were transferred to gel vacutainers (yellow-toped) for the collection of serum and EDTA vacutainer for hematological profiling of the lion. The samples were allowed to clot and the clotted blood was then centrifuged at 3500 rpm for 5 minutes. The supernatant, clear straw color fluid (serum) was aspirated using a pasture pipette and transferred into Eppendorf tubes. The serum samples thus collected were stored at -20 degrees until serological analysis was performed (Samaha HA, El-Gohary AH 1993).
Similarly, 150 blood collection strategy was adopted in human subjects either having contact with large felids or not. These human subjects were veterinarians, para-vets, caretakers, housekeeping staff, maintenance staff, food suppliers, and managerial staff. Data regarding each sampled human subject were entered in data capture form where entries included name, job responsibility, sex, age, job duration, duty hours, family history of abortion, birth abnormalities, and personal hygiene. These individuals were included in the study with prior written consent to participate in the study. Services-authorized phlebotomists were hired to collect blood samples from these human subjects along with data related to age and blood group was also collected.

Experiment 1: Seroprevalence of *T. gondii* in large felids and zookeepers using LAT:
Serum samples of large felid were analyzed for anti-toxoplasma antibodies using the Latex Agglutination test (LAT) (Dubey et al. 1999; Ahmad et al. 2015) at dilutions 1:16, 1:64, 1:128, and 1:256 in 96-well microtiter plates. Latex agglutination test was performed using LAT kit (Intek UK Ltd) with 81% specificity and 99% sensitivity (Taylor et al. 1997) available in the local market. The test was performed according to the procedure described by the kit manufacturer. Test results were interpreted as follows;

- Negative 1:16 sera indicate absence of immunity
- Positive 1:16 sera indicate residual or non-specific immunity
- Positive titers from 1:32 to 1:128 are due to acquired or evolving immunity
- Positive titers equal to or higher than 1:256 suggest possible recent contact.

Experiment 2: Seroprevalence of *T. gondii* in large felids using Enzyme-Linked Immunosorbent assay (ELISA):
All sera samples of large felids were subjected to ELISA for further confirmation (Basso et al. 2013). ELISA test was performed using an ELISA kit provided by the Department of Parasitology, UVAS Lahore. The details of the ELISA protocol are as follows.
For protein coating, the rSAG1 antigen was diluted to a concentration of 0.125 µg/ml in 50 mM carbonate buffer (pH 9.6) made from a 1M carbonate stock solution. Then 100 µl of the diluted rSAG1 solution was added to each well of the ELISA plate and the plate was incubated overnight at 4°C to ensure proper antigen coating on the well surface. After the overnight incubation, the plate was washed three times with 0.001M phosphate-buffered saline (PBS) containing 0.05% Tween 20 to remove unbound antigens. Subsequently, 200 µl of 4% Bovine Serum Albumin (BSA) solution was added to each well to prevent nonspecific antibody binding. Afterward, a serum test was done by adding primary antibodies, diluted 1:50, to each well followed by incubating the plate at 37°C for 2 hours to allow specific antigen-antibody binding. The plate was then washed three times with 0.001M PBS containing 0.05% Tween 20 to remove unbound primary antibodies. After that 100 µl of secondary antibodies (conjugated with Alkaline Phosphate - AP) was added to each well and the plate was incubated at 37°C for 2 hours to enable proper binding of secondary antibodies to primary antibodies. In the next step, 100 µl of the substrate solution (2-amino-2-methyl-1-propanol (AMP)) was added to each well. That substrate reacted with the enzyme (AP) conjugated to the secondary antibodies and resulted in a color change. Finally, 100 µl of 1M NaOH was added to each well to halt the enzyme-substrate reaction.
To find out the presence and concentration of the antigen in the sample the Optical Density (OD) values were measured at 405 nm wavelength using an ELISA reader after a 15-minute incubation with the substrate. Interpretation of results were done by analyzing the OD values to determine the

presence and quantity of the specific antigen *(*rSAG1) in the samples. The higher OD values corresponded to the higher antigen concentrations.

Experiment 3: Hematological profiling of large felids and associated human subjects:

Blood samples preserved with EDTA from LAT-positive and negative lions and zoo keepers were collected and processed to investigate the impact of *T. gondii* infection on the hematological parameters of the study population. We randomly selected 20 *T. gondii* positive and negative lions irrespective of their age, gender, and geographic location. Red Blood Cells (RBCs), White Blood Cells (WBCs), neutrophils, lymphocytes, monocytes, and eosinophil counts were measured and compared. The same approach was adopted in the human study group. However, permission was sought from workers as well as the administration of zoological gardens in human groups before sample collection. Additionally, an association of four blood groups (A, B, AB, and O) with the occurrence of *T. gondii* infection was also estimated in the latter group.

Statistical analysis:

The statistical analysis for qualitative data was performed using the chi-square test (χ^2) while quantitative data was analyzed using the t-test or Wilcoxon rank sum test in R statistical language. Descriptive statistics of quantitative data were also calculated. The differences were considered statistically significant at <0.05.

RESULTS

The study estimated the overall prevalence of *T. gondii* 21.1% and 29.7% in large felids using LAT and ELISA respectively. Besides, the prevalence in human subjects was 31.3% using LAT. The details of the prevalence are described hereunder.

Results of Experiment 1:

LAT-based seroprevalence of T. gondii in large felids in zoological gardens of Punjab:

The overall prevalence of *T. gondii* in large felids of private zoological gardens was 14.4% as shown in table 1. The prevalence of Toxoplasma in wild felids was examined throughout several private zoological parks located in several cities throughout Punjab. According to the research, there were interesting differences between each city's prevalence rates for lions, tigers, leopards, and pumas. Two out of every eight lions and two out of every five tigers in Gujranwala tested positive for *T. gondii*, whereas there were no positive cases found in leopards or pumas. The prevalence was lower overall in Sialkot, where there were no lions and just one out of every four tigers tested positive. Multan demonstrated a greater frequency among tigers, with 25% (3/12) testing positive, compared to lions and leopards, which had 14.2% (1/7) and 0% (0/5) positive cases, respectively. With 14.2% (1/7) and 10% (1/10) lions and tigers testing positive, respectively. Jhelum showed a somewhat steady prevalence, whereas there were no positive cases among leopards. Lions did not test positive in Faisalabad, but tigers and pumas did, with 25% (1 /4) and 100% (1/1) positive cases, respectively as shown in Table 1.

LAT-based prevalence of T. gondii in large felids of public zoological gardens:

Prevalence was considerably different in public zoological gardens. Lions in Lahore Zoo displayed a higher prevalence, with 50% (11/22) testing positive, while lions in Safari Garden had 30% (9/30) positive results. Bahawalpur had 14.2% (1/7) positive lions, DG Khan had 50% (1/2) positive lions, Kamalia Park had 11.11% (1/9) positive lions, and Lohi Bher showed no positive samples out of 2 lions (0%) tested. For tigers, Lahore had 18.18% (2/11) positive cases, Safari Zoo Lahore had 20% (1/5) positive cases, and Bahawalpur had 25% (1/4) positive cases. As for leopards, 0% (0/3) tested positive in Lahore Zoo, and Safari Zoo Lahore had no positive samples out of 2 leopards (0%). Likewise, in the case of pumas, 1 positive sample was found in Safari Zoo Lahore. These findings are comprehensively described in Table 1.

We also studied the prevalence of toxoplasma in different species of large felids at different titers.

The results showed that prevalence did not vary significantly (p <0.05) when titers were changed. However, the odds of having a non-significant percentage change varied among different species (table 2).

LAT-based Prevalence of T. gondii in human subjects associated with large felids of zoological gardens in Punjab:

We tested 150 human subjects belonging to the categories of keeper, managers, meat vendors, purchaser, supervisor, and veterinarian for *T. gondii* infection using LAT and found 47 (31.3%) positive samples as shown in Table 3. The highest percentage of positive results were found in supervisors (60%), followed by veterinarians (40%), keepers (32.8%), and mean vendors (20%). No positive cases were found in managers (n = 5) and purchasers (n = 5). There was also no significant difference (p < 0.05) among the prevalence of toxoplasmosis in different categories of human subjects as shown in Table 3.

Results of Experiment 2:

ELISA-based prevalence of T. gondii in large felids:

According to the ELISA results, the overall prevalence of *T. gondii* increased to 29.7% in large felids (table 1). In lions at private zoos, the prevalence became double (25%) as compared to LAT-based prevalence. Besides, the prevalence in lions of public zoos was 40.1%. A total of 104 lions were tested, and 37 tested positive for *T. gondii*, representing a prevalence rate of 35.6%. None of the 12 leopards tested positive both in private and public zoological gardens. Interestingly, there was no change in the prevalence rate of *T. gondii* in leopards, puma, and tigers and the results were the same as found by LAT as shown in Table 1.

Results of Experiment 3:

Hematological profiles of toxoplasma positive and negative lions:

The impacts of *T. gondii* infection on the hematological profiles of lions were also quantified and analyzed. The lions were divided into two groups: those who tested positive for *T. gondii* infection and a control group who tested negative. The lions that tested positive for *T. gondii* infection displayed significant changes in several important hematological markers. The counts of WBCs (22.2 ± 1.17), neutrophils (14.2 ± 0.75), and monocytes (3.25 ± 0.21) significantly increased (p < 0.05) in *T. gondii* positive lions as compared to hematological profiles in lions tested negative for *T. gondii* as shown in Table 4. The remaining parameters, i.e., RBCs, lymphocytes, eosinophils, and hemoglobin were also affected but not significantly (Table 4). The box and whisker plot show the range of changes in the hematological profiles in toxoplasma-positive and negative lions (Figure 2). The results of toxoplasma positive lions were indicated with red color while the negative ones with blue color. The median value is adequate in all the samples whether positive or negative. However, those parameters that vary significantly have their plots at different levels giving a quick interpretation at first glance (figure 2).

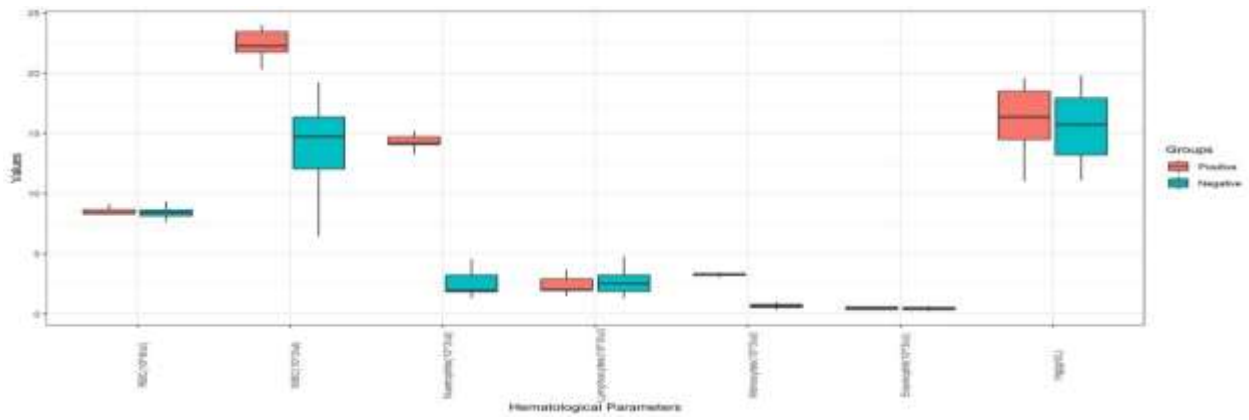

Figure 2: Box and whisker plot showing hematological profile values of wild felids which are positive and negative for toxoplasmosis

Hematological profiles of toxoplasma positive and negative human subjects interacting with large felids

The results of hematological profiles of *T. gondii* positive and negative human subjects were almost like the results of lions with the exceptions of eosinophils and neutrophils. WBCs (16.7 ± 1.7), lymphocytes (61 ± 2.4), and eosinophils (7.40 ± 0.98) were found significantly ($p < 0.05$) increased as shown in Table 4. The box and whisker plot showed the median line was not very adequate in all the parameters and there was also dispersion as indicated by standard deviation values given in Table 4. These graphs provide a quick overview of the results (figure 3).

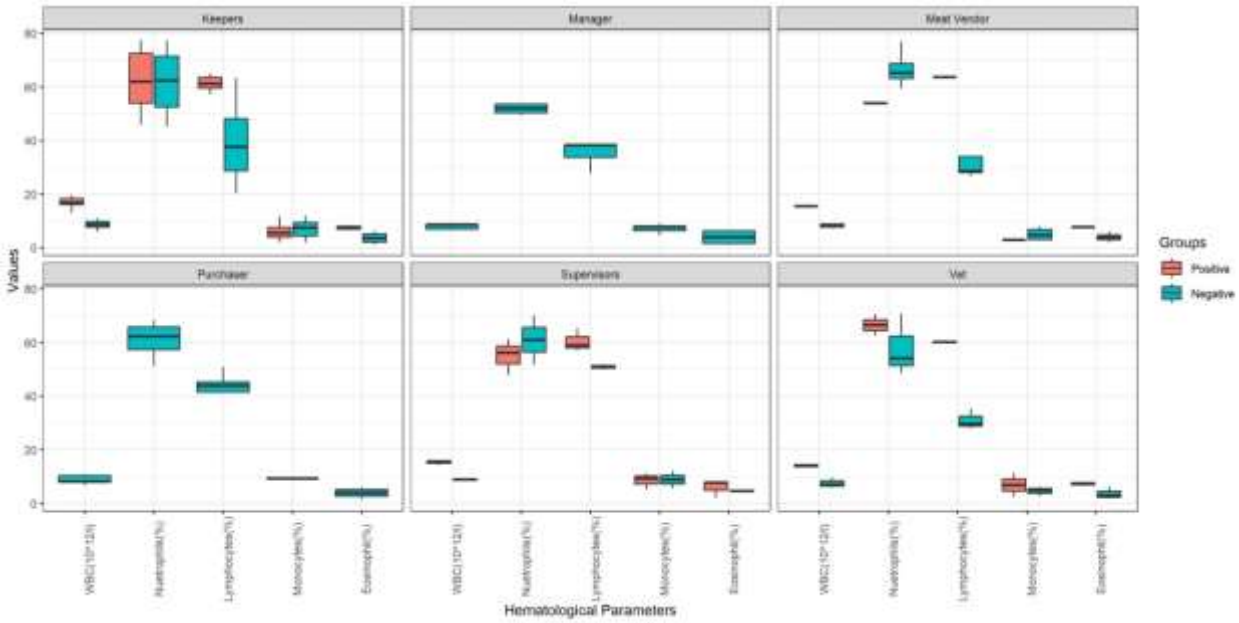

Figure 3: Box and whisker plot showing hematological profile values of humans which are positive and negative for toxoplasmosis based on LAT

Blood Group association with the occurrence of T. gondii infection in human subjects:

We investigated the potential association of blood groups with the occurrence of *T. gondii* infection. Thirteen of the 38 people (34.2%) with blood type A were found to have *T. gondii* infection. There were 28 people with AB blood group, 38 with A, 55 with B, and 28 with O blood group. Out of these, 42.8% of people with AB, 34.2% with A, 43.6% with B, and 10.7% with O blood groups were found positive for *T. gondii* infection. There was a significant difference ($p < 0.05$) of toxoplasmosis prevalence among different blood groups. The odds of having toxoplasmosis were 6.45, 6.25, and 4.33 times higher of people with B, AB, and A blood groups, respectively as compared to the odds in people with blood group O. (Table 5).

Discussion

The main goal of the study was to determine the seroprevalence of *T. gondii* in the fascinating wild felids. This study provided scientific evidence of existence of toxoplasmosis in these captured wild animals in public and private zoological gardens of the Punjab. The prevalence rate obtained through LAT and ELISA were different (21.1% vs 29.7%) (table 1). However, this variation was only in the samples of lions 22.2% versus 35.5% when determined through LAT and ELISA, respectively. In the rest of the animals, the prevalence rate was the same. The reason behind can be due to a low number of available samples in the species other than lions. Moreover, ELISA is more sensitive and specific than LAT, allowing it to detect lower antibody concentrations and deliver

more reliable results. Since the LAT is a quick test, it might have lesser sensitivity and miss some positive cases (Garcia et al. 2006).

The prevalence rate of *T. gondii* in private (18.4%) and public (30.3%) zoological gardens were also different (table 1). This difference was also found primarily due to the prevalence differences of toxoplasmosis in lions. However, these results underscore the importance of understanding the factors that influence the transmission dynamics of the parasite in wild felids. The higher prevalence was found in the public sector zoos and the reasons can be traced to different managemental practices in different types of zoos. Thus, more studies are required to pinpoint these managemental differences and their association with the occurrence of *T. gondii*.

As far as the prevalence rate in human subjects associated with these zoological gardens and large felids was concerned, 48 out of the 150 samples were found positive by LAT (table 3). It indicates the existence of antibodies to this parasitic infection (Shin et al. 2009). These 48 positive samples may have been exposed to *T. gondii* at some time in their lives. *T. gondii* can infect people in several ways, including eating raw meat contaminated with the parasite's cysts (BBQ), drinking water tainted with oocysts from infected cats, or passing the parasite congenitally from an infected mother to her unborn child (Sroka et al. 2010; Sroka et al. 2018; Sroka et al. 2019). It's crucial to understand that a positive LAT test does not always indicate an infection that is active. It merely indicates that the individual has ever been exposed to the parasite and has developed immunity to it (Hassanain et al. 2011). To distinguish between acute and chronic infections and to determine the infection's present state, further tests, such as IgM and IgG antibody testing or PCR, could be required. Hence, future studies with these tests and PCR are essential to find out the active infection status in human subjects.

Our analysis of hematological parameters in lions and human subjects revealed interesting patterns. Lions that tested positive for *T. gondii* through ELISA showed an increase in monocyte count, neutrophil count, and total white blood cell count (table 4). Whereas the human subjects also showed the same findings except for lymphocytes which were also found raised in these cases. These alterations in hematological parameters suggest an inflammatory response and immune activation, which are characteristic features of *T. gondii* infection. The findings align with previous research indicating that the parasite can elicit immune responses in infected animals, leading to changes in their blood cell counts (Dubey et al. 2010). The same explanation applies to the human subjects as well. The rise in monocyte count in subjects positive for *T. gondii* is a sign that the body was activating an immune defense against the infection. These monocytes are essential to the body's fight against intracellular diseases like *T. gondii*. The immune system detects the threat posed by the parasite when it enters the body and releases monocytes to capture and eliminate the pathogen. The neutrophils also increased because these cells constitute the first line of defense against all infections. Therefore, their rise also indicates an active and recent infection in positive cases. With the exception of lymphocytes, the same findings in human subjects were reported in a recent study by (Faieq and Al-Hadraawy 2024). Lymphocytes are an essential part of the adaptive immune system that helps the body recognize and get rid of specific infections like *T. gondii*. The rise in lymphocyte counts shows that the immune system is actively detecting the parasite's existence and generating an adaptive immune response to successfully manage the infection. On the whole, the correlation found between Toxoplasma infection and hematological changes in human subjects underscores the zoonotic potential and harmful impacts of *T. gondii* (Omaima I. Mahmood 2023).

An interesting part of our research was finding out the association of blood groups with the occurrence of T. gondii infection in human subjects. There are multiple explanations for the observed trends in *T. gondii* infection rates across various blood groups. Specific antigens found in blood type A may affect how the immune system reacts to *T. gondii* infection. Expression of the glycoconjugates of the ABO blood group system and route of infection of *T. gondii* occurs in the gastrointestinal tract (GIT). This may build an association between the two (Rodrigues et al. 2011). In our study, there was a significant association between the two (Table 5). However, there are studies that either support this association like (Neamah and Abdullah 2021), or negate any

association (Rodrigues et al. 2011). So, this association may vary from age, gender, profession, and health status of the human subjects and more studies will clarify the status of this association.

In conclusion, this study reports the existence of *T. gondii* infection in large felids and associated human subjects for the first time in Pakistan. The prevalence rate obtained through LAT (21.1%) and ELISA (29.7%) were different. The highest prevalence was found in lions, followed by pumas and tigers whereas no positive case was found in leopards. Apart from species, this prevalence rate also varied in private (18.4%) and public (30.3%) zoological gardens. Moreover, human subjects associated with these zoological gardens also had positive cases (32%). Hematological analysis in lions and humans showed a significant ($p < 0.05$) rise in the WBCs, monocytes, and eosinophils. The study highlights the severity of T. gondii infection in zoological gardens of Punjab in large felids and the zoonotic potential of this parasite. It also fills the gap in research in this area, target species, and pathogen and aims to catch the attention of the researchers, veterinarians, and policy makers. Above all, it provides the foundation for future studies using more sophisticated techniques in wider areas and species.

Declaration:

The authors declare no conflict of interest.

Acknowledgement

None

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Table 1: Prevalence of *T. gondii* in large felids of at various public and private sector zoological gardens based on LATEX Agglutination test and ELISA

Species	Private sector zoological gardens						Public sector zoological gardens					
	Garden	Total samples	LATEX results		ELISA results		Garden	Total samples	LATEX results		ELISA results	
			Positive samples	Prevalence (%)	Positive samples	Prevalence (%)			Positive samples	Prevalence (%)	Positive samples	Prevalence (%)
Lions	Gujranwala	8	2	25	3	37.5	Lahore zoo	22	9	40.9	11	50
	Sialkot	5	0	0	0	0	Safari Garden	30	7	30	12	40
	Multan	7	1	14.28	3	42.9	Bahawalpur	7	1	14.3	1	14.3
	Jehlum	7	1	14.28	2	28.6	DG Khan	2	1	50	1	50
	Faislabad	5	0	0	0	0	Kamalia Park	9	1	11.11	3	33.3
	-	-	-	-	-	-	Lohi Bher	2	0	0	0	0

	Total	32	4	12.5	8	25	Total	72	19	32.9	29	40.3
Tigers	Gujranwala	5	2	40	2	40	Lahore zoo	11	2	18.18	2	18.18
	Sialkot	4	1	25	1	25	Safari Zoo Lahore	5	1	20	1	20
	Multan	12	4	25	4	25	Bahawalpur	4	1	25	1	25
	Jehlum	10	1	10	1	10	-	-	-	-	-	-
	Faislabad	4	1	25	1	25	-	-	-	-	-	-
	Total	35	9	22.8	9	22.8	Total	20	4	20	4	20
Leopards	Gujranwala	5	0	0	0	0	Lahore zoo	3	0	0	0	0
	Jehlum	2	0	0	0	0	Safari Zoo Lahore	2	0	0	0	0
	Total	7	0	0	0	0	Total	5	0	0	0	0
Pumas	Gujranwala	1	0	0	0	0	Safari Zoo Lahore	2	1	50	1	50
	Faisalabad	1	1	100	1	100	-	-	-	-	-	-
	Total	2	1	50	1	50	Total	2	1	50	1	50

Table 2: LATEX based prevalence at different concentrations of sera

Breeds	**No. of Sera Tested**	**No. (%) of Cats Tested Positive at Titer**				**OR**	**CI (95%)**	***p*-Value**
		1:16	1:64	1:128	1:256			
Lion	104	49	23	21	10	Referent		
Leopard	12	0	0	0	0	16.31	64.89 to NA	0.989
Puma	4	2	2	0	0	-1.26	3.42 to 0.9	0.22
Tiger	55	15	13	9	5	-0.09	0.85 to 0.71	0.827

Table 3: LAT-based prevalence and Wilcoxon rank sum test data of T. gondii in zoo keepers and human subjects interacting with large felids in public and private zoological garden of Punjab

Category	**Total**	**Positive**	**Prevalence (%)**	*p*- value
Keepers	125	41	32.8	0.2
Manager	5	0	0	
Meat Vendor	5	1	20	
Purchaser	5	0	0	
Supervisors	5	3	60	
Vet	5	2	40	
Grand Total	150	47	31.3	

Table 4: Wilcoxon rank sum probability test values and descriptive statistics of hematological profile of Lions and human subjects tested positive and negative for *T. gondii*

Characteristics	**Lions**			**Human subjects**		
	Positive	**Negative**	***p*-value**	**Negative**	**Positive**	***p*-value**
RBC (10^6/ul)	8.34 ± 0.79	8.39 ± 0.55	>0.9	-	-	-
WBC (10^3/ul)	22.2 ± 1.17	14.7 ± 3.3	<0.001	8.6 ± 1.4	16.7 ± 1.7	<0.001

Neutrophils (10^3/ul)	14.2 ± 0.75	1.9 ± 1.85	<0.001	62 ± 9.7	62 ± 9.8	0.6
Lymphocytes (10^3/ul)	1.99 ± 0.71	2.50 ± 0.88	0.6	38 ± 11.5	61 ± 2.4	<0.001
Monocytes (10^3/ul)	3.25 ± 0.21	0.64 ± 0.4	<0.001	7.50 ± 2.8	5.50 ± 2.76	0.14
Eosinophil (10^3/ul)	0.44 ± 0.11	0.46 ± 0.12	>0.9	3.80 ± 1.6	7.40 ± 0.98	<0.001
Hemoglobin (g/dL)	15.8 ± 2.9	15.4 ± 2.6	>0.9	-	-	-

Table 5: Association of blood groups with occurrence of T. gondii infection in human subjects

	A		**AB**		**B**		**O**	
	Negative	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Keepers	23	10	13	10	26	22	18	3
Manager	0	0	1	0	1	0	3	0
Meat Vendor	1	1	0	1	1	0	1	0
Purchaser	1	0	1	0	2		1	0
Supervisors	0	1	0	1	0	2	1	0
Vet	0	1	1	0	1	0	1	0
Grand Total	25	13 ()	16	12	31	24	25	3
Percentage (%)		34.2		42.8		43.6		10.7
χ^2	9.8449							
p value	0.02							
OR		4.33		6.25		6.45		R