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Research Article

Epidemiological Survey of *Toxoplasma gondii* and Associated Risk Factors in Ruminant Species of the Khyber Pakhtunkhwa Province of Pakistan

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Toxoplasma infection is one of the most common human parasitic diseases. During 2018-2020, in the rural areas of three districts of Pakistan, we surveyed a total of 451 animals, belonging to different asymptomatic ruminant species, to determine the prevalence *of Toxoplasma gondii* antibodies. We used ELISA assay as well as recorded some associated risk factors contributing to its transmission. IgM antibodies were detected in 17% and IgG in 13.4% of ruminant samples with the highest percentage, 10% for IgM and 8.6% for IgG in sheep. A strong significant association was found between antibodies and different species (IgM, $\chi^2 = 29.280$, $P = .000$, and IgG, $\chi^2 = 22.580$, $P = .000$), respectively. Infection with *T. gondii* seems mainly associated with different geographic features and the presence of cats in the environment, low hygiene water systems and livestock that are mostly dependent on outdoor drinking and grazing. There was no significant association between IgM and age grouping ($\chi^2 = 6.660$, $P = 0.840$ nor for IgG ($\chi^2 = 8.136$, $P = 0.43$). The results of this study may be considered the starting point to promote the awareness about parasitic infections in ruminants in Pakistan in order to prevent this infection from further spreading.

1. Introduction

Toxoplasma infection is a common human parasitic disease worldwide, and it is estimated that the prevalence in people is about 1-2 billion [1]. Toxoplasma infection is a cosmopolitan zoonotic disease caused by a coccidian protozoan, *Toxoplasma gondii*, which mostly affects humans, mammals, and birds [2]. *T. gondii* causes many severe diseases, for example, chorioretinitis, serious congenital pathologies like cerebral calcification, microcephaly, and seizer disorders. Most of the congenital infections are believed to have asymptomatic and spontaneous abortion or stillbirth [3], and in immunocompromised people, it is cause of myocarditis, pneumonitis, and encephalitis [4].

The ratio of toxoplasma infection is even common in developed countries. For example, in the USA, 400–4000

infants are born with congenital toxoplasmosis annually and record complications like bipolar disorder, schizophrenia, and obsessive-compulsive disorder that are linked to *T. gondii* [5]. Different regions of the world have different frequencies of infection because of differences in culture, eating systems, and the types of management of livestock [6, 7]. Countries where food is thoroughly cooked have less seroprevalence (10–40%) [8].

Sheep and goats, and other small ruminants, are mostly infected by *T. gondii* as compared to cattle and buffalo due to a comparatively weak immune system [9]. Toxoplasma infection is also a public health issue owing to its transmission to humans by ingestion of undercooked meat containing tissue cysts, or by consuming food or drink contaminated with oocysts, or through accidental ingestion of sporulated oocysts from the environment [10–12].

The definitive hosts of this parasite are domestic cats and various other species of wild felids, while the intermediate hosts are mammals and birds [13]. Mammal meat with infected *T. gondii* is the most common source of infection for humans [14] Meat from small ruminants have a high chance of infection especially in those countries where the consumption of sheep and goat meat is part of the culinary tradition [15–17]. Milk from infected animals is another infection route [18].

In rural areas of Pakistan, children with allergies to cow/buffalo milk are consuming small ruminant milk, such as goat. The prevalence of *T. gondii* in human population of Pakistan ranges from 12 to 28% [19, 20]. *T. gondii* rates are higher in pregnant women, 63% from Punjab, 38% from Khyber Pakhtunkhwa Province, and 48% prevalence from Azas Jammu and Kashmir [21].

Toxoplasmosis causes great economic losses in ruminants, especially in sheep, cattle, and goats by causing early embryonic death, fetal, neonatal death, abortion, stillbirth, death, and reduced flock milk production [22–24]. In the Charsada district of Pakistan, *T. gondii* is present in 17.3% of buffalo and up to 40% of sheep, as determined using a latex agglutination test [25]. *T. gondii* was recorded in sheep with 44.13% and 42.28% in goats in the district of Mardan using indirect hemagglutination antibody (IHA) test [26]. Different serological tests recorded about 41% seropositivity of *T. gondii* in sheep in the Khyber Pakhtunkhwa Province [27]. Despite the high influence of the parasite in animal and human health, the epidemiological information about toxoplasma infection is scarce in Pakistan. Therefore, the knowledge about the seroprevalence of *T. gondii* in ruminants is of interest in order to implement future strategies on public health programs and to clarify the role of livestock as a source of infection in three main districts of Khyber Pakhtunkhwa province of Pakistan.

2. Materials and Methods

2.1. Study Area. Three districts were selected for the study including Peshawar, Mardan, and Charsadda of Khyber Pakhtunkhwa province, Pakistan (Figure 1). Peshawar is located from 33°-44 to 34°-15 North latitudes and 71°-22 to 71°-42 East longitudes, Mardan which is located from 34°-05′ to 34°-32 north latitude and 71-48 to 72°-25 East latitude having total of 1632 km2 area, and finally Charsadda which is located between 34-030 and 34-380 north latitudes and 71-280 and 71-530 east longitudes. There is a temperate climate. In summer, the mean maximum temperature in Peshawar district exceeds 40°, while the mean minimum temperature is 25°C. During winter, the maximum is 18.35°C, while the mean minimum temperature is 4°C. The average annual precipitation level is about 400 millimeters, while the highest annual rainfall recorded of 904.5 millimeters, and the relative humidity varies from 46% to 76% from June through August. In Mardan, the temperature reaches its maximum in the month of June, i.e., 41.5°C. The mean minimum temperature in the month of January is 2.1°C. In August, the maximum rainfall is 125.85 mm. In December, the maximum humidity has recorded about 73.33%. The tract is generally wet, moist, and humid and this could be due to irrigation and cultivation, whereas district Charsadda has an annual average rainfall of 16.5 cm [28].

2.2. Blood Specimens. The present study was carried out from 2018 to 2020 in the rural area of three districts, Mardan-Charsadda and Peshawar of Pakistan, where we surveyed a total of $n = 451$ formers. Before sampling, we arranged a meeting with the local specialist officers for livestock in each district. Random blood samples were taken from ruminants in different. The majority of samples were from sheep (167), goat (126), cow (100), and buffalo (58) (Table 1). About 2 mL of blood was collected from the jugular vein through a sterile syringe from each ruminant. The serum was separated and stored at -20°C until used. Sera were extracted from blood samples by centrifugation at 2000 × g for 10 min.

2.3. Questionnaire Survey. Moreover, we also prepared a questionnaire in collaboration with local specialists. We surveyed all possible farmers from whom we collected samples. The questionnaire was available in Urdu, a national language of Pakistan. The purpose of the survey was to find the association of different risk factors with the *T. gondii* transmission in ruminant. Respondents were interviewed for questions such as type of species they have, location, hygienic status, cat in the vicinity, drinking water either indoor or outdoor, and were livestock free-living or caged. The following investigation received ethical approval by the farmers.

2.4. Enzyme-Linked Immunosorbent Assay. ELISA kits (Bio-ELISA Toxo-IgM and IgG kits) were used for Ruminant Serum Toxoplasmosis and the detection of anti-*T. gondii* antibodies (purchased from Biokit, S.S. Barcelona Spain) and used according to the supplier's instructions.

2.5. Statistical Analysis. We first choose IgM as a dependent variable while keep gathered gender, age, location, and others as independent variables in order to evaluate if there is a significant association between these variables and possible antibodies for *T. gondii* in ruminant species. The same process was repeated for IgG antibody. Statistical analysis of frequencies was calculated using the chi-square test (χ^2). We also run a binary logistic regression while keeping the IgM and IgG antibodies as dependent variables separately to evaluate the possible risk factors such as hygienic system, water

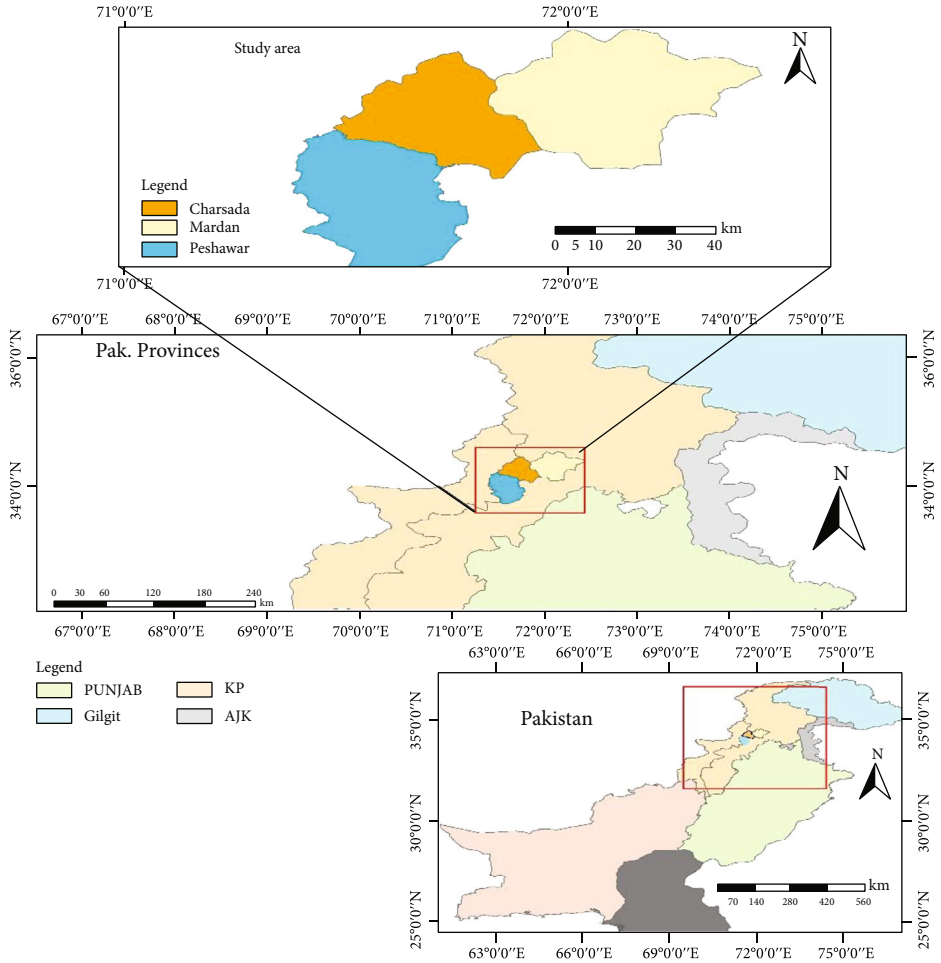

Figure 1: Different study areas in the Khyber Pakhtunkhwa Province of Pakistan.

Table 1: Total number of sera examined along with antibody positivity from different species in the study area.

Species	Total samples	IgM positive	IgG positive
Sheep	167	49	39
Goat	126	13	10
Cow	100	10	8
Buffalo	58	5	4
Total	451	77	61

intake, cat in the vicinity, and living lifestyle. All the analyses were run through SPSS version 25 for windows, and the differences were considered statistically significant at <0.05.

3. Results

All samples were tested for *T. gondii* using ELISA commercial kits. There was some variation among both the antibodies in overall samples, for example, the IgM antibodies were found in 17.0% and IgG in 13.4% of the total collected samples. For that location, the seropositivity of *T. gondii* for IgM and IgG was 10.8% and 10.1% at Mardan and 3.7% and 2.2% at Peshawar, and only 2.4% and 1.1% was recorded at Charsadda district, respectively. Therefore, the places were remained significant for IgM and IgG antibodies ($\chi^2 = 101.181$, $P = .000$, $\chi^2 = 22.804$, $P = .000$), respectively. Among all ruminants, the youngest age group, up to one year, was most infected (11% for IgM and 9.5% for IgG), and other age groups remained less infected for both antibodies. However, there was no significant association we recorded nether for IgM ($\chi^2 = 6.660$, $P = 0.840$ nor for IgG ($\chi^2 = 8.136$, $P = 0.43$) in different age groups, respectively.

For individual species, we recorded 10% of sheep with IgM positive antibodies and 8.6% for IgG. Similarly, the antibodies in cow with the manner that the IgM was higher with 2.8% while only 2.2% of IgG were found. However, in goat, IgM was recorded lower with 2% and slightly higher IgG with 2.2% was noted. In buffalo, the IgM was again higher with 1.1% and lower IgG was recorded with 0.8%. There was a strong significant association between different species and positivity for both IgM and IgG ($\chi^2 = 29.280$, $P = .000$, and $\chi^2 = 22.580$, $P = .000$), respectively. Tables 2 and 3.

Moreover, binary logistic regression revealed that cats in the vicinity, water intake, and hygienic system were strongly associated with the transmission of *T. gondii* in ruminants. Association of these risk factors with different antibodies such as IgM and IgG is given in Tables 4 and 5.

Table 2: Possible risk factors associated with higher IgM antibody of *Toxoplasma gondii* prevalence in Ruminants using chi-square analysis.

Variables	Categories	Total	IgM positive (%)	Chi-square test value	*P* value
Gender	Male	190	34 (17)	.157	.692
	Female	261	43 (16.4)		
Age	Up to one year	242	51 (21)	6.660	0.84
	One to two years	166	19 (11.4)		
	Three years and above	42	7 (16)		
Location	Peshawar	135	17 (12.5)	101.181	0.006
	Mardan	213	49 (23)		
	Charsadda	103	11 (10.6)		
Species	Cow	100	13 (13)	29.280	.000
	Sheep	167	49 (29)		
	Goat	126	10 (7.9)		
	Buffalo	58	5 (8.6)		
Cat in the vicinity	Yes	240	54 (22.5)	10.671	.001
	No	211	23 (10.9)		
Hygienic system	High	74	6 (8)	13.644	.001
	Moderate	179	23 (12.8)		
	Low	198	48 (24)		
Living	Free	83	10 (12)	1.814	.178
	Caged	368	67 (18)		
Water intake	Indoor	312	38 (12)	17.446	.000
	Outdoor	138	39 (28)		

Table 3: Possible risk factors associated with higher IgG antibodies of *Toxoplasma gondii* prevalence in ruminants using chi-square analysis.

Variables	Categories	Total	IgG positive (%)	Chi-square test value	*P* value
Gender	Male	190	26 (15)	.007	.933
	Female	261	35 (13.4)		
Age	One to five months	242	43 (17.7)	8.136	0.43
	Five to eighteen months	166	14 (8.4)		
	Eighteen and above	42	4 (9.5)		
Location	Peshawar	135	10 (7.4)	22.804	.000
	Mardan	213	46 (21.5)		
	Charsadda	103	5 (4.8)		
Species	Cow	100	10 (10)	22.580	.000
	Sheep	167	39 (23)		
	Goat	126	8 (6)		
	Buffalo	58	4 (6)		
Cat in the vicinity	Yes	240	48 (20)	18.386	.000
	No	211	13 (6)		
Hygienic system	High	74	3 (4)	11.958	.003
	Moderate	179	20 (11)		
	Low	198	38 (19)		
Living	Free	83	10 (12)	.190	.663
	Caged	368	51 (13.8)		
Water intake	Indoor	312	27 (8.6)	20.861	.000
	Outdoor	138	34 (24.6)		

Table 4: Associated possible risk factors with IgM antibody of *Toxoplasma gondii* prevalence in ruminants using binary logistic model.

Variable	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B)	
							Lower	Upper
Hygienic system	-.716	.201	12.641	1	.000	.489	.329	.725
Water intake	-1.006	.266	14.311	1	.000	.366	.217	.616
Cat in the vicinity	.771	.279	7.627	1	.006	2.162	1.251	3.736
Living lifestyle	-.605	.380	2.535	1	.111	.546	.259	1.150
Constant	4.711	1.084	18.879	1	.000	111.214		

Table 5: Association of possible risk factors with IgG antibody of *Toxoplasma gondii* prevalence in ruminants using binary logistic model.

Variable	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B)	
							Lower	Upper
Hygienic system	-.752	.230	10.733	1	.001	.471	.301	.739
Water intake	-1.171	.294	15.871	1	.000	.310	.174	.552
Cat living in the vicinity	1.258	.338	13.845	1	.000	3.517	1.813	6.821
Living lifestyle	-.236	.395	.357	1	.550	.790	.364	1.714
Constant	4.038	1.169	11.938	1	.001	56.722		

4. Discussion

In order to evaluate the seroprevalence of *T. gondii* in ruminants, we investigated three districts of Pakistan. Infection caused by *T. gondii* is a serious threat to humans due to its complicated nature and transmission, while also has influence on economic growth as it is responsible for negative impacts on reproductive efficiency in farm ruminants worldwide [29, 30]. Since observation of cyst directly from the tissue is difficult, we used serological techniques that appear to identify the presence of *T. gondii* [31].

The use of ELISA has been widely documented in epidemiological studies for the detection *T. gondii* antibodies in ruminants [32, 33]. Our study found a seroprevalence for *T. gondii* of about 17% for IgM and 13.4% for IgG. The antibodies were not always aligned, and this could be due to the serum IgG and IgM immune assays that are used to differentiate chronic and acute infection and population surveillance of *T. gondii* [34]. It is pertinent that the specificity and sensitivity of different serological tests used to detect *T. gondii* antibodies in cattle sera has not been determined, because viable *T. gondii* has been rarely isolated [15].

Our study show contradiction with reports from Spain (41%) [35], Serbia (76.3% in cattle, and 84.5% in sheep) [36], Greece (39.72%) [37], Italy (92%) [38], and Brazil (71%) in cattle [39], where there was a high proportion of ruminants exposed to the parasite. Nevertheless, the prevalence rate recorded in the current study was higher in respect to data from Lara State, Venezuela (6.3%) for goats [40], China (4.4%) for sheep [41], and India (3.2%) for goats [42]. Thus, the present study is consistent with the idea that different cultures, foods, and geography are important factors regulating the spread of *T. gondii* in different regions [43]. Differences in prevalence rates between countries may also be due to different husbandry methods used in these regions [35, 44]. Further, it can be related to differences in techniques used in each study to monitor the *T. gondii* antibody [34]. Perhaps, the climate is another important factor contributing to this [45].

We also recorded that the antibodies of *T. gondii* were different in different species. For example, sheep was the most affected animal species by *T. gondii* from all three districts, and this corroborates with a study recently reported from Magnolia [46] with prevalence of 34.8% in sheep and 32% in goats, from Ghana [47] with 33.2% in sheep and 26.8% in goat, and from Greece [48] with 48.6% in sheep ad 30.7% in goat using ELISA. Thus, among different species, sheep is more likely to be infected. Nevertheless, our study show contrasts where *T. gondii* in sheep was 11.2% compared to 25.4% in goats and also of a low prevalence in adult sheep and goats [49]. Consequently, the result of our and other studies revealed that sheep and goats are the species most often infected with *T. gondii*; however, cattle and buffaloes are considered to have lower rates of infection given the fact large ruminants are more resistant to *T. gondii* [9, 50].

Environment, geography, presence of cats, the rearing system, and age all have vital as major risk factors in the distribution of *T. gondii* infection [7, 51, 52]. In this study, the high prevalence was observed from the District Mardan when compared to District Peshawar. The high right in District Mardan might be due to the high density of ruminants [53], and it may be due to cats in the vicinity [51]. Cat populations are definitive hosts of *T. gondii*, which may impact and control the spread of pathogens in the environment [54, 55], and a dry climate is another key fact that influences the sporulation of oocysts in the environment [56]. The current study found that all three different districts had different hygienic standards, drinking water type, and type of confinement, ecological conditions, and annual rainfall, changes in the habits of consumers [57, 58].

As an animal ages, its cumulative likelihood for exposure increases. Given the fact, the age of animals plays an important role in the prevalence rate of *T. gondii* infection in animals [26, 49]. Among both, the sheep and goats from the group up to one year were highly seropositive as compared to the 1-2 years age group. This could due to the age groups one year and less had not properly maternal passive immunity remaining. In the present study, no correlation was found among sex groups in ruminants, while there were in water drinking places such as we recorded higher infection in animal drinking water outdoor than indoor. Therefore, this could be considered a risk factor for ruminants while drinking outdoor.

5. Conclusions

The seropositivity of *T. gondii* was higher in sheep, and Mardan was found to be somewhat affected among the three districts. This is what environment and geography play an important role and are considered major risk factors in the distribution of *T. gondii* infection. This assumed that the given regions have some percentage of infection, however, lower from other parts of Pakistan. Therefore, there is advance screening performance that needs to investigate the disease continuously in order to prevent such a spreading in the regions. Also, there needs to be more education for farmers as well as proper screening at slaughterhouses which is essential for the prevention of *T. gondii* in humans.

Data Availability

The data supporting he findings of this study are available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

All authors have equal contribution in the study.

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