Original Article

Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan

Nisar Ahmad, Mazhar Qayyum

Department of Zoology, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan

Abstract

Introduction: Toxoplasmosis is a widespread zoonosis that causes significant economic losses due to abortions and other developmental disabilities in livestock animals. The objectives of the current study were to determine the prevalence and associated risk factors of *Toxoplasma gondii* infection in cattle and buffaloes in northern parts of Punjab, Pakistan, where no such work previously existed.

Methodology:Serum samples obtained from 400 cattle and 422 buffaloes present on different private and government-owned farms were tested for anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM antibodies by using enzyme linked immunosorbent assay (ELISA). Additional data, including water sources, hygienic status at the farm, management practices, size of the herd, and presence of cats in the vicinity of the farm were obtained using a questionnaire in surveys and interviews.

Results: The overall prevalence of infection was 19.75% (79/400) in cattle and 15.16% (64/422) in buffaloes. IgG antibodies were found in 75 (18.75%) cattle and 58 (13.74%) buffaloes, while IgM antibodies were found in 9 (2.25%) cattle and 10 (2.37%) buffaloes. Seroprevalence was significantly higher in females and older animals of both species. Seroprevalence was found to be associated with poor hygienic conditions, extensive management practices, and presence of cats in the surrounding areas. No difference of seroprevalence was observed with respect to different breeds, location, water source, and herd size.

Conclusion: The present study found that *T. gondii* is prevalent in large ruminants in northern Punjab, and may have important implications for the livestock industry and public health.

Key words: *Toxoplasma gondii*, cattle, buffaloes, Punjab, Pakistan, IgG, IgM.

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Introduction

Toxoplasmosis is a cosmopolitan zoonotic infection caused by a coccidian protozoan *Toxoplasma gondii*, which affects a wide range of hosts including humans, mammals, and birds [1]. The main route of transmission to livestock animals is through ingestion of sporulated oocysts. Wild and domestic cats are the principal reservoir where these oocysts are produced during the sexual stage of the lifecycle. The infection is considered nonexistent or rare in the absence of cats [2]. Livestock animals, when infected during pregnancy, suffer from parasitemia, which can infect the placenta and fetus and ultimately result in fetal resorption, miscarriage, death, or mummification. Often the young ones are born, but die shortly after birth [3]. In addition to being hazardous to livestock animals, the *T. gondii* infection is also important due to its zoonotic implications [4]. Congenital abnormalities in humans, such as microcephaly, hydrocephaly, chorioretinitis, convulsion, cerebral calcification, epilepsy, blindness, deafness, and mental retardation may occur if the mother acquires infection during pregnancy [5]. In addition to congenital anomalies, *T. gondii* also causes severe neuropathologic infections in immunocompromised hosts, such as AIDS and cancer patients receiving chemotherapy [6].

Although *T. gondii* can multiply in bovine tissue, it is usually eliminated; however, beef can be considered to be a minor source of *T. gondii* infection [7]. A study from Turkey found *T. gondii* tissue cysts in frozen buffalo meat [8]. Cattle and buffaloes are an important source of meat and milk for Pakistani people. The risk of toxoplasmosis is high in countries like Pakistan due to a lack of modern farming techniques. Several studies conducted in different parts of the world, including those conducted in Morocco [9], Thailand [10], Iraq [11], Iran [12], and Trinidad [13], have reported a prevalence of toxoplasmosis in cattle and buffaloes.

Keeping in view the significance of the infection, there is a substantial need to regularly monitor the

infection rate of toxoplasmosis in different livestock species [14]. Despite being important for livestock animals and humans, the epidemiological information about this parasite is scarce in Pakistan. Therefore, the present study was designed to determine the prevalence of toxoplasmosis and associated risk factors contributing to its transmission in cattle and buffaloes in four districts of the Pothwar Plateau region in the northern parts of Punjab province.

Methodology

Study area

The present study was conducted in four districts of the Pothwar Plateau region, including Rawalpindi, Jhelum, Chakwal, and Attock. Pothwar Plateau is located between 32°30′ to 34° northern latitudes and 71° 45′ to 73° 45′ eastern latitudes in the northern parts of Punjab province. Consisting of a population of 7.5 million individuals, the plateau covers an expanse of 23,160 square kilometers. The area is usually arid, and agriculture mainly depends on rainfall, which varies between 350 mm to 500 mm annually. The average temperature is around 7.9°C in winter and 30.6°C in summer.

Sampling and serology

Cattle and buffaloes included in the study were being reared in the study area at various government-owned and private farms. The data were collected from 15 farms located in the study area. A total of 400 cattle and 422 buffalo samples were tested for the infection between January and December 2012. Three breeds of cattle were included in the study (Desi, Red Sindhi, and Jersey), while all buffaloes were of the Nili Ravi breed. The blood samples were collected in 5 mL vacutainers without anticoagulants and were promptly transported to the Parasitology Laboratory, PMAS-Arid Agriculture University, Rawalpindi, where they were centrifuged at 3,000 rpm for 15 minutes to obtain serum. Age was either estimated based on dental information or obtained from animal records.

Questionnaire surveys

Additional information regarding source of water, presence of cats, herd size, hygienic conditions at the farm, and farm management was obtained using a questionnaire in a survey of farms and interviews of the persons working on the farms. Hygienic condition was categorized as high, moderate, and low. *High* referred to farm that were cleaned every day, *moderate* referred to farms that were cleaned after two days, and *low* referred to farms that were cleaned after more than two days. Indoor water sources included different water troughs present inside the farm, while outdoor water sources included water from running springs or ponds. Animals were placed in three categories based on the management system of the farm to which they belonged. In intensive management systems, animals are kept in large confinements, with large amounts of input in the form of feeding and labor. Animals are normally not sent to pastures for grazing. Contrarily, extensive management systems provide very low input, as animals are sent for grazing in pastures. In semi-intensive management systems, animals are supplied with intensive feeding, and are also allowed to graze in natural areas (Table 1).

Collection of climatic data

To determine the seasonal distribution of toxoplasmosis, positive results from acute disease phases (IgM-positive cases) were compared with the climatic data. Daily climatic data (maximum temperature, minimum temperature, rainfall, and relative humidity) were collected from the meteorological department of Pakistan.

Enzyme-linked immunosorbent assay

Commercial ELISA Kits (ID Screen Toxoplasmosis Indirect, ID-VET Company, Grabels, France) and Toxo IgM ELISA Neobiolab were used for the detection of IgG and IgM antibodies, respectively. Results were interpreted according to the manufacturer's instructions.

Statistical analysis

Univariate and multivariate logistic regression models were applied to determine the association between toxoplasmosis and various risk factors (*i.e.*, locality, sex, age, breed, source of water, presence of cats, herd size, hygienic conditions on farms and farm management) using SPSS version 11.5. Crude and adjusted odds ratios were calculated by comparing non-infected and infected animals.

Ethical issues

The project was approved by the ethical committee of Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi.

Table 1. Univariate and multivariate analysis of risk factors associated with *T. gondii* infection in cattle and buffaloes

Risk Factors	Categories	Infected (%)	Univariate		Multivariate	
			OR (95% CI)	P value	AOR (95% CI)	P value
Species	Buffaloes*	64/422 (15.16)	1		NC	
	Cattle	79/400 (19.75)	1.38 (0.96–1.98)	0.08		
Breeds	Nili Ravi* (buffaloes)	64/422 (15.16)	1		NC	
	Desi (cattle)	36/192 (18.75)	1.29 (0.82–2.02)	0.26		
	Red Sindhi (cattle)	19/98 (19.38)	1.35 (0.76–2.37)	0.30		
	Jersey (cattle)	24/110 (21.82)	1.56 (0.92–2.64)	0.09		
Gender	Male*	27/242 (11.16)	1		1	
	Female	116/580 (20)	1.99 (1.27–3.12)	**0.002**	1.74 (1.52–2.14)	**0.021**
Locations	Rawalpindi*	30/211 (14.22)	1		NC	
	Attock	38/191 (19.9)	1.50 (0.89–2.53)	0.13		
	Chakwal	39/231 (16.88)	1.23 (0.73–2.06)	0.44		
	Jhelum	36/189 (19.05)	1.42 (0.84–2.41)	0.19		
Age (months)	<24*	16/195 (8.21)	1		1	
	25–48	63/369 (17.07)	2.30 (1.29–4.11)	**0.003**	1.94 (1.69–2.22)	**0.018**
	>48	64/258 (24.81)	3.69 (2.06–6.62)	**<0.001**	2.99 (2.68–3.42)	**<0.001**
Water source	Indoor*	75/440 (17.04)	1		NC	
	Outdoor	88/402 (21.90)	1.36 (0.97–1.92)	0.08		
Hygienic status	High*	35/299 (11.71)	1		1	
	Moderate	47/296 (15.88)	1.42 (0.89–2.28)	0.14	0.92 (0.58–1.42)	0.24
	Low	61/227 (26.87)	2.77 (1.75–4.39)	**<0.001**	1.72 (1.47–2.08)	**<0.01**
Farm management	Intensive*	44/396 (11.11)	1		1	
	Semi–intensive	63/257 (24.51)	2.60 (1.70–3.97)	**<0.001**	2.12 (1.68–3.12)	**<0.001**
	Extensive	46/169 (27.21)	2.99 (1.89–4.75)	**<0.001**	2.72 (1.64–3.34)	**<0.001**
Cats in vicinity	No*	41/332 (12.35)	1			
	Yes	102/490 (20.82)	2.65 (1.66–4.25)	**<0.001**	1.59 (1.11–2.12)	**0.004**
Size of herd	<10*	17/110 (15.45)	1		NC	
	11–30	55/317 (17.35)	1.15 (0.63–2.08)	0.65		
	31–50	49/259 (18.92)	1.28 (0.70–2.33)	0.48		
	>50	22/136 (16.17)	1.06 (0.53–2.10)	0.23		

* reference category, OR: odds ratio, AOR: adjusted odds ratio; CI: confidence interval; NC: not calculated

Table 2. IgG and IgM positive or negative cattle and buffaloes

Categories	Cattle		Buffaloes	
	N	%	N	%
IgG (-) and IgM (-)	321	80.25	358	84.83
IgG (+) and IgM (-)	70	17.50	54	12.80
IgG (-) and IgM (+)	4	1.00	6	1.42
IgG (+) and IgM (+)	5	1.25	4	0.94
Total IgG (+)	75	18.75	58	13.74
Total IgM (+)	9	2.25	10	2.37
Overall positives	79	19.75	64	15.17
Total samples	400		422	

Results

In a total of 400 cattle and 422 buffaloes, 79 (19.75%) cattle and 64 (15.17%) buffaloes were found to be seropositive. The overall positive samples included both IgG and IgM positive cases. IgG antibodies were found in 75 (18.75%) cattle and 58 (13.74%) buffaloes, while IgM antibodies were found in 9 (2.25%) cattle and 10 (2.37%) buffaloes. Five cattle and four buffaloes were positive for both IgG and IgM antibodies (Table 2). Despite the high percentage of infection in cattle, there was no significant difference in infection between the two species. All three breeds of cattle did not differ significantly with respect to prevalence of the infection. Both species showed a similar trend of seroprevalence with respect to different risk factors. Prevalence was higher in female animals of both species and was also higher in older age groups.

No significant differences of acute or chronic infection in different localities of the study area were observed. The number of seropositive animals was significantly higher in farms with poor hygienic conditions. Overall seroprevalence was also significantly higher in animals reared in extensive management systems. The presence of cats in the vicinity of the farm also stood out as a significant contributing factor for the spread of the infection. Different water sources and herd sizes did not show significant variations in seroprevalence (Table 2).

The comparison of climatic data collected during different months and acute infection (IgM positive cases) in animals during the same period did not indicate significant fluctuations. Nevertheless, acute infection was found most frequently in the monsoon season (July to September), with high rainfall and moist and warm conditions (Figure 1). This trend was not observed when chronic infection was compared with climatic data.

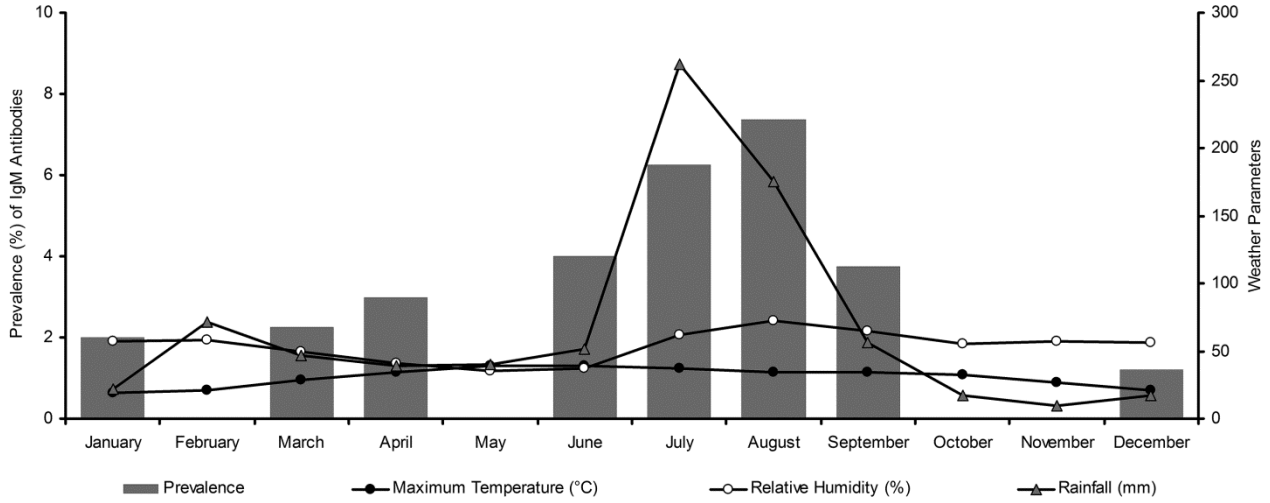
Discussion

There has been increasing interest to determine the prevalence of toxoplasmosis in food animals because of their role in the transmission of the infection to humans by ingestion of meat and milk [15]. Demonstration of IgM and IgG antibodies is a major diagnostic tool for toxoplasmosis. Detection of IgM antibodies is more important, as they appear shortly after infection; however, they diminish in quick time. IgG antibodies persist for the entire life span. Both IgM and IgG antibodies can be detected by ELISA, immunofluorescence antibody test (IFAT), and microscopic agglutination test (MAT).

Very few studies have been conducted in all of Pakistan to determine the prevalence of *T. gondii* infection in food animals. The risk factors contributing to the prevalence of the infection have never previously been studied in a country where livestock contributes 12% of the gross domestic product. We therefore investigated the prevalence and associated risk factors of toxoplasmosis in cattle and buffaloes, which are an important source of meat and milk in the country.

There are limited reports worldwide on the seroprevalence of *T. gondii* infection in cattle and buffaloes [16]. The observed seroprevalence in cattle in the current study was lower compared to 25%

Figure 1. Correlation between weather parameters and prevalence of acute infection in cattle and buffaloes in northern Punjab, Pakistan



seroprevalence reported in southwestern parts of Pakistan [17], 22.3% in Thailand [10], 93.5% in Turkey [18], and 71.3% in Iran [19]. Comparatively, lower seroprevalence rates of 4.8%, 10.75%, 2.60%, 3.86%, and 5.70% have been found in Iran [20], Egypt [21], Malaysia [22], Korea [23], and China [24], respectively. Varying rates of seroprevalence in buffaloes have also been reported by various researchers in several countries, including 20.4% in Afghanistan [25], 3% in Vietnam [26], 8.8% in Iran [12], 30% in Iraq [11], 2.9% in India [27], and 7.8% in Trinidad [13]. Various risk factors (age, sex, breed, diagnostic test, and climatic variations) may contribute to the differences in seroprevalence in the current study and other studies of the world.

We did not find any significant difference of seroprevalence in both species and their breeds or in different localities of the study area. This suggests an equal susceptibility to the infection in both species and their breeds in the entire study area.

Previously, it has been reported that female animals are more likely to get protozoan infections as compared to males [28]. This hypothesis was strengthened as we observed significantly higher seroprevalence of *T. gondii* infection in female animals of both species and breeds as compared to males. These differences may be attributed to the reduction in immunity in female animals during pregnancy and lactation [29,30]. We also observed 75% of acute cases of toxoplasmosis in pregnant and lactating females. Similar findings have also been reported previously in cattle [18] and water buffaloes [31]. An increase in both chronic and acute phases of toxoplasmosis was observed in older animals. The higher seroprevalence found in animals older than four years of age may be due to their continuous exposure to risk factors, as the infection, once acquired, renders animals seropositive for life. The increase in the acute phase of the disease in older animals is due to the reduction of immunity with age. The increase in seroprevalence in older animals has also been reported previously in cattle and buffaloes [24,27]. The lack of any significant difference in seroprevalence in different localities may be attributed to the similarity in climatic conditions and management systems.

The farms with poor hygienic conditions also showed a significantly higher number of seropositive animals. This is due to the fact that proper cleaning at farms reduces the risk of contamination of food and water with oocysts, which minimizes the risk of toxoplasmosis [32]. The number of positive animals was also higher in farms that raised animals extensively or semi-intensively as compared to intensively managed farms. Animals fed extensively have a greater risk of coming in contact with oocysts present in cat feces, which enhances the risk of acquiring *T. gondii* infection [33]. A similar trend has also been reported in sheep in previous studies [34,35,36]

The current study also showed a higher number of positive animals in farms where cats were present in the vicinity as compared to farms where no cats were present. These results are due to the fact that the presence of, and close contact with, cats is a very important factor in the epidemiology of toxoplasmosis. Cats shed millions of oocysts in the environment, which could be ingested by animals along with food and water [37]. Direct contact with cats is less important than the presence of cats in the vicinity because cats' shedding of oocysts is the source of infection, rather than direct contact with cats [37]. Other studies have also found a significant association of the prevalence of toxoplasmosis with the presence of cats in the vicinity. A study from Poland reported that the presence of free-roaming cats is an important risk factor for the transmission of the infection in goats [38]. Similar findings in sheep and goats have also been reported in Ghana and Brazil [34,39]. Seroprevalence was significantly higher in farms where cats had access to the water and feed of the livestock. A significant increase in seroprevalence in cattle due to the presence of stray cats in the vicinity was also reported more recently in Malaysia and Brazil [22,40]. In the present study, the lack of association between seroprevalence and herd size suggests an absence of horizontal transmission between animals.

The high prevalence of acute toxoplasmosis observed between July and September may be attributed to the higher monsoon rainfalls. Moist conditions, high rainfall, and warm temperature favor the survival of oocysts in an environment [41] (Figure 1). The time period between July and September is ideal for the survival of oocysts in Pothwar region due to warm, moist temperatures and high percentages of relative humidity.

Abortions and neonatal deaths in livestock animals are important problems worldwide [7]. *T. gondii* is the most common cause of abortion in livestock animals in various countries [42]. Toxoplasmosis rarely shows any sign and symptoms; it is very difficult, therefore, to diagnose clinically [43]. *T. gondii* can only pass to the fetus if the infection is acquired during pregnancy. Protection during this short period of risk time may

reduce the risk of abortion and neonatal mortalities in livestock species.

Conclusions

The results of the present study not only found that toxoplasmosis is a widespread infection in large ruminants in the region, but also determined the different risk factors contributing to its transmission. The presence of cats in the vicinity, extensive management systems, and poor hygienic conditions stood out as the main risk factors. This information may serve as a road map for building control strategies to minimize the risk of this important zoonosis to farm animals and humans.

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Corresponding author
Nisar Ahmad (PhD Scholar)
Department of Zoology,
Pir Mehr Ali Shah, Arid Agriculture University
Shamsabad, Rawalpindi, Pakistan
Phone: +92 345 5929549
Email: nisarahmed11@gmail.com

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