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Seroprevalence and Spatial Distribution of Toxoplasmosis in Relation to Various Risk Factors in Small Ruminants of Punjab, India

Deeksha Pandit[1], Mandeep Singh Bal[1], Paramjit Kaur[1], L.D. Singla[1], Vishal Mahajan[2], R.K. Setia[3]

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ABSTRACT

Background: *Toxoplasma gondii* is an obligatory apicomplexan, intracellular, ubiquitous, eurykaryotic zoonotic pathogen that infects all warm blooded animals and recognized worldwide as one of major causes of infectious abortions and reproductive failure in small ruminants.

Methods: In the present study, 823 small ruminants (245 sheep, 578 goats) evaluated for *T. gondii* infection by ELISA to explore seroprevalence and spatial distribution of toxoplasmosis from different agro-climatic zones of Punjab.

Result: A high seroprevalence (40.58%) of *T. gondii* recorded in small ruminants being higher in sheep (42.85%) than goat (39.60%). Seropositivity of toxoplasmosis in sheep and goat with history of abortion was found to be 63.80 and 50.00%, respectively. Goats with the history of abortion were 1.72 times more at risk (CI =1.15-2.56) to *T. gondii* infection as compared to animals without the history of abortion (CI =0.39-0.86). Similarly, sheep with the history of abortion were 2.74 times more at risk (CI =1.31-5.71) to toxoplasmosis as compared to sheep without abortion history (CI= 0.17-0.76). Goat and sheep farms with frequent access to cats were 2.03 times (CI =1.05-3.90) and 4.04 times (CI =1.78-9.14) more at risk, as compared to farms with limited cats access. Spatial distribution and predictive seroprevalence analysis indicate possible risk of toxoplasmosis in whole of the state with higher possibility of disease in agroclimatic zones III, IV and V.

Key words: ELISA, Risk factors, Seroprevalence, Small ruminants, Spatial distribution, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii,* is a cosmopolitan zoonosis affecting the warm blooded animals including man as intermediate hosts and cat as a definitive host (Celik *et al*. 2018). Medical importance of toxoplasmosis was first recognized in 1939 after diagnosed in a congenitally infected baby (Wolf, 1939). Among the three infective stages, tachyzoites are transmitted vertically through placenta, blood transfusion or milk contamination, bradyzoites by ingestion of meat from infected animal and the sporozoites by feed or water contaminated with sporulation of oocysts (Dubey and Thulliez, 1993, Singla *et al.,* 2014).

Toxoplasma gondii is recognized worldwide as one of major causes of infectious abortions and reproductive failure in small ruminants (Dubey *et al.,* 2011, Hussein *et al.,* 2001). Seroprevalence of 25.3 and 30.3% for sheep and goats respectively has been reported from northern India (Chhabra *et al.,* 1985) with scanty reports on exposure of ruminants to *T. gondii* infection from Punjab state (Sharma *et al.*, 2008, Kalambhe *et al.,* 2017).

Toxoplasmosis can be diagnosed by direct smear, immuno-histochemistry, sero-molecular techniques, however, for epidemiological survey immunodiagnostic tests are more reliable due to the non-availability of detectable stages in secretions, excretion and blood. Among the immunological tests, ELISA is widely employed to assess the exposure of small ruminants to *Toxoplasma* infection as it bears high sensitivity, specificity and reliability (Puije *et al.*, 2000).

As toxoplasmosis results in still birth and abortions in small ruminants causing severe economic losses to owners (Hussein *et al.,* 2001), 3.27 and 1.28 lakh of goat and sheep population respectively of Punjab (Anonymous, 2012) is at risk to exposure of toxoplasmosis. Hence the aim of study was to explore the seroprevalence and spatial distribution of toxoplasmosis in small ruminants in the region.

[1]Department of Veterinary Parasitology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 001, Punjab, India. [2]Animal Disease Research Centre, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 001, Punjab, India. [3]Punjab Remote Sensing Centre, The Punjab Agricultural University Campus, Ludhiana-141 001, Punjab, India.

Corresponding Author: Vishal Mahajan, Animal Disease Research Centre, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 001, Punjab, India. Email: mahajanv17@gmail.com

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MATERIALS AND METHODS

Study area

Study was conducted in five different agro-climatic zones of Punjab state ZONE I (sub-mountain undulating zone / Western Himalayas sub humid), ZONE II (undulating plain zone /Northern Plain Zone Dry sub humid), ZONE III (central plain zone/ Northern Plain Zone sub arid), ZONE IV (Western Plain Zone) and ZONE V (Western Zone) (Gulati *et al.,* 2017). Research work was conducted in Department of Veterinary Parasitology, Animal Disease Research Centre, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana from June 2018 to June 2019.

Sample collection

Sera samples (823) from goat (n=578) and sheep (n=245) collected from different villages selected by using survey tool box software, falling under different districts representing all the agro climatic zones of Punjab state. The study was approved by Institutional Animal Ethics Committee (protocol number GADVASU/2018/IAEC/46/04 (Reference No. IAEC/ 2018/1153-1188 Dated 28.09.2018).

Serological assay

Serum samples were evaluated by ELISA to detect anti *Toxoplasma* IgG antibodies using commercial ELISA kit (Prio CHECK *Toxoplasma* Ab SR Kit) according to manufacturer's instructions.

Risk factors evaluation

Based on information obtained by animal owners at the time of sample collection, various risk factors (species, agroclimatic zones, age, history of abortion, access of cats to farm, grazing managements and gender) in relation to seroprevalence of *T. gondii* in small ruminants were evaluated.

Spatial distribution

In order to predict the disease at un-sampled locations, the data of each district was linked with district boundary in GIS and the Inverse Distance Weighted (IDW) method in Arc GIS 10.4 was used for spatial prediction of disease (Sharma *et al.* 2019). Inverse Distance Weighted is a type of deterministic method for multivariate interpolation with a known scattered set of points. The assigned values to unknown points were calculated with a weighted average of the values available at the known points.

Statistical analysis of data

Statistical analysis of the data was done by chi-square test to evaluate the risk factors associated with the prevalence of toxoplasmosis using SPSS 16.0 software for windows.

RESULTS AND DISCUSSION

Seroprevalence and spatial distribution

Results revealed an overall seropostivity as 40.58% (n =334) for *T. gondii* antibodies in small ruminants in Punjab state. Spatial distribution of data showed highest prevalence in district Barnala (54.80%) and lowest in S.B.S. Nagar (11.76%) (Table 1, Fig 1). Species wise highest seroprevalence for *T. gondii* in goats and sheep was seen in districts Sangrur (58.49%) (Table 1, Fig 2) and Fazilka (100%) (Table 1, Fig 3) respectively.

Table 1: Seroprevalence of *Toxoplasma gondii* in small ruminants of Punjab state.

Districts	Samples Collected			Total Positive Samples			Seroprevalence (%)		
	Goat	Sheep	Total	Goat	Sheep	Total	Goat	Sheep	Total
Pathankot	8	8	16	4	0	4	50	0	25
Gurdaspur	15	3	18	4	0	4	26.60	0	22.22
Hoshiarpur	48	6	54	17	4	21	35.41	66.66	38.80
Amritsar	30	10	40	14	1	15	46.66	10	37.50
SBS Nagar	14	3	17	2	0	2	14.20	0	11.76
S.A.S.Nagar	8	11	19	0	8	8	0	72.72	42.10
TaranTaran	11	8	19	3	0	3	27.77	0	15.70
Jalandhar	11	7	18	5	0	5	45.45	0	27.77
Faridkot	30	13	43	17	4	21	56.66	30.76	48.80
Moga	54	15	69	15	3	18	27.77	20	26.08
Barnala	11	20	31	9	8	17	81.81	40	54.80
Ludhiana	60	10	70	23	7	30	38.33	70	42.80
Sangrur	53	6	59	31	0	31	58.49	0	52.50
Patiala	46	10	56	22	6	28	47.82	60	50.00
Muktsar	28	33	61	16	17	33	57.14	51.51	54.09
Bhatinda	44	21	65	15	7	22	34.09	33.33	33.84
Mansa	30	34	64	12	20	32	40.00	58.82	50.00
Ferozpur	17	13	30	5	6	11	29.41	46.15	33.33
Fazilka	60	14	74	15	14	29	25.00	100	39.18
Total	**578**	**245**	**823**	**229**	**105**	**334**	**39.60**	**42.85**	**40.58**

Zone-wise highest seroprevalence was recorded in Zone IV (45.78%) followed by Zone III (41.14%), Zone V (39.18%), Zone II (32.40%) and lowest in Zone I (25.00%) (Table 2, Fig 4). Predictive seroprevalence of toxoplasmosis at un-sampled locations based on the spatial distribution indicated small ruminant population in the districts adjoining Haryana and Rajasthan states at more risk, while animals of districts adjoining to the Himalayan region are at lower risk of toxoplasmosis (Fig 5, 6 and 7).

The present study, a first systematic report on spatial seroepidemiology of toxoplasmosis with overall seroprevalence of 40.58%, indicated wide exposure of small ruminants to *T. gondii* in Punjab. However, previous single study on seroprevalence of toxoplasmosis in 186 sheep examined from Punjab, showed low level of antibodies of *T. gondii* (Sharma *et al.,* 2008). High seroprevalence of *T. gondii* in small ruminants from adjoining Punjab province of Pakistan (Ahmad *et al.,* 2015) is indicative of similar geoclimatic conditions in both neighboring nations. High seroprevalence in sheep and goats, indicated that the meat-producing animals may be a great threat to human population if their meat is consumed undercooked. Further, there is possibility of economic losses to farmers as toxoplasmosis is responsible for abortion, stillbirth and neonatal mortality in sheep and goats (Dubey *et al.,* 2011).

Associated risk factors

Sheep were at apparently higher risk of *T. gondii* infection (OR =1.14, 95% CI: 0.84 -1.54, P=0.431) as compared to goats (OR=0.87, 95%CI: 0.63-1.19, P= 0.431) with an overall seropositivity in sheep 42.85% and 39.60% in goats (Table 2). Among zones (I toV) seroprevalence ranged from 25-45.78%, being non significantly highest in zone IV.

Apparently higher seropostivity in goats of > 1 year (39.70%) (OR= 1.02, CI= 0.65-1.59, P = 0.92) was seen as compared to < 1 year of age (OR = 0.97, CI =0.62-1.52, P = 0.92). Similarly in sheep animals > 1 year of age were apparently at more risk to toxoplasmosis (OR=1.01, CI= 0.57-2.10, P= 0.70) as compared to animals < 1 year of age (OR = 0.90, CI =0.46-1.76, P= 0.70).

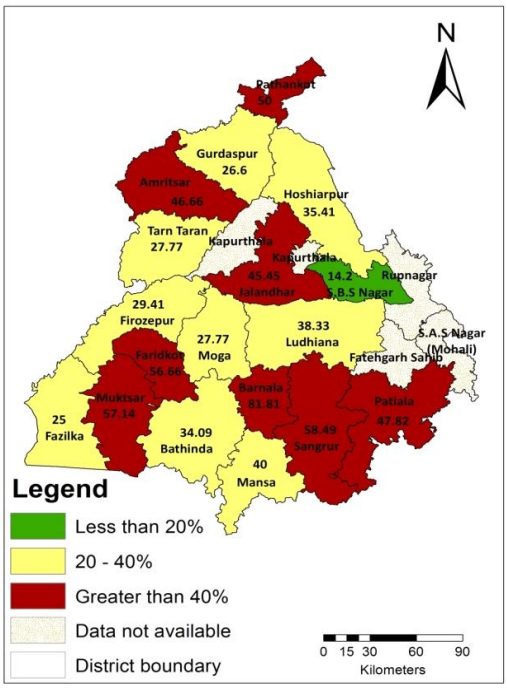

Fig 2: District wise seroprevalence of *T. gondii* in goats.

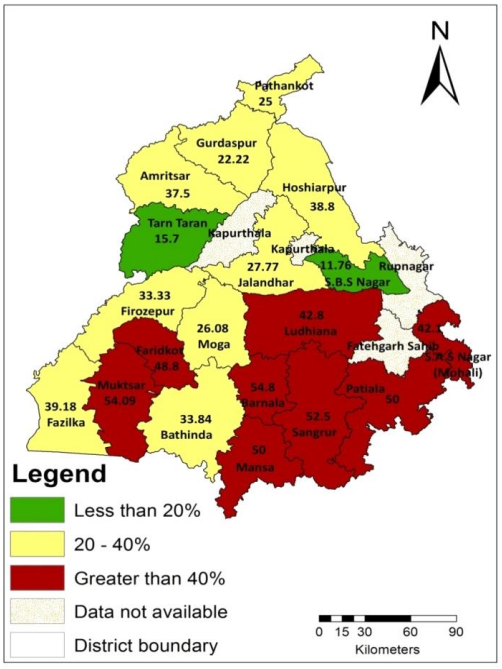

Fig 1: District wise seroprevalence of *T. gondii* in small ruminants.

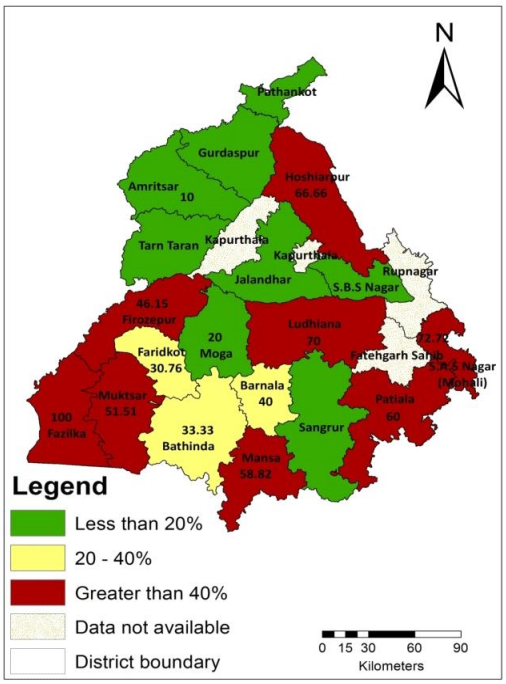

Fig 3. District wise seroprevalence of *T. gondii* in sheep.

Table 2: Risk factors evaluation in relation to seroprevalence of *T. gondii* in small ruminants.

Risk factor		Category	Total samples	Positive samples	Seroprevalence (%)	OR	95% CI	P Value	χ^2 value
Species		Goat	578	229	39.60	0.87	0.63-1.19	0.431	0.620
		Sheep	245	105	42.85	1.14	0.84-1.54		
Agroclimatic		Zone I	16	4	25.00	0.69	0.21-2.31	0.55	0.355
Zone		Zone II	108	35	32.40	1.44	0.43-4.78		
		Zone III	435	179	41.14	0.48	0.15-1.50	0.20	1.598
		Zone IV	190	87	45.78	0.40	0.12-1.27	0.11	2.586
		Zone V	74	29	39.18	0.52	0.15-1.75	0.29	1.141
Age	Goats	Less than 1 Year	97	38	39.17	0.97	0.62-1.52.	0.92	0.010
		Greater Than 1 Year	481	191	39.70	1.02	0.65-1.59		
	Sheep	Less than 1 Year	44	18	40.90	0.90	0.46-1.76	0.70	0.083
		Greater Than 1 Year	201	87	43.28	1.10	0.57-2.1		
History of	Goats	Yes	124	62	50.00	1.72	1.15-2.56	0.008	8.141
Abortion		No	454	167	37.20	0.58	0.39-0.86		
	Sheep	Yes	36	23	63.80	2.74	1.31-5.71	0.006	7.622
		No	209	82	39.23	0.36	0.17-0.76		
Access of cats	Goats	Frequent Access	527	216	41.40	2.03	1.05-3.90	0.03	4.668
to Farm		Limited Access	51	13	25.00	0.49	0.25-0.94		
	Sheep	Frequent Access	202	97	48.01	4.04	1.78-9.14	0.01	11.354
		Limited Access	43	8	18.60	0.24	0.11-0.56		
Grazing	Goats	Grazing only	530	215	40.56	1.65	0.87-3.16	0.12	2.391
Managements		Grazing + Stall Fed	48	14	29.16	0.60	0.32-1.15		
	Sheep	Grazing only	217	95	43.77	1.40	0.62-3.18	0.42	0.659
		Grazing + Stall Fed	28	10	35.71	0.71	0.31-1.62		
Gender	Goats	Female	572	229	40.03	INF	NAN	0.06	3.978
		Male	6	0	0	0	-		
	Sheep	Female	241	105	43.50	INF	-	0.08	3.05
		Male	4	0	0	0	-		

OR= Odds ratio; *95%CI; Statistically significant variables are indicated by bold typing.

Risk factor analysis indicated that goats with history of abortion were 1.72 times (OR=1.72, CI=1.15-2.56, P=0.008) and sheep were 2.74 times (OR=2.74, CI=1.31-5.71, P=0.006) more at risk to *T. gondii* as compared to animals with no history of abortion. The seropostivity in goats from farms having frequent access to cats was high (41.40%) to toxoplasmosis as compared to farms having limited access (25.00%) revealing 2.03 times more at risk (OR= 2.03, CI= 1.05-3.90, P= 0.03). Similarly, in sheep higher seropostivity was observed in animals with frequent cat access (48.01%) and were 4 times more at risk to toxoplasmosis (OR= 4.04, CI= 1.78-9.14, P=0.01) as compared to animals belonging to farm with limited cat access.

Goats with history of grazing only, were 1.65 times higher (OR= 1.65, CI = 0.87-3.16, P= 0.12) at risk to *T. gondii* infection (40.56%) as compared to animals with grazing

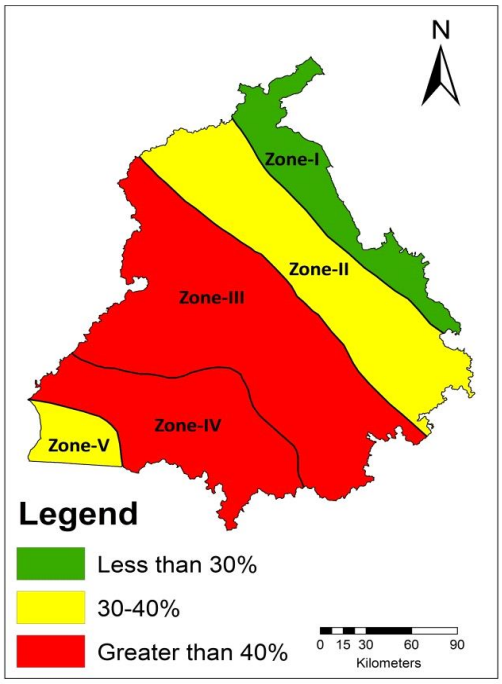

Fig 4: Zone wise seroprevalence of *T. gondii* in small ruminants.

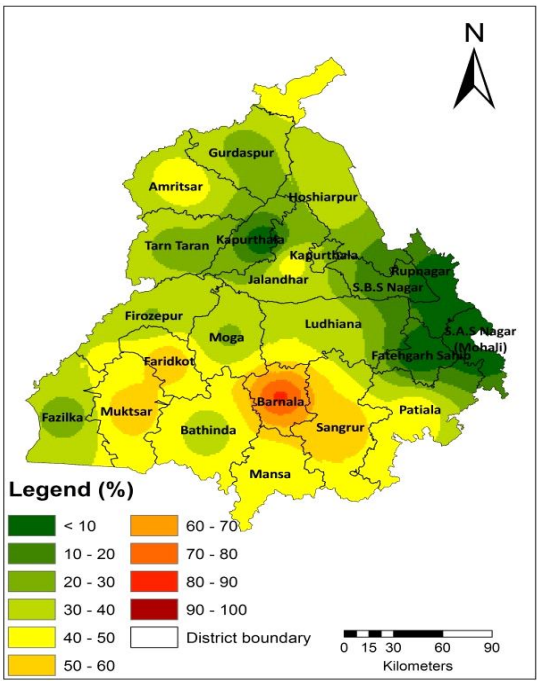

Fig 6: Predictive seroprevalence of *T. gondii* in goats.

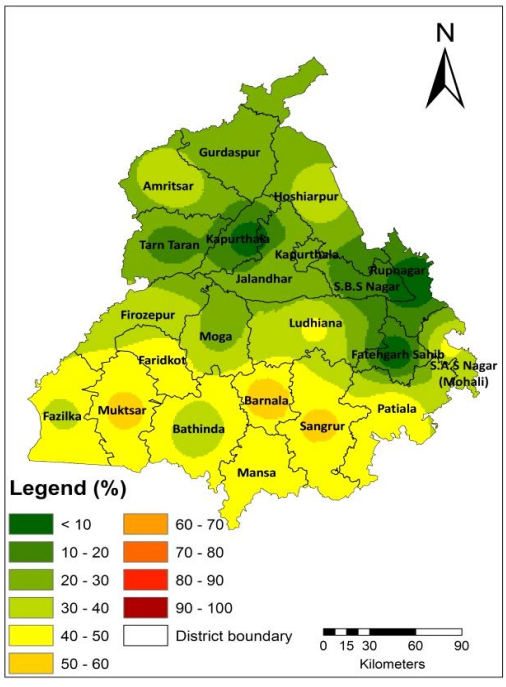

Fig 5: Predictive seroprevalence of T. gondii in small ruminants.

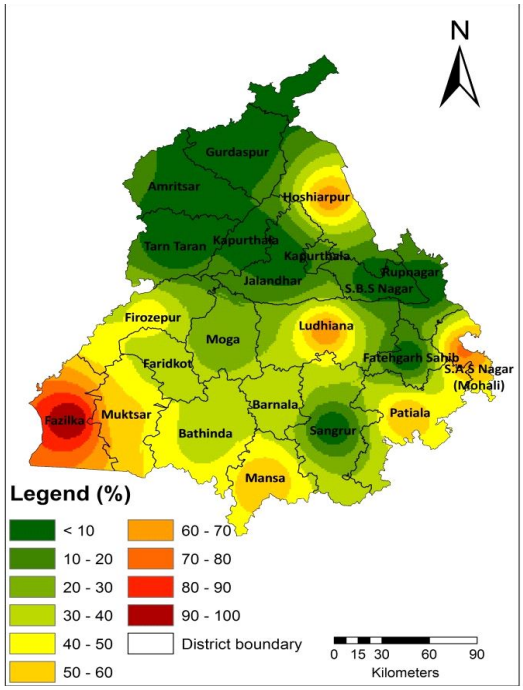

Fig 7: Predictive seroprevalence of T. gondii in sheep.

coupled with stall feeding (29.16%). Similar pattern of seropositivity in sheep (43.77%) with history of only grazing found 1.40 times (OR=1.40, CI= 0.62-3.18, P= 0.42) at higher risk of toxoplasmosis as compared to other animals with grazing coupled with stall feeding (35.71%). Gender-wise more seroprevalence was observed in females in both goats (40.03%) and sheep (43.50%) as compared to males as none of the animal was found seropositive.

As in the present study sheep being more susceptible to disease as compared to goats, previous studies from Brazil (Pinheiro *et al.,* 2009), Egypt (Ghazi *et al.,* 2006) and Pakistan (Ahmed *et al.,* 2016) also indicated sheep at higher risk of toxoplasmosis. It might be due to the fact that samples from sheep were collected from grazing system, while some of sampled goats were reared in the intensive system. Overall higher seroprevalence in sheep could be due to the possibility of picking up infective oocysts from ground as compared to goats as sheep is a ground grazer and goats are browser. On the contrary, Ahmed *et al.* (2016) reported higher infection rate in goats as compared to sheep.

Higher seroprevalence in Zone III, IV and V adjoining to Rajasthan and Haryana states as compared to Zone I and II adjoining to Himachal Pradesh may be related to higher temperature, lower precipitation and lower altitude compared to the absence of *T. gondii* at other locations (Kantzoura *et al.,* 2013). Previous workers reported the toxoplasmosis is more prevalent in humid and damp areas than in dry and hot environmental conditions (Dubey and Thulliez, 1993, Singh and Nautiyal, 1991). It was observed that majority of small ruminant farmers in zones III-V, were nomads, while majority of animal owners of zones I and II reared their animals in fixed position due to availability of ample fodder. One possible reason for more seroprevalence in zones 3, 4 and 5 could be the more movement of animals in these zones and more sale and purchase of animals with adjoining states Haryana and Rajasthan. An increased in the *T. gondii* antibodies level with higher age in both sheep and goats could be possible as older animals are more exposed to pathogens than the younger ones (Li *et al.* 2016, Othman and Al-Azuheir, 2014, Puije *et al.*, 2000). Previously similar observations were reported by Lashari and Tasawar, (2010), Tasawar *et al.* (2011) and Ramzan *et al*. (2009).

Significantly higher sero-prevalence observed in animals having history of abortion indicates that *T. gondii* could be an important abortifacient agent in small ruminants.

Presence and frequent access of cats to small ruminant farms as a significant risk factor for higher seroprevalence may be due to fact that cats being the definitive hosts of the parasite play a vital role in infecting intermediate hosts by shedding oocysts in the environment (Lopes *et al.*, 2010). One study from Poland revealed the presence of free-roaming cats as important risk factor for the transmission of toxoplasmosis in goats (Neto *et al.,* 2008).

More seroprevalence observed in animals reared on grazing system corroborates with the published reports on the assessment of different type of feeding practice revealing animals being raised extensive system to be more prone to the infection (Lopes *et al.*, 2010, Wang *et al.,* 2011).

Higher seroprevalence of toxoplasmosis in females than males is consistent with various reports published globally (Ramzan *et al.,* 2009, Othman and Al-Azuheir, 2014). However, on the other hand few workers (Ntafis *et al.,* 2007, Caballero-Ortega *et al.,* 2008) reported no association between gender and seropositivity.

CONCLUSION

High seroprevalence of *T. gondii* in small ruminants and history of abortion, access to cats as major risk factors at ruminant farms of Punjab state, indicate possible risk of zoonotic infection to people in the region. Spatial distribution and predictive seroprevalence data analysis also indicate possible risk of toxoplasmosis in whole of the state with higher chances of disease occurrence in districts adjoining Haryana and Rajasthan states.

Conflict of interest

Authors declare there is no conflict of interests regarding the publication of this research paper.

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