DOI: 10.7589/2017-09-228

Journal of Wildlife Diseases, 55(1), 2019, pp. 129–135

Seroprevalence and Risk Factors of *Toxoplasma gondii* in Wild Birds of Punjab Province, Pakistan

Ahmad Naveed,[1] Shahzad Ali,[1,8] Haroon Ahmed,[2] Sami Simsek,[3] Muhammad Rizwan,[1] Imdad Kaleem,[2] Muhammad Asif Gondal,[2] Asghar Shabbir,[2] Fahad Pervaiz,[2] Muhammad Aamir Khan,[4] Muhammad Shahid Nadeem,[5] Muhammad Sohail Afzaal,[6] and Sajid Umar[7] [1]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, 75550, Pakistan; [2]Department of Biosciences, COMSATS University Islamabad (CUI), 75500, Pakistan; [3]Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, Elazig, 23000, Turkey; [4]Department of Management Sciences, COMSATS University Islamabad (CUI), 75500, Pakistan; [5]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, 21577, Saudi Arabia; [6]Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, 75500, Pakistan; [7]Department of Pathobiology, Faculty of Veterinary Sciences, Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi, 75500, Pakistan; [8]Corresponding author (email: shahzad.ali@uvas.edu.pk)

ABSTRACT: *Toxoplasma gondii* is a protozoan parasite of veterinary and human public health importance for which birds act as an intermediate host. No information is available about the epidemiology of *T. gondii* in wild birds of Pakistan. The present study was designed to determine the seroprevalence and risk factors associated with *T. gondii* antibodies in wild birds of District Kasur, Punjab Province, Pakistan. A total of 200 wild birds of 28 species were captured from four tehsils (administrative subdistricts of districts) of the district Kasur and their serum samples screened for the presence of *T. gondii* antibodies using a latex agglutination test (cut-off value: 1:64). Twenty-five (13%) individual birds and 13 (46%) of the bird species were seropositive for *T. gondii* antibodies. There were statistical differences in *T. gondii* prevalence between adults and young (15% and 7%, respectively, P=0.001) and healthy and sick (11% and 50%, respectively, P=0.000) while there were not differences between genders, sites, urbanicity, and tehsils. The present study provides evidence of *T. gondii* antibodies in wild birds of Pakistan.

Key words: Pakistan, seroprevalence, *Toxoplasma gondii*, wild birds.

Toxoplasma gondii is an intracellular protozoan which infects all warm-blooded animals including marine mammals, livestock, humans, and birds (Dubey 2010; Dubey et al. 2010; Gennari et al. 2014; Tidy et al. 2017). Because they act as a reservoir of many infections, birds are important sources of many infections, including toxoplasmosis, for other animals, especially felids. The seroprevalence of toxoplasmosis and the associated risk factors for the disease were determined in cats, dogs (Ahmad et al. 2014), sheep, and goats (Ahmed et al. 2016) in different areas of Pakistan. Every year, a billion birds are eaten by felids, especially by stray cats (Cray 2011; Loss et al. 2013; Ali 2016). Furthermore, tissue cysts (the infective form of *T. gondii*) are present in the muscle tissues of the intermediate hosts, including birds (both wild and captive), and ingestion of meat from infected birds is considered an important source of infection in humans (Hladikova et al. 2008; Sakikawa et al. 2012). Contamination of environmental matrices (i.e., water, soil, vegetables, raw meats, and fruits) with *T. gondii* has been reported from Pakistan (Ajmal et al. 2013; Khan et al. 2013), but no information is available about *T. gondii* infection of wild birds. The present study aimed to determine seroprevalence and risk factors associated with *T. gondii* in wild birds of District Kasur, Punjab, Pakistan.

Our study was approved by the Animal Ethics Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan (Dr. no. 597). Wild birds from which blood samples were collected were handled according to instructions and guidelines set by the Animal Ethics Committee of University of Veterinary and Animal Sciences.

Our study was conducted in District Kasur situated in the province of Punjab, Pakistan. District Kasur is comprised of four tehsils (an area of land with a city or town that serves as its administrative center). The tehsils, Pattoki, Chunian, Kot Radha Kishan, and Kasur, have an area of about 4,796 km^2 (Fig. 1). Geographically, the study area lies from 31°21′47″N to 30°39′47″N and from

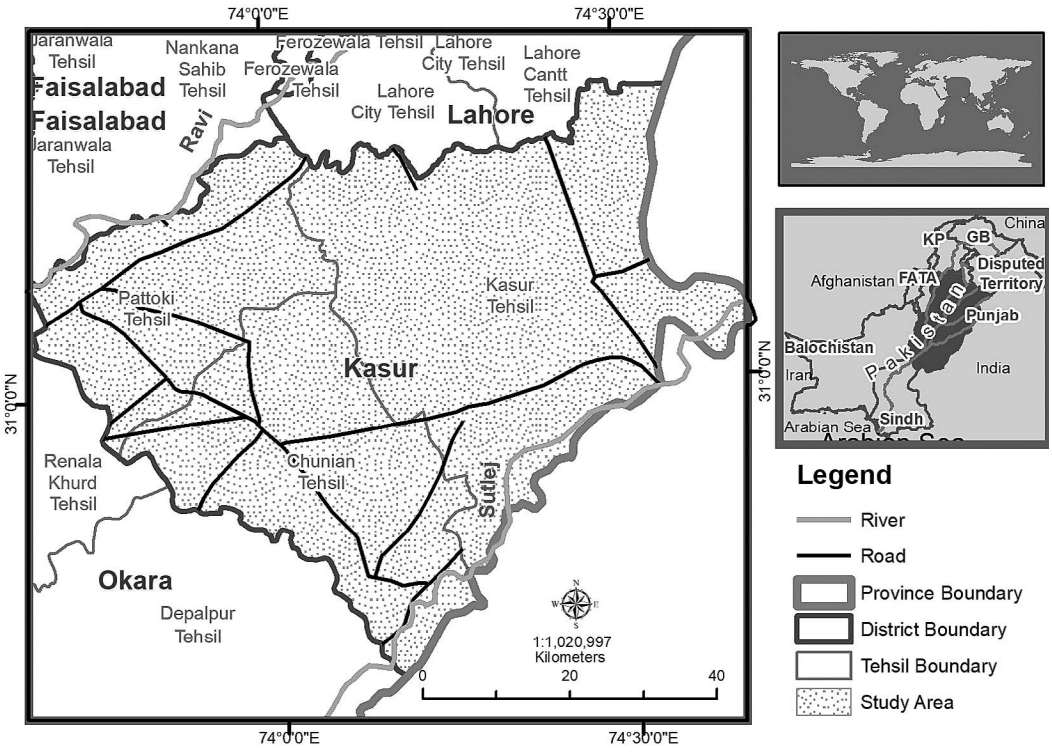

FIGURE 1. Study area map of four tehsils (Pattoki, Chunian, Kot Radha Kishan, and Kasur) in District Kasur, Punjab, Pakistan. A total of 200 wild birds of 28 species were captured from these four tehsils for sampling and testing for *Toxoplasma gondii* using a latex agglutination test. FATA=Federally Administered Tribal Areas; KP=Khyber Pakhtunkhwa; GB=Gilgit-Baltistan.

74°35′06″E to 73°37′23″E. Five sites were selected from each tehsil of District Kasur. Land use sites selected for capturing of wild birds were: 1, Crops; 2, Land; 3, Populated area; 4, Trees; and 5, Water body (Fig. 2). Ten samples were collected from each site. In this way, 50 samples were collected from each tehsil, with a total of 200 samples collected from the four tehsils.

Twenty-eight common wild bird species, categorized as of Least Concern by the International Union for Conservation of Nature (2016), were sampled from 1 November 2016 to 28 February 2017 (Table 1). Each bird species was identified using a field guide to birds of Pakistan (Grimmett et al. 2008). Blood samples were collected from basilic veins with disposable syringes and were slowly transferred to a screw-capped, sterile test tube without anticoagulant to avoid hemolysis. The sample was left to clot for about an hour. The blood samples were centrifuged at 1,372 × G for 5 min. Serum was stored at −20 C until further processing. Data related to age, gender, health status, tehsils, urbanicity, and sites were collected on the blood-sampling day. Birds that had dull coloration, low weight, fluffed feathers, and loss of appetite were considered ill and were not sampled.

A latex agglutination test was performed according to manufacturer's instructions (Antec Diagnostic Products, Bridport Dorset, UK). Initially, a serum sample from each bird was serially diluted twofold in phosphate-buffered saline from 1:2 to 1:8. A sample found positive at 1:2 to 1:8 serial dilutions was tested at higher, doubling dilutions 1:16, 1:32, 1:64, etc.

A negative reaction indicated the absence of *T. gondii* antibodies. A clear positive

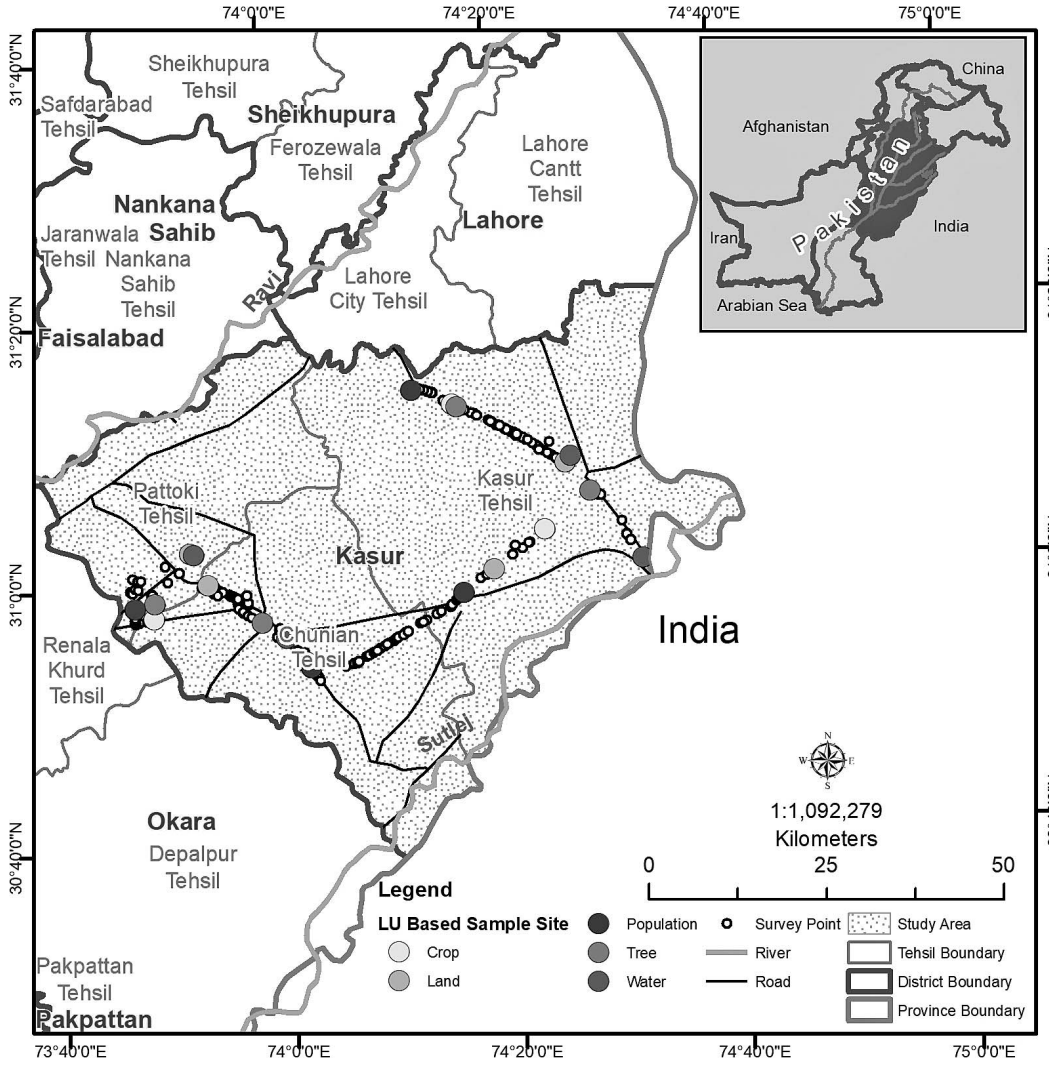

FIGURE 2. Showing the landscape classifications of sampling sites in four tehsils (Pattoki, Chunian, Kot Radha Kishan, and Kasur) in District Kasur, Punjab, Pakistan. A total of 200 wild birds of 28 species were captured for sampling and testing for *Toxoplasma gondii* using a latex agglutination test. The landscape classifications were: Crops, Land, Populated area, Trees, and Water body. Kot Radha Kishan Tehsil is not shown in the map of District Kasur because it was a part of Kasur Tehsil at the time of sampling and is now independent. It is located between Kasur Tehsil and Chunian Tehsil.

reaction indicated the presence of *T. gondii* antibodies equal to or greater than 4 IU/mL, which reflected either a past infection or an evolving infection. In these cases, the final titer was determined. Sensitivity of the test was 3–7 IU/mL; normal levels in adults were significantly less. The data were subjected to chi-square statistical analysis using the Statistical Package for Social Sciences® version 20.0 software (IBM, Armonk, New York, USA). Results were tested against $P \leq 0.05$ for significance.

We found 13% (25/200) of serum samples to be seropositive for *T. gondii* antibodies (Table 1). A total of 28 wild bird species were captured and serum samples were tested using latex agglutination test. A total of 46% (13/28) wild bird species were seropositive for

TABLE 1. Seroprevalence of *Toxoplasma gondii* in a total of 200 wild birds of 28 species captured from four tehsils (administrative subdistricts of districts) of the district Kasur, Punjab Province, Pakistan. Serum samples were screened for the presence of *T. gondii* antibodies using a latex agglutination test (cut-off value: 1:64).

Species	Scientific name	No. of samples		
		Tested	Positive	%
Bank Myna	*Acridotheres ginginianus*	15	0	0
Common Myna	*Acridotheres tristis*	11	2	18
White-breasted Waterhen	*Amaurornis phoenicurus*	9	0	0
Indian Pond Heron	*Ardeola grayii*	8	0	0
Spotted Owlet	*Athene brama*	9	3	33
Cattle Egret	*Bubulcus ibis*	13	0	0
Crow Pheasant	*Centropus sinensis*	6	0	0
Brown Rock Chat	*Cercomela fusca*	2	0	0
Rock Pigeon	*Columba livia*	4	0	0
House Crow	*Corvus splendens*	17	6	35
Common Quail	*Coturnix coturnix*	8	0	0
Rufous Treepie	*Dendrocita vagabunda*	5	0	0
Black Drongo	*Dicrurus macrocercus*	9	1	11
Common Snipe	*Gallinago gallinago*	4	1	25
Common Moorhen	*Gallinula chloropus*	5	1	20
Indian Silverbill	*Lonchura malabarica*	4	2	50
Scaly-breasted Munia	*Lonchura punctulata*	1	0	0
White Wagtail	*Motacilla alba*	11	1	9
House Sparrow	*Passer domesticus*	9	1	11
Baya Weaver	*Ploceus philippinus*	5	0	0
Rose-ringed Parakeet	*Psittacula krameri*	9	0	0
Red Vented Bulbul	*Pycnonotus cafer*	11	3	27
Eurasian Collared Dove	*Streptopelai decaocto*	10	2	20
Asian Pied Starling	*Sturnus contra*	3	0	0
Common Starling	*Sturnus vulgaris*	3	0	0
Common Greenshank	*Tringa nebularia*	1	0	0
Common Babbler	*Turdoides caudatus*	2	1	50
Jungle Babbler	*Turdoides striatus*	6	1	17

T. gondii antibodies (Table 1). Two species of wild birds, the Common Babbler (*Turdoides caudatus*) and the Indian Silverbill (*Lonchura malabarica*), both had the highest seroprevalence for *T. gondii* antibodies at 50% while the lowest seroprevalence (9%) was observed in the White Wagtail (*Motacilla alba*). The seroprevalence of *T. gondii* in the remaining species ranged from 11% to 35% (Table 1).

The risk factors for *T. gondii* are summarized in Table 2. There was a significant difference (P=0.001) in the prevalence of *T. gondii* in age groups (15% in adults and 7% in young). A significant difference (P<0.001) was also found in prevalence between healthy (11%) and sick birds (50%). Variables such as gender (P=0.389), sites (P=0.454), urbanicity (P=0.749), and tehsil (P=0.441) were not verified as risk factors for *T. gondii* infection in wild birds of Pakistan.

Toxoplasma gondii is a single celled, intracellular protozoan parasite that can cause mortality in certain bird species (Dubey 2010). No information is available about *T. gondii* seroprevalence in wild birds in Pakistan. Like other warm-blooded animals, birds also serve as important intermediate hosts of *T. gondii* and become a source of infection for cats preying on them, after which cats release oocysts (Loss et al. 2013).

TABLE 2. Risk factors associated with *Toxoplasma gondii* in a total of 200 wild birds of 28 species captured from four tehsils (administrative subdistricts of districts) of the district Kasur, Punjab Province, Pakistan.

Variable	Category	No.		Chi-square value	*P* value
		Tested	Seropositive (%)		
Age	Adult	138	21 (15)	7.16	<0.001[a]
	Young	62	4 (7)		
Gender	Male	76	10 (13)	0.742	0.389
	Female	124	15 (12)		
Health Status	Healthy	182	16 (11)	28.37	<0.000[a]
	Sick	18	9 (50)		
Tehsil	Pattoki	50	3 (6)	2.697	0.441
	Chunian	50	8 (16)		
	Kot Radha Kishan	50	7 (14)		
	Kasur	50	7 (14)		
Urbanicity	Urban	45	4 (9)	0.102	0.749
	Rural	155	21 (14)		
Sites	Land	40	6 (15)	3.657	0.454
	Crops	40	7 (18)		
	Water Body	40	2 (5)		
	Trees	40	5 (13)		
	Populated area	40	5 (13)		

[a] Differences between categories are significant.

The prevalence of antibodies in our study area might be due to multiple factors including feeding habits of wild birds because during feeding they are more exposed to their environment. The prevalence of *T. gondii* oocysts has been confirmed from environmental samples such as soil, vegetables, water, fruits, milk, milk products, raw meat, and bird's seminal fluid (Jimenez-Coello et al. 2012; Afonso et al. 2013; Ajmal et al. 2013; Khan et al. 2013). We found that 46% (13/28) of wild bird species were seropositive for *T. gondii* in the present study. The relatively high seropositivity for *T. gondii* in Pakistan may be due to sampling diverse species.

For the Eurasian Collared Dove (*Streptopelai decaocto*), 20% (2/10) of serum samples were seropositive for *T. gondii* infection in our study. However, deaths of doves due to the presence of *T. gondii* infection were reported in France (Rigoulet et al. 2014). We suggest that any birds from Pakistan with signs related to *T. gondii* should be investigated for the causative agent.

In the case of House Crows (*Corvus splendens*), we found 35% (6/17) of serum samples to be positive for antibodies against *T. gondii*. The higher seropositivity of toxoplasmosis in crows that we found might be due to the scavenging on infected carrion. A fatal role of *T. gondii* in the failure of the reintroduction of an endangered crow species, the Hawaiian Crow ('Alalā; *Corvus hawaiiensis*) in Hawaii is well documented (Work et al. 2000).

We found that 33% (3/9) of Spotted Owlets (*Athene brama*) were seropositive against *T. gondii* antibodies. A possible reason for a high seroprevalence in owls might be because they feed on small rodents, which could be carriers of *T. gondii*. The seropositivity of small rodents for *T. gondii* is well documented in Pakistan (Ahmad et al. 2012).

For the House Sparrow (*Passer domesticus*), 11% (1/9) of serum samples were positive for *T. gondii* antibodies, which may be due to their ground-feeding habits. The titer of *T. gondii* antibodies was high in this species, possible evidence of oocyst contamination in the environment. House Sparrows have maximum

interaction with humans in Pakistan where cats are also present, so the risk of infection of *T. gondii* is increased for humans and cats. Other bird species were also found to be seropositive for *T. gondii*, which is evidence of a wide range of intermediate hosts for the parasite.

In our study, the seroprevalence of *T. gondii* was higher in adult wild birds as compared to young wild birds. That was probably due to more interaction of adult birds with environmental matrices (water, soil, food, etc.), multiplying the risk of infection. Another important variable that was statistically different was health status, with the seroprevalence of *T. gondii* being higher in sick birds (50%) compared to healthy birds (11%). Our results showed that there were no significant differences in the seroprevalence of *T. gondii* by gender, urbanicity, sites, and tehsils in wild birds. This might have been because sharing of the same food by both sexes of birds at different locations means that they are equally exposed to *T. gondii*. Any bird found dead or sick should be investigated for the presence of *T. gondii* using culture and molecular tools for the prevention and control of cross-species transmission of the parasite.

Our study provides evidence that *T. gondii* is present in multiple wild bird species in Pakistan. Health status and age were determined to be major risk factors associated with the seropositivity of *T. gondii* in our study area. Although we only sampled bird species of least conservation concern, there is a need to evaluate the role of *T. gondii* in conservation of vulnerable and near-endangered wild birds in Pakistan. Moreover, the present study may provide primary data for the control of *T. gondii* in wild birds to minimize the load of *T. gondii* in livestock and human beings to also improve human and veterinary public health.

The authors thank the institutions that provided technical support for the development and implementation of this study. This work was supported by the Higher Education Commission, Pakistan via Project HEC (FD)/2015/4942.

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Submitted for publication 17 September 2017.
Accepted 31 January 2018.