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Research Article
Snapshot View of Intestinal Parasites in Wild Birds of Chitwan District, the Southcentral Lowlands of Nepal

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Abstract

Background and Objectives: Intestinal parasites of wild birds often cause fatal diseases such as schistosomiasis, dermatitis, cyclosporiasis, diarrhea, etc. in humans and domestic animals in developed and underdeveloped countries. Therefore, the aim of this study was to determine prevalence of intestinal parasites of wild birds in Chitwan District. **Materials and Methods:** This cross-sectional study was conducted during 15th January to 16th July, 2018. The collected feces were preserved in 2.5% of potassium dichromate solution for their routinely examination with the aid of electric photography compound microscope. **Results:** During study period, 345 fecal samples from wild birds roosting at 6 different localities of Chitwan District were collected. Among positive samples (n = 318, 92%), schistosomatids (trematodes), *Capillaria* spp. (nematodes) and *Balantidium* spp. (protozoans) were the most prevalent parasites. A total of 60% (n = 191), 29% (n = 91) and 11% (n = 36) samples contained 1, 2 and 3 types of parasites, respectively. High prevalence of helminthes known to occur among wild birds corresponds to the suitable temperature for the growth of secondary hosts and feces collected mostly from migratory birds with higher chances of parasitic infection. **Conclusion:** *Balantidium* spp., *Cryptosporidium* spp. and *Cyclospora* spp. (protozoans) and *Hymenolepis* spp., *Raillietina* spp., *Capillaria* spp. and *Ascaridia galli* (helminthes) known in wild birds during this study can infect humans, domesticated, pet, zoo and wild animals. Therefore, it is essential to conduct more sophisticated study to prepare the profile of parasites of veterinary and public health importance.

Key words: Helminthes, intestinal parasites, parasitic zoonoses, protozoan, secondary hosts

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are several pathogenic parasites causing serious impacts on wildlife (e.g., fishes, birds, terrestrial and aquatic mammals), domestic animals, captive birds and humans[1-4]. Diverse intestinal parasites such as protozoans, trematodes, cestodes, acanthocephalans and nematodes infect wild and domesticated birds distributed widely in different geological landscapes and climatic conditions[2,3,5-8]. This influences on health of animals and humans because most of the birds are definitive and/or alternative hosts for several parasites.

The omnivorous birds are prone to be infected with intestinal parasites because their foods comprise of several vectors and/or intermediate hosts (e.g., annelids, insects (e.g., ants), crustaceans (e.g., *Cyclops*), mollusks (e.g., snails), fishes (e.g., cyprinids), amphibians, reptiles and small mammals) of parasites and those foods are contaminated with feces of animals and soil[3,9-11]. Therefore, wild birds, specially the migratory birds, are mostly infected with parasites due to their foods containing infective stages of parasites and exposure to different environment (such as soil, water, etc., contaminated with feces)[6,12]. Consequently, this increases chance of transmission of parasitic diseases globally keeping poultry industry, zoos and human health at the risks of exposure to dreadful diseases that may result in deaths of humans and animals widely[13]. Hence, this study has public health concerns and socioeconomic values.

There are limited studies on wild bird parasites in Nepal. Gupta and Pandey[14] studied intestinal parasites in vultures and Shrestha *et al.*[15] and Mitchell and Dick[16] documented ectoparasites of vultures and other birds, respectively, in this country. This study discloses wild birds' intestinal parasites which often cause fatal diseases such as schistosomiasis, dermatitis, cyclosporiasis, diarrhea, etc., in domestic animals and humans in developed and underdeveloped countries. Herein, intestinal parasites (i.e., protozoans, trematodes, cestodes and nematodes) in wild birds in Chitwan District are listed. This documentation is crucial because it can provide essential information on parasites and for the prevention and control of parasitic zoonoses.

MATERIALS AND METHODS

This was a descriptive cross-sectional study of wild bird intestinal parasites involving fecal samples collected during 15th January to 16th July, 2018 in Chitwan District, southcentral lowlands of Nepal. Hence, a control was not included in this study.

Study area: Chitwan District having tropical and the sub-tropical climates occupies 2,238.39 km^2 and adjoins Nawalpur District in the West, Bihar State of India in the South, Makawanpur and Parsa Districts in the East and Gorkha and Dhading Districts in the North. It lies between 27°21'45'' to 27°52'30'' North latitude and 83°54'45'' to 84°48'15'' East longitude and extends between 100-815 m a.s.l. (above sea level) where at least 630 species of birds are distributed[17]. Its varied topography between the Mahabharat and the Siwalik ranges is dissected by numerous permanent and seasonal water systems. Further, the most fertile alluvial floodplain lands, forests, rivers and lakes in Chitwan District demonstrate one of the economically important areas of Nepal. The diverse climate, topography, bird species and greater economical activities in Chitwan District provide opportunities to flourish different parasites causing epidemic outbreak.

This study confined in 6 places located at approximately 190 m a.s.l. in Chitwan District (Table 1). These sites possess tropical climate, which is characterized by high temperature and extreme variability in precipitation with humid summer (with about three-fourth of the annual rainfall) and dry winter (with scanty rainfall).

Collection of fecal samples: About 0.5-2 g of feces from each bird were collected by spreading plastic sheets under the trees where wild birds used to roost for night and day hours in study sites (Table 1). Also, the wild birds' droppings were collected from the top of freshly voided fecal mass directly from the grounds using scalpel. All fecal samples were preserved in 2.5% potassium dichromate solution in air-tight plastic vials and coded using tags to classify and analyze samples. Then, samples were carried to laboratory as early as possible. Before and after collection of samples, all possible precautions and hygienic measures such as wearing apron, hand gloves, disinfection of slides were taken to avoid contamination.

This study was approved by Research Management Cell, Birendra Multiple Campus, Bharatpur, Chitwan District, Tribhuvan University.

Identification of intestinal parasites: Samples were routinely examined using photographic electric compound microscope (model number: HL 10, company: Coslab, country: India) preparing thick and thin films on slides. Oocysts, cysts and trophozoites of protozoans and eggs and larvae of trematodes, cestodes (including body segments in case of cestodes) and nematodes were photographed by using ScopeImage 9.0 Image Processing Software for identification. Generic and specific names were confirmed comparing

morphological characteristics of intestinal parasites of birds available elsewhere[18-26]. Parasitic specimens were carefully differentiated from non-parasites using published laboratory guidelines[26].

Analysis of data: Positive fecal samples were analyzed using percentage, tables and photographs. Infection rate was calculated dividing number of positive samples by total samples and quotient was multiplied by 100. Percent values were round-figured. Single, double and triple infections were defined based on 1, 2 and 3 types of parasites present in those fecal samples, respectively.

RESULTS

Positive fecal samples: Out of 345 fecal samples of wild birds, 92% (n = 318) samples contained at least one type of intestinal parasite and 8% (n = 27) contained no parasitic infections. Among positive samples, 60% (n = 191), 29% (n = 91) and 11% (n = 36) samples contained one, two and three types of parasites, respectively. In location-wise analysis of parasites, samples collected from Chepang Taal located in Ratnanagar Municipality, ward number 4 had the highest (97%, n = 116) and premises of Aroma English Secondary School had the lowest (78%, n = 35) positive samples. The samples collected from school premises represented parasites of birds inhabiting terrestrial habitats and the rest samples represented semi-aquatic habitats (Table 1).

Diversity of intestinal parasites infecting wild birds: From positive samples, 15 genera and one species belonging to protozoans, trematodes, cestodes and nematodes were identified (Table 2, Fig. 1a-o). Protozoans included *Balantidium* spp. (Fig. 1a), *Cyclospora* spp. (Fig. 1b), *Cryptosporidium* spp. (Fig. 1c) and *Eimeria* spp. (Fig. 1d). Trematodes included *Clinostomum* spp. (Fig. 1e), *Echinostoma* spp. (Fig. 1f), *Schistosoma* spp. (Fig. 1g) and *Trichobilharzia* spp. (Fig. 1h). Cestodes included *Bothriocephalus* spp. (Fig. 1i), *Choanotaenia* spp. (Fig. 1j), *Diphyllobothrium* spp. (Fig. 1k), *Hymenolepis* spp. (Fig. 1l) and *Raillietina* spp. (Fig. 1m). Similarly, nematodes included *Ascaridia galli* (Fig. 1n) and *Capillaria* spp. (Fig. 1o).

Prevalence of intestinal parasites: Among all, nematodes were the most prevalent parasites (35%, n = 110) followed by trematodes (28%, n = 86), cestodes (25%, n = 79) and protozoans (14%, n = 43). The most prevalent parasites were *Capillaria* spp. (13%, n =41) followed by *Trichobilharzia* spp. (11%, n = 34), *Balantidium* spp., *Schistosoma* spp. and *Ascaridia* spp. (each 7%, n = 23) and *Raillietina* spp. (6%, n = 20) (Table 2).

DISCUSSION

This study reveals noticeably high prevalence of intestinal parasites (92%, n = 318/345, Table 1) representing one species, 15 genera, 15 families and 10 orders (Table 2) in wild birds. Alike to finding in this study, Hoque *et al.*[27] also reported preponderance of cases with single infection among domestic and wild birds in Bangladesh. Mixed infections known in this study was comparable to the reports of two species of intestinal parasites in four ducks (20%, 4/25) and triple infection in one duck (4%, 1/25)[9]. Although several studies report intestinal parasites in wild birds in other countries[2,5,6,8-10,28,29], there are limited literature describing wild bird intestinal parasites in Nepal[14]. Therefore, findings in this study may be a basis for the sophisticated study to understand contribution of wild bird parasites to zoonoses. Therefore, knowledge on wild bird parasites and the host–parasite relationships should be updated periodically to understand the impact of parasites on the host and any possible health risks due to parasitic infections in free-ranging birds.

Table 1: Study sites and fecal samples collected from wild birds in Chitwan district (herein, "Taal" = lake)

Study sites (from where feces were collected)	Habitat types of birds	Common names of birds (observed while sample collection)	Sample size	Positive sample size	Percentage
Tikauli Taal (Ratnanagar Municipality, ward number 4)	Semi-aquatic	Heron, egret, darter, cormorant, water hen, crake, king fisher, stork, eagle	20	17	85
Chepang Taal (Ratnanagar Municipality, ward number 4)	Semi-aquatic	Darter, cormorant, open bill, ibis, lesser adjutant	120	116	97
Darai Taal (Khairahani Municipality, ward number 8)	Semi-aquatic	Darter, cormorant, open bill, ibis, egret, stork, eagle, heron, king fisher	60	56	93
Bote Taal (Khairahani Municipality, ward number 8)	Semi-aquatic	Lapwings, heron, egret, snipe, stilt, jacana	30	27	90
Thulo Ratpi River bank (Khairahani Municipality, ward number 8)	Semi-aquatic	Bar-headed goose, ruddy shelduck, pintail, teal, cormorant, darter, pochard	70	67	96
Aroma English Secondary School premises (Bharatpur Municipality, ward number 10)	Terrestrial	Crow, coucal, mynah, sparrow, cockoo, drongo, magpie, robin, doves, shrike, oriole, starling and others	45	35	78
Total			345	318	92

Table 2: Intestinal parasites of wild birds of Chitwan District, southcentral Nepal

Parasite types	Order	Family, subfamily	Genus/species	Prevalence [percentage (positive sample size)]
Kingdom Protista (herein, Protozoans)				
Class: Litostomatea	Vestibuliferida	Balantidiidae	*Balantidium* spp.	7.34 (23)
Class: Apicomplexa	Eucoccidiorida	Cryptosporidiidae	*Cryptosporidium* spp.	3.66 (12)
Class: Apicomplexa	Eucoccidiorida	Eimeriidae	*Cyclospora* spp.	0.92 (3)
Class: Apicomplexa	Eucoccidiorida	Eimeriidae	*Eimeria* spp.	1.00 (3)
Class: Apicomplexa	Eucoccidiorida	-	-	0.92 (3)
Kingdom Animalia (herein, Phylum 1: Platyhelminthes (flatworms))				
Class: Trematoda (flukes)				
	Diplostomida	Clinostomidae	*Clinostomum* spp.	2.75 (9)
	Plagiorchiida	Dicrocoeliidae	-	1.83 (6)
	Plagiorchiida	Echinostomatidae	*Echinostoma* spp.	0.92 (3)
	Plagiorchiida	Opisthorchiidae	-	0.92 (3)
	Digenea	Schistosomatidae	*Schistosoma* spp.	7.34 (23)
	Digenea	Schistosomatidae	*Trichobilharzia* spp.	11.01 (34)
			Trematode species	2.75 (9)
Class: Eucestoda (tapeworms)				
Subclass: Cestoda (larvae have six hooks on the scolex (head))				
	Pseudophyllidea	Bothriocephalidae	*Bothriocephalus* spp.	0.92 (3)
	Cyclophyllidea	Dilepididae	*Choanotaenia* spp.	1.83 (6)
	Diphyllobothriidea	Diphyllobothriidae	*Diphyllobothrium* spp.	0.92 (3)
	Cyclophyllidea	Hymenolepididae	–	0.92 (3)
	Cyclophyllidea	Hymenolepididae	*Hymenolepis* spp.	0.92 (3)
	Cyclophyllidea	Davaineidae (Raillietidae)	*Raillietina* spp.	6.42 (20)
			Cestode species	12.84 (41)
Phylum 2: Nemathelminthes/Nematoda (roundworms)				
Class: Secernentea				
	Ascaridida	Ascarididae	*Ascaridia galli*	4.59 (15)
	Ascaridida	Ascarididae	*Ascaridia* spp.	2.75 (9)
Class: Aphasmidea				
	Trichinellida	Capillariidae, Capillariinae	*Capillaria* spp.	12.84 (41)
			Nematode species	14.68 (46)

Balantidium spp. infecting wild birds in this study sites (Table 2, Fig. 1a) also infected game birds in Bangladesh[8]. *Balantidium* spp. are cosmopolitan and highly transmissive protozoan parasites and immunocompromised individuals appear to be less resistant to balantidiosis[23]. *Cyclospora* spp., a coccidian parasite, known from wild birds from Chitwan District (Table 2, Fig. 1b) also infected community people in Nepal[30]. *Cryptosporidium* spp. infecting wild birds in this study (Table 2, Fig. 1c) also infected zoo birds[31] and domestic ducks[9]. Alike to our findings, *Balantidium coli* and *Cryptosporidium* spp. infected domestic and wild birds in New Zealand[32]. Wild birds in these study areas were also infected by *Eimeria* spp. (Table 2, Fig. 1d). Similar infections in chickens by *Eimeria* spp. were reported from Chitwan District[33]. Therefore, characterizing all possible sources of contaminations with *Balantidium* spp., *Cryptosporidium* spp., *Eimeria* spp. and *Cyclospora* spp. to humans and domesticated animals is essential elsewhere including Nepal like developing countries.

Among the helminthes, *Capillaria* spp. were the most prevalent parasites (13%, n = 41) followed by *Trichobilharzia* spp. (11%, n = 34), *Schistosoma* spp. and *Ascaridia* spp. (each 7%, n = 23) and *Raillietina* spp. (6%, n = 20) (Table 2). *Hymenolepis* spp. (Fig. 1l), *Raillietina* spp. (Fig. 1m), *Capillaria* spp. (Fig. 1o) and *Ascaridia galli* (Fig. 1n) infection known to occur in this study also infected domesticated chickens elsewhere[32,34,35], domesticated guinea fowls in Nigeria[28], domestic, wild and birds in Bangladesh[8,27], swallows in Iran[36], pet birds in Italy[31], zoo birds in India[37] and vultures in Chitwan, Nepal[14]. Helminth infections may affect flight performance and predatory effectiveness of wild birds[38] and cause reduction in egg production and overall growth in domesticated birds[7]. Moreover, *A. galli* infections may cause reduction in egg production and overall growth of birds[7] and play a role in transmission of *Salmonella* infections[39,40]. Therefore, poultry industries and zoos should not be exposed to wild birds to avoid potential cause of dreadful diseases in poultry industries and zoos and associated economic loss.

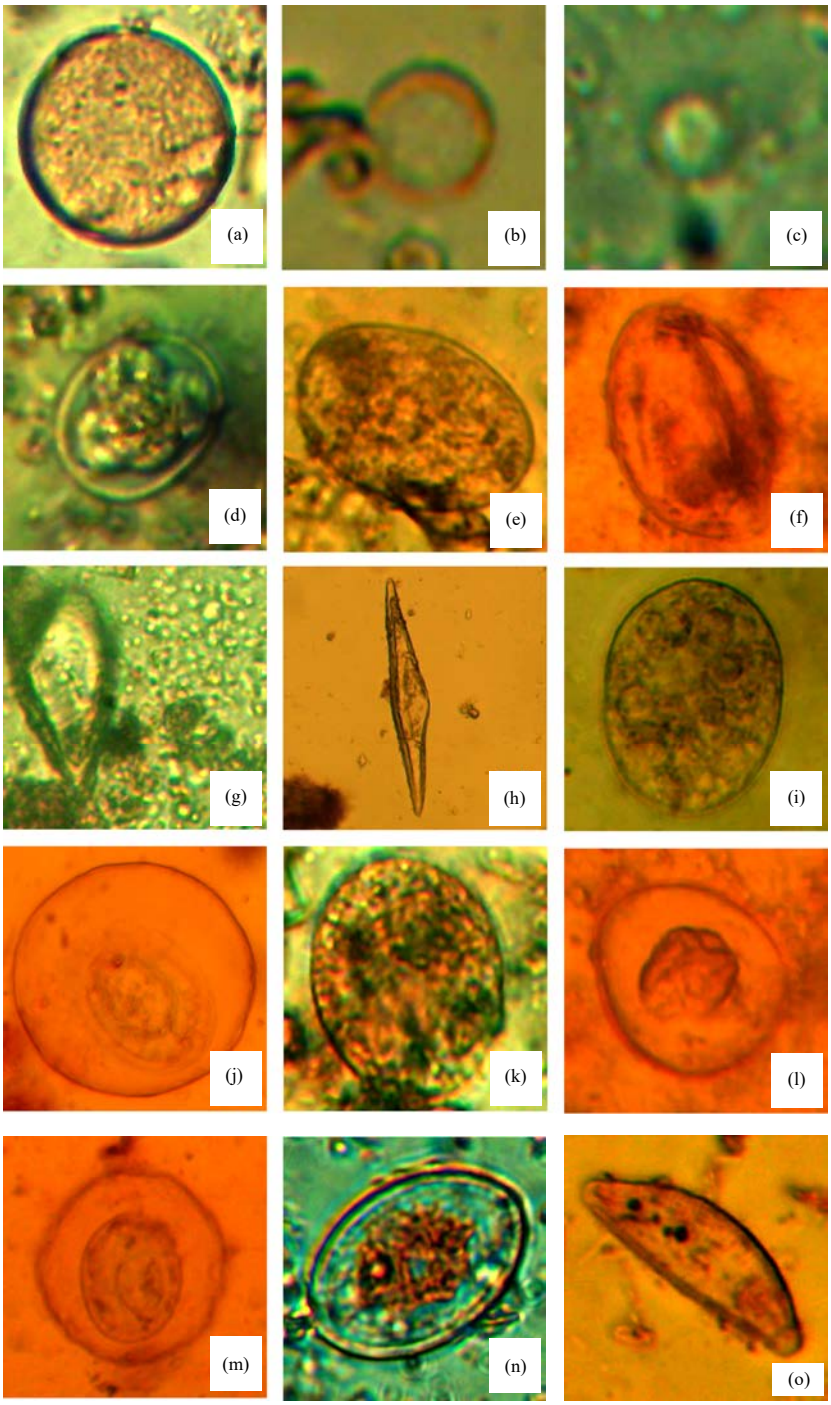

Fig. 1(a-o): Light photomicrograph of protozoans, (a) Unstained cyst of *Blantidium* spp., (b) Unstained oocyst of *Cyclospora* spp., (c) Unstained oocyst of *Cryptosporidium* spp., 1000X magnification, (d) Unstained oocyst of *Eimeria* spp., 400X magnification, unstained eggs of trematodes, (e) Operculated eggs released by the adult *Clinostomum* spp., (f) Egg of *Echinostoma* spp., (g) Egg of *Schistosoma* spp., (h) Egg of *Trichobilharzia* spp., all observed in 400X magnification), unstained eggs of cestodes, (i) *Bothriocephalus* spp., (j) *Choanotaenia* spp., (k) *Diphyllobothrium* spp., (l) *Hymenolepis* spp., (m) *Raillietina* spp., all observed in 400X magnification) and unstained eggs of nematodes, (n) *Ascaridia galli* and (o) *Capillaria* spp., both observed in 400X magnification) (with a background of fecal sediment debris) observed in thin/thick smear of fecal samples collected from wild birds in Chitwan District, southcentral Nepal

Trematodes often infected those wild birds (28%, n = 97) (Table 2). This higher prevalence of trematodes corresponds to the consumption of snails (intermediate hosts) by birds[3,41,42]. Therefore, the wild and domesticated animals including humans are susceptible to the infection by trematodes widely. *Clinostomum* spp. (digenean trematode parasite) infected wild birds in Chitwan District also infested herons from other countries[43,44]. Schistosomatids infecting wild birds in this study area (Table 2) may infect humans causing dermatitis[41,42]. Cosmopolitan distribution of some migratory birds (Table 1) and their exposure to several parasitic infections owing to migratory behavior and the optimum climatic conditions might be the cause of high trematode infection rate in this study area. There are outbreaks of schistosomes and *Trichobilharzia* spp. infections among humans in the USA due to migratory geese birds and snails[41]. Similar outbreak of dermatitis may occur in Nepal as dermatitis-causing trematodes i.e., *Trichobilharzia* spp. (Fig. 1h) and *Schistosoma* spp. (Fig. 1g) infected wild birds in Chitwan District. Therefore, adopting proper prevention strategies against dermatitis is essential in this area. For the effective prevention strategies, wild bird parasites should be studied comprehensively using more sophisticated methods.

This shows importance of knowing parasitic burden of wild birds to develop strategies for prevention of zoonoses and conservation of endangered birds and other wildlife. Therefore, documentation of intestinal parasites in this study has great significance because several zoonotic diseases cause public health, veterinary, economic and social problems in many developing countries including Nepal. Hence, periodic surveillance of the parasitic fauna and assessment of their infection in areas where migratory birds often visit is essential.

Because of financial and time constraints, we could not use the most effective methods such as measurement and staining for identifying intestinal parasites. The quantity of organisms per specimen could be increased if the number of effective techniques for diagnosis and identification of parasites in the available stool samples were involved. Hence, prevalence and diversity of intestinal parasites in the wild birds can be expected greater than this report. *Cryptosporidium* could not be differentiated from *Isospora* oocyst in fecal samples [24]. Probably, *Isospora* occysts might be identified as *Cryptosporidium* oocysts.

CONCLUSION

This study reveals high prevalence of nematode, trematode and cestode infections in wild birds. The majority of wild birds harbored multiple intestinal parasites. These birds may serve as important source of infection by helminthes (particularly, *Ascaridia* spp., *Capillaria* spp. and Schistosomatids) and protozoans (*Balantidium* spp. and *Cryptosporidium* spp.) to other wildlife, zoo birds and commercial birds in Chitwan District and vicinity. Therefore, further in-depth study of parasitic infection in wild birds and their impact on human life and domesticated animals is essential to understand the mode of transmission of intestinal parasites in migratory birds (considering their feeding behavior) to other wildlife and humans. This study also reveals the need of refined studies on the distribution of intestinal parasites among individuals of the migratory and non-migratory birds with the potential of causing zoonoses. Mass education is essential to minimize the risk of transmission of wild bird parasites to animals and humans through contaminated foods and water. Thus, adequate funding supports should be allocated to prepare profile of zoonotic parasites of medical and veterinary importance to ensure well-being of humans, domestic animals and wildlife elsewhere.

SIGNIFICANCE STATEMENT

Since Chitwan District has several lakes and river systems where thousands of migratory birds visit routinely, our snapshot view of intestinal parasites in wild birds indicates risks of protozon and helminth infections in humans and animals in this region of Nepal. In so far as our knowledge, no similar study was conducted in present study sites. Therefore, this study paves the way to sophisticated study to plan prevention strategies against zoonotic diseases.

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