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SHORT COMMUNICATION

Gastrointestinal parasites of zoonotic importance detected in porcine faeces in Chitwan National Park, Nepal

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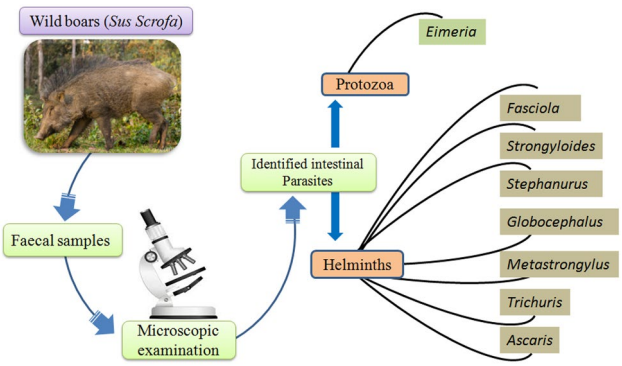
Abstract

Wild boar (*Sus scrofa)* is considered to be a potential source of zoonotic parasites. Wild boars are found at considerable number in and around the Chitwan National Park (CNP). The information regarding their intestinal parasites is limited. A cross-sectional study was carried out to determine the prevalence of gastrointestinal parasites in wild boars in CNP. A total of 100 fresh fecal samples were subjected to microscopic examination using direct smear, floatation and sedimentation method. Overall, 95% fecal samples were found positive for at least one parasite. Prevalence of protozoan parasites was found comparatively higher (70%) followed by nematode (56%) and trematode (12%). Nine gastrointestinal parasites such as *Eimeria* sp. (70% without micropyle and 40% with micopyle), *Fasciola* sp. (12%), *Strongyloides* sp. (56%), strongyle-type nematodes (49%), *Stephanurus* sp. (44%), *Globocephalus* sp. (38%), *Metastrongylus* sp. (12%), *Ascaris* sp. (7%) and *Trichuris* sp. (6%). were recorded. *Eimeria* sp. exhibited the highest prevalence while *Trichuris* had the least prevalence. This study has provided baseline information regarding the diversity of gastrointestinal parasites in wild boars. It requires continuous study at molecular level to explore other species of parasites and verify their zoonotic potential.

Graphical abstract



Keywords Chitwan · Gastrointestinal parasites · Prevalence · Wild boars

Introduction

Wild boars (*Sus scrofa*), characterized by the high fecundity rate, are one of the most widely distributed mammals in the world. Their abode extends from the Western Europe and the Mediterranean basin to Eastern Russia, Japan and South-east Asia (Massei et al. 2015). Also known as 'Bandel' in Nepali, wild boar is native to India, Nepal, Burma, Western Thailand and Sri-lanka. This species is widely distributed

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across Nepal including within all protected areas of lowland Tarai and parts of protected areas in the highland Churia to Annapurna ranges, and also occurs extensively outside protected areas (Jnawali et al. 2011). In conservation status, it has been considered as least concern in global and national contest because of its wild distribution range and an abundance in number (IUCN 2011).

Wild boars like domestic pigs are omnivorous and consume various foods like plant roots, seeds, barks, fungi, animal matters like insects, small amphibians, reptiles, and even carcasses. They have diverse feeding behaviours such as browsing, grazing, rooting and preying, and consume whatever diet is abailable (Ballari and Barrios-Garcia 2014). Domestic pigs and wild boars are susceptible to the similar types of pathogens. Wildlife itself represents a potential reservoir of various pathogens which can infect domestic animals and humans (Ferroglio et al. 2011). As wild boars are considered as potential reservoirs of parasites including helminthes (Meng et al. 2009; Dodangeh et al. 2018), they can transmit diseases to other wild animals, domestic animals and even humans (Jankowska-Mąkosa et al. 2019).

Wild boars host a variety of protozoan parasites such as *Balantidium coli*, *Trichomonas suis, Entamoeba polecki*, *Entamoeba suis, Iodamoeba butschlii*, *Blastocystis*, *Giardia*, *Cryptosporidium* and *Toxoplasma gondii* which may be transmitted to humans from wild boars (Solaymani-Mohammadi et al. 2004; Seifollahi et al. 2016). Similarly, helminthes parasites identified from them are *Ascaris*, *Ascarops*, *Cysticercus tenuicollis, Dicrocoelium dendriticum*, *Globocephalus*, *Gongylonema*, *Hyostrongylus rubidus*, *Macracanthorhynchus hirudinaceus*, *Metastrongylus*, *Nematodirus*, *Oesophagostomum dentatum*, *Physocephalus sexalatus*, *Stephanurus dentatus*, *Strongyloides*, *Taenia hydatigena*, *Trichinella*, *Trichuris suis*, etc. (Meng et al. 2009; Senlik et al. 2011; Silva and Müller 2013; Yagoob et al. 2014; Okoro et al. 2016; Mansouri et al. 2016).

Although the gastrointestinal parasites of domestic pigs are well documented, the information is scanty in relation to the prevalence of GIPs in wild boars. Wild boars are considered an important biotic factor in relation to parasite epidemiology as they frequently change their habitats, disseminate the parasites in large areas and several species of parasites specific to them have also been reported from other wild animals, domestic animals, and even from humans (Panayotova-Pencheva and Dakova 2018). In and around CNP, there are some sporadic reports on crop raiding and even attacks on humans by the wild boar (Lamichhane et al. 2018). These animals may contaminate the water resources and agricultural lands and subsequently increase the chances of transmission of their parasites to human settlements. The main objective of this study was to explore the prevalence of potentially zoonotic GIPs in wild boar in CNP.

Materials and methods

Study area

Established in 1973, Chitwan National Park (27°30′0″ N; 84°20′0″ E) is situated in south central subtropical lowland of inner Tarai of Nepal extending within an area of 952.63 km^2. In altitude it ranges from about 100 m (300ft) in the river valleys to 815 m (2674ft) in the Churia hills. UNESCO has declared CNP a World Heritage Site in 1984 considering its unique ecosystem of international significance. The park consists of a diversity of ecosystems- including the Churia hills, Ox-bow lakes, and the flood plains of the Rapti, Reu and Narayani Rivers. CNP shares its eastern boundary with the Parsa Wildlife Reserve. With the tropical and subtropical forests, the average temperature in this area is about 25°C during October to February and rises up to 43°C during March to June. CNP has been home to a total 75 species of mammals belonging to 24 families, and several other animals. The study area comprises Tikauli forest from Rapti river to the foothills of the Mahabharat extending an area of 175 km^2 with humid subtropical monsoon and high humidity throughout the year (DNPWC 2017).

Sample collection

Ethical approval was obtained from the authorities of CNP for sampling fecal samples. During April to May 2017, fresh faecal samples were collected in the morning between 7 and 9am with the help of research assistants. Wet faecal deposits with presence of mucous were considered as fresh fecal samples. Using sterilized disposable gloves about 10 g freshly laid faecal sample was picked up and kept in sterilized vial with 2.5% potassium dichromate as the preservative. Each sample was observed macroscopically for its consistency, and if any adult worms or proglottids were present. Collected samples were carried in ice to the Parasitology Laboratory at the Central Department of Zoology, Tribhuvan University, Kathmandu and stored at 4°C until examination.

Laboratory analysis

All samples were processed through saline and iodine wet mount, and floatation and sedimentation techniques were applied to concentrate the protozoan cyst/oocyst and helminth eggs as described by Soulsby (2012) and Zajac and Conboy (2012) with slight modification. In floatation technique, approximately 3gm of each faecal sample was placed in a separate beaker and added with 42ml of water, gently mixed with the help of sterilized wooden spatula and filtered through tea strainer. The filtrate was poured into falcon tube and centrifuged at 1000rpm for 5min. After decanting the

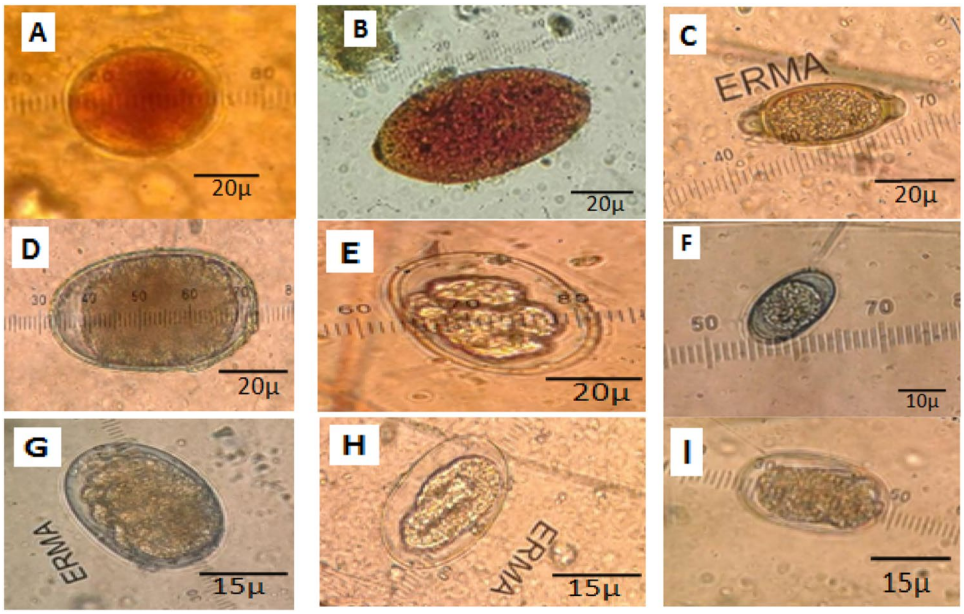

Fig. 1 Gastrointestinal parasites identified from wild boars. The photomicrographs of the images were taken under 400×magnification. **A.** Egg of *Ascaris* sp.; **B.** Egg of *Fasciola* sp.; **C.** Egg of *Trichuris* sp.; **D.** Egg of *Stephanurus* sp.; **E.** Egg of *Globocephalus* sp.; **F.** Oocyst of *Eimeria* sp.; **G.** Egg of strongyle nematode; **H.** Larvated egg of *Strongyloides* sp. and **I.** Egg of *Metastrongylus* sp

supernatant, the sediment was mixed with saturated sodium chloride (NaCl) solution followed by centrifugation. The saturated NaCl solution (floating fluid) was added to develop a convex meniscus at the top of each falcon tube and one drop of methylene blue was added to stain nuclear structure of protozoan cysts/oocysts. A cover slip was placed on top of the falcon tube. The preparation was left undisturbed for half an hour, the cover slip was transferred on to a slide and examined under high power (×400).

In sedimentation technique, about two gram of faecal sample was thoroughly homogenized with 12ml of normal saline, centrifuged at 12,000rpm for five minutes and the supernatant was discarded. The tube was filled with 10ml of 10% formalin and 3ml ethyl acetate, centrifuged at 12,000rpm for five minutes and the sediment was examined under microscope (×400). The oocyst, eggs and larvae were identified on the basis of morphological characters. The intensity of parasite infection was determined by counting the number of oocysts and eggs per high field microscopic field and categorized as low (≤2), mild (2–5), moderate (5–10) and heavy (≥10).

Results

The results revealed that 95% of the faecal samples were found positive for at least one parasitic stage. Overall ten genera of GIPs were identified. It includes *Eimeria* sp. [(40% with micropyle, and 70% without micropyle)], *Fasciola* sp. (12%), *Ascaris* sp. (7%), *Stephanurus* sp. (44%), *Strongyloides* sp. (56%), strongyle-type nematodes (49%), *Metastrongylus* sp. (12%), *Trichuris* sp. (6%) and *Globocephalus* sp. (38%) (Fig. 1). There was highly significant difference between the genus wise prevalence of GIPs in wild boars

Table 1 Prevalence and infection intensity of GIPs in wild boars (n = 100)

Identified parasites		Prevalence (%)	Intensity of infection			
			Light	Mild	Moderate	Heavy
Protozoa	*Eimeria* sp. (without micropyle)	40	18 (45%)	10 (25%)	7 (17.75%)	5 (12.5%)
	Eimeria sp. (with micropyle)	70	34 (48.57%)	17 (24.28%)	12 (17.14%)	7 (10%)
Trmatode	*Fascioloa* sp.	12	8 (66.66%)	4 (33.33%)	–	–
Nematodes	*Ascaris* sp.	7	4 (57.14%)	3 (42.85%	–	–
	Stephanurus sp.	44	10 (22.72%)	13 (29.54%)	6 (13.63%)	15 (34.09%)
	Trichuris sp.	6	4 (66.66%)	2 (33.33%)	–	–
	Metastrongylus sp.	12	2 (16.66%)	7 (58.33%)		3 (25%)
	Globocephalous sp.	38	8 (21.05%)	12 (31.57%)	10 (26.31%)	8 (21.05%)
	Strongyloides sp.	56	18 (32.14%)	12 (21.42%)	8 (14.28%)	18 (32.14%)
	Strongyle-type eggs	49	18 (36.73%)	7 (14.28%)	10 (20.40%)	14 (8.57%)

($\chi^2 = 208.34$, df = 9, $P < 0.05$). Only one genera of trematode namely *Fasciola* sp. (12.63%) was recorded. Similarly among the nematodes, *Strongyloide*s sp. (58.94%) showed the highest prevalence followed by strongyles (51.57%), *Stephanurus* sp. (46.31%), *Globocephalus* sp. (40%), *Metastrongylus* sp. (12.63%), *Ascaris* sp. (7.36%) and *Trichuris* sp. (6.31%) (Table 1).

Of the total parasite positive faecal samples, double infection showed the highest rate followed by multiple (50.53%), triple (20%) and single infection (10.53). *Eimeria* sp. was seen to have high intensity while most of the positive samples had light intensity of *Fasciola* sp. while in most of the nematodes like *Stephanurus* sp. *Strongyloides* sp., strongyle-type nematodes, *Metastrongylus* sp. and *Globocephalus* sp. had heavy intensity.

Discussion

There is lack of comprehensive studies and very limited data on the prevalence of intestinal parasites in wild boar in Nepal. This study revealed that 95% faecal samples were found to be positive for at least one type of parsite oocysts or eggs. The higher prevalence of GIPs in our study is comparable to that of Jarvis et al. (2007) who quoted that none of all the examined carcasses of wild boars from Central Spain and those imported from France were free of helminths. Likewise, Dodangeh et al (2018) reported about 62% infection during necropsy examination. Overall *Eimeria* sp. was found to be the most prevalent parasite in this study which is worth comparing with Pilarczyk et al (2004) who documented 58.5% prevalence of *Eimeria* in wild boar in northeast Poland. However, Moretta et al (2010) and Tomass et al (2012) reported low prevalence of *Eimeria* sp. High prevalence of *Eimeria* species in our study area may be attributed to the overcrowding of other wild animals and overlapping habitat. The variation might be related to the environmental factors and sampling season.

Among helminths, *Strongyloides* sp., strongyle-types, *Stephanurus* sp. and *Globocephalus* sp. showed the high prevalence. *Strongyloides* worms may be present in a host as a parasitic and free living form in the soil as they have direct life cycle and causes infection by ingestion of contaminated vegetation and drinks with the larva of this species (Staphen and Gareth 2003). The present prevalence rate of *Strongyloides* sp. was higher than the earlier reports (Mudim et al. 2004; Moretta et al. 2010; Dadas et al. 2016). Since wild boars are associated with feeding of earthworms, beetles, bugs and numerous larvae which contribute as intermediate or paratenic hosts for various helminthic fauna and also habitat overlap, and competition for food also contribute the higher prevalence of *Strongyloides* in animals (Ezenwa 2002). Similarly, the prevalence of *Globocephalus* sp. in this study was higher than reported by Senlik et al (2011) and Dadas et al (2016) who respectively recorded 22% and 74% prevalence, and the prevalence of *Stephanurus* sp. might be due to the high persistant rainfall and high humidity.

Furthermore, *Fasciola* sp., *Metastrongylu*s sp., *Ascaris* sp. had similar prevalence as reported by Begum et al (2014). However, a study from Northen Ethopia indicates low prevalence just only 1.8% (Tomass et al. 2012). Factors such as presence of reservoir hosts, presence of snail intermediate host, and ability of *Fasciola hepatica* to colonise and to adopt new hosts affects its spread in livestock in a specific area. The prevalence regarding *Metastrongylus sp*. was found to be similar to Mudim et al. (2004) while Yagoob et al (2014) and Mansouri et al (2016) found higher prevalence of 66.6%, 34% and 68% respectively. The difference in prevalence rate might be caused by variation in geographical distribution of different earthworm species which form a part of diet of wild boars and act as intermediate hosts for these parasites. *Ascaris* sp. and *Trichuris* sp. are the most important and common gastrointestinal worms in pigs. Both are more common in growing pigs than in adult ones (Lee 2012). The prevalence rate of *Ascaris* sp. and *Trichuris* sp. was nearly similar as reported by Boral et al. (2009) whereas Eijck et al. (2005), Mundim et al. (2004), Nur-E-Azam et al (2015) and Dadas et al (2016) recorded higher prevalence. However, neither proglottids nor eggs of cestodes were encountered in this study.

The reasons for higher percentage of overall positivity of parasitism in wild pigs might be associated to their diversified feeding activities, distribution in the peripheral regions in the wild, consumption of different types of intermediate host animals, consumption of feed materials contaminated by excreta of the co-existing wild animals that most often share the habitat in the wild environment, and absolute lack of health care related measures.

Conclusions

The prevalence and diversity of parasites especially intestinal helminths has been recorded very high among the wild boars. This study has at least provided a base line data on the intestinal parasites in the wild boars inhabiting CNP area. As the habitat in CNP used by the wild pigs is overlapping, a comparative and comprehensive study of GIPs is necessary in order to find the zoonotic potential and probalble risk among other wild mammals and humans.

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Author contributions All authors equally contributed to the study in conceptualization, sample collection, laboratory analysis, interpretation, writing manuscript, editing the draft and approval of the final manuscript.

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Declarations

Conflict of interest Authors declare that there is no conflict of interest.

Ethical approval The present study was conducted after obtaining the permission from Chitwan National Park authorities, and none of the animal was harmed.

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