Prevalence and Burden of Gastrointestinal Parasites in Stray Cattle of Kathmandu Valley

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Research Article

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Abstract

Gastrointestinal parasites (GIPs) are ubiquitous among cattle resulting severe infection. Prevalence of GIPs in stray street cattle may pose risk of dissemination of parasites of zoonotic importance. This study was conducted to determine the prevalence of GIPs in stray cattle of Kathmandu valley. Hundred (n=100) freshly voided dung samples were collected from eight places. The samples were processed using concentration method for microscopic examination, and modified McMaster technique for quantification of mean eggs/oocysts per gram of feces (EPG/OPG). Results revealed that 72% of the cattle were found positive for one or more species of GIPs and nine genera of GIPs were recorded (*Eimeria*, *Ostertagia*, *Haemonchus*, *Trichostrongylus*, *Capillaria*, *Trichuris*, *Toxocara*, *Fasciola* and *Paramphistomum*). The prevalence of parasitic infection was higher in male (73.68%) than in female (69.76%). The prevalence was found to be highest in adults (63.89%) followed by heifers (27.78%) and calves (8.33%). Approximately 76% of the cross breed and 65% local breed of cattle were positive for parasitic infection. The parasites differed both in prevalence and intensity, *Eimeria* sp. being the most prevalent (27%) with highest intensity (858.02 OPG $\pm$ 63.46 SD). To our information, this is the first research of its kind in relation to stray cattle in Nepal. Our findings reveal that there is burden of helminth infections of zoonotic and socioeconomic importance in the straycattle. Therefore, it warrants regular inspection, relevant preventive measures and molecular detection of parasites.

Introduction

One of the major impediments for commercial livestock production in tropical and subtropical countries is intestinal parasitic infections (Hoste et al. 2006; Kakar et al. 2008). Almost all ages of cattle are infected by a variety of groups of intestinal parasites, however the mortality associated with it is very less. The gastrointestinal parasites (GIPs) cause reduced weight gain, digestive disturbances, lowered production, impaired reproductive performance, mortality (Raza et al. 2007). Reduced output of animal products, by products, manure and traction directly affect in the role of cattle in ensuring food security most particularly in developing countries (Rajakaruna and Warnakulasooriya 2011).

The problems posed by gastrointestinal parasitism are often neglected and overlooked since most of the infected cattle show little obvious signs during their reproductive life however the effects are gradual and chronic (Raza et al. 2010). The most important predisposing factors of helminth infestation in cattle are grazing habits (Brito et al. 2013), climate (Fox et al. 2012), nutritional deficiency (de Gier et al. 2014), pasture management (De Mendonca et al. 2013), immunological status (Mabbott 2018; Weatherhead et al. 2020), presence of vector (Otranto et al. 2013), intermediate host (Chubb et al. 2010), the number of infective larvae and eggs in the environment (Adedipe et al. 2014). Favorable environmental condition such as temperature, humidity and several others play an important role for the growth of GIPs, and grasslands are one of the main sources of gastrointestinal parasitic diseases to animals (Mondal et al. 2000) from where parasites get transmitted to one another by fecal-oral route. The parasites of the digestive system particularly small intestine and large intestine infest grazing animals (Taylor et al. 2007). Therefore, grazing land play important role to make animal healthy. In general, younger animals and animals under stress are most likely to show signs of parasitism (Sahoo et al. 2002).

Rampant movement of stray cattle in the streets and other public places mostly around temples, under bridges, around the river banks and even in the main road are a common observation in Kathmandu valley. Prevalence of GIPs in stray street cattle may pose risk of dissemination of parasites of zoonotic importance. Therefore, this research was conducted to determine the prevalence and intensity of gastrointestinal parasites along with susceptibility of groups (i.e., local vs cross breed or male vs female or calves vs heifer vs adult) in stray cattle of Kathmandu valley.

Materials And Methods

Study area

Kathmandu district is located in Bagmati province (Fig.1) with an area of 395 km² and 1,744,240 inhabitants (NPHC 2011). Kathmandu valley is the capital and largest city of Nepal standing at an altitude of 1400masl, characterized by moderately hot during March to April. The maximum temperature varied from 25.3°C to 28.2°C and minimum 8.2°C to 11.7°C, and the relative humidity varied from 53% to 61% during the study period (Anonymous 2019).

Methods

Sample collection and preservation:

The minimum sample size for this study was determined by using Thrusfield (2005) formula. Ethical approval was obtained from Tribhuvan University, animal welfare policy was strictly followed and the cattle were not harmed by any means. During March to April, 2019, 100 fresh dung samples (approximately 5-10gm from each animal) were collected from eight locations immediately after defecation using sterilized wooden applicator, stored in plastic container with 2.5% potassium dichromate as preservative and labeled with specific identification number. While sampling the cattle were classified as calf (0-1 years), heifer (1-3 years), adult (≥ 3 years), local breed and cross breed with the aid of trained veterinary technician. The collected samples were immediately fetched to the Parasitology Laboratory of Central Department of Zoology, Tribhuvan University and stored at 4°C until examination.

Questionnaire survey

Questionnaire survey was conducted among forty people generally vendors, local shopkeepers, street shop owners in and around the sampling area. It mainly included queries regarding the total number of roaming cattle in the sampling spot, their regular place of living, approximate distance travelled for finding food and water, types of food materials, sources of water, chances of food being supplied by local people and mixing up with other stray animals.

Identification and quantification of GIPs

Qualitative procedures like normal saline and iodine wet mount, flotation and sedimentation techniques were used with slight modification. At least, two smears were prepared from each sample for each technique to identify eggs and oocysts based on their morphological characters (Soulsby 1982; Foreyt 2001; Taylor et al. 2007; Zajac and Conboy 2011). The size of coccidian oocysts and helminth eggs were measured using calibrated ocular micrometer under 400x magnification and compared with the available data (Foreyt 2001; Zajac and Conboy 2012). Parasite positive faecal samples were subjected to McMaster technique with slight modification to quantify the eggs/oocysts per gram (EPG/OPG) of faecal sample (Levecke et al. 2011). The degree of infection was classified as light (50-799EPG/OPG), moderate (800-1200EPG/OPG) and higher (>1200 EPG/OPG) according to Urquhart et al. (1994).

Data analysis

The data were entered into the MS-Excel 2010 and statistical analysis was performed using “R”, version 3.5.2 software package with Chi-squared test to compare the differences between the groups, and mean values were compared by Duncan's multiple range test (DMRT). In all cases 95% confidence interval (CI) and $p < 0.05$ was considered for statistically significant association.

Results

Prevalance of GIPs

Of the one hundred samples examined, 72% cattle were found to be positive for one or more GIPs. A total of nine genera of parasites were identified. *Eimeria* (27%) with highest prevalence was the only parasite witnessed among protozoans. Among nematodes, *Ostertagia* (9%) had the maximum prevalence followed by *Haemonchus* (7%), *Trihcostrongylus* (5%), *Capillaria* (3%), Strongyle-type (3%), *Trichuris* (2%) and *Toxocara* 1% (Table 1). Similarly, *Fasciola* (11%) and *Paramphistomum* (4%) were observed among trematodes, and none of the cestodes were recorded. The photographs of the identified coccidian oocysts and helminth eggs have been illustrated (Fig. 2).

The results revealed that the parasite prevalence was higher in male (73.68%) than in female (69.76%), but there was no statistical significance ($p > 0.986$) (Table 2). Prevalence of GIPs was recorded to be 8.33%, 27.78% and 63.89% in calves, heifers and adults respectively (Table 3). The prevalence was statistically significant ($p < 0.025$) in relation to the age of stray cattle. Regarding breed-wise analysis of GIPs prevalence, 64.86% ($n=24$) of local breeds and 76.19% ($n=48$) of cross breeds revealed positive result for parasitic infection (Table 2). There was no statistical significant association of infection between local breeds and cross breeds ($p = 0.738$).

Area-wise prevalence of GIPs shows that highest percentage was observed in Kirtipur area (83.33%) followed by Kalanki (80%) and Gaushala (73.33%). *Eimeria* was recorded in all the sampling sites with its highest prevalence in Budhanilkantha (42.85%). None of the trematodes were recorded from Budhanilkantha and Naksal area (Table 4).

Single and mixed infection of GIPs

Out of 72 cattle shedding parasite oocysts and/or eggs, 36.11% (n=26) were infected with single parasites where *Fasciola* was dominant, and 68.89% (n=46) had mixed infection which consisted of *Eimeria* plus *Fasciola* being the most common. Among mixed infection, a great majority of cattle had duplet infection (86.95%) where the association of *Eimeria* was maximum followed by *Fasciola*, and minority (13.04%) had triplet infection (Table 5).

Intensity of GIPs

Approximately 58% of the stray cattle were found to have lower, 33 % moderate and only 8% higher level of infection of GIPs. The higher intensity of infection was noticed only for *Eimeria*, and helminths had lower to moderate intensity. The highest mean OPG (858.02±63.46) was recorded in *Eimeria* while among the nematodes, *Capillaria* had the highest mean EPG (544.44±19.24), *Haemonchus* and *Trichuris* had almost same mean EPG, and among trematodes *Fasciola* had highest mean EPG (408.33±62.51) (Table 1).

Behavioral observation of stray Cattle

The questionnaire survey and field observation have provided us some important information. The stray cattle did not have a fixed location as they were often witnessed moving for a long distance for finding food and water, and some of them were even found travelling more than 5 km distance. They generally rely on vegetable garbage thrown from the vegetable markets and leftover food available along with other wastes. They also graze along the river banks and other open fields along the road side. Sometimes, they are also fed by the local people, pedestrians, pilgrims, and restaurant owners. Great majority of the stray cattle take rest during day and night under bridges, some under the small sheds or tents outside the shop most often accompanied by the stray dogs, and others are found in the open places along the road side, river and wherever possible even during sweltering hot summer days and the time of heavy rain fall.

Discussion

The finding of our study revealed that 72% of the stray cattle roaming at various regions in Kathmandu valley had burden of gastrointestinal parasite infection which is in agreement with prevalence of gastrointestinal parasites in cattle and buffaloes in Jabalpur, India (Markole et al. 2016) and 75.46% from Bangladesh reported by Ilyas et al. (2016). Yet, prevalence observed in our study was quite lower than 95.5% prevalence reported from Southern Ghana (Squire et al. 2013), 86.9% prevalence among dairy cattle reported from Taiwan (Huang et al. 2014) and 84.8% prevalence reported from Dhaka, Bangladesh (Rashid et al. 2015). In contrast, Jittapalapong et al. (2011) and Gunathilaka et al. (2018) have reported relatively lower prevalence in domesticated cattle managed in intensive farms at the rate of 46.6 % and 11.56%, respectively. This variation in finding might be due to the difference in the number of fecal sample examined from free ranging and semi-intensive cattle, season of sampling and climatic condition of the study area. Similar type of prevalence study was reported by Shirale et al. (2008), Marskole et al. (2016), Takeet et al. (2016) and Dogo et al. (2017).

Fasciola was found to have highest prevalence (11%) among all the helminth parasites recorded. However, nematode topped the overall prevalence (30%) followed by *Eimeria* (27%) and trematodes (15%). The overall prevalence of fascioliasis was 51% in cattle examined in Mahottari and Dhanusha (Yadav et al. 2015). Based on molecular phylogenetic analysis *Fasciola* flukes have been reported from eight districts of Nepal including Bhaktapur and Kavrepalanchok (Shoriki et al. 2014). Our finding is worth comparing with Choubisa and Jaroli (2013) who reported 15.18% prevalence of *Fasciola* spp. in various domestic ruminants and 18.46% in cattle. The prevalence of *Fasciola* spp. was higher (16.67%) than that of *Paramphistomum* spp. (9.37%) in yak in lower Mustang, Nepal (Acharya et al. 2016) while Maitra et al. (2014) recorded 12.4% prevalence of various species of paramphistomes in domestic ruminants. The prevalence of trematodes in our study is remarkable, but there is not much information regarding the presence of suitable intermediate molluscan hosts for larval development in our study area. It might be because the stray cattle may have harbored such flukes for a long time since when the cattle were taken by the farmers. The overall prevalence of helminth infection (46%) noted in this study is almost closer to that of Adedipe et al. (2014) while it differs from those reported by Leon et al. (2019) who found 22.3% prevalence of *Fasciola* and 16.5% of strongylid nematodes with 17.4% of *Eimeria*. Heidari et al. (2014) reported nine species of *Eimeria* with overall infection rate of 8.25%. A very high prevalence of *Eimeria* (94.7%) was reported from dairy cattle in Costa Rica (Jimenez et al. 2007) while it was lower among the female cattle in Bali, Indonesia (Indraswari et al. 2017). Such variations may be attributed

to the anthelmintic treatment history, body conditions of cattle and management system (Bacha and Haftu 2014). But in our study there is neither record of anthelmintic treatment nor other previous information as all the stray cattle were sampled from various sites where they were found gathered, roaming, and feeding whatever was available. This might also cause variation in the prevalence of GIPs in cattle. Our study has recorded *Toxocara*, a common worm found in canine and feline hosts, in one of the cattle. This might be the consequence of their close association with dogs, and probable dog-faecal contamination to the cattle food.

The overall gastro-intestinal parasitic infection was similar between male and female cattle. This result is in concordance with Marskole et al. (2016) and Maharana et al. (2016). Male and female grazing on the same grass land or forest, sharing the same shed and supplied with same grains and grass, that might have provided equal chance of transmission of any parasitic diseases (Bacha and Haftu 2014). The prevalence rate of GIPs regarding age-wise classification in calves (43%), heifers (76%) and adults (77%) appears to be similar (i.e., rate of infection growing with growing age) to that of Bacha and Haftu (2014) as both have similar study design. Similarly, the higher prevalence recorded in the adult cattle could be the result of their prolonged exposure to the infective stages of GIPs. We have also noticed more mixed infections than single infection among older cattle. The younger cattle were characterized by the presence of majority of duplet infection than triplet. The mixed infection has been considered to be a main cause of morbidity and reduced production from the livestock (Bersissa et al. 2011) and at the same time it accelerates the host susceptibility to other parasitic infections (Wang et al. 2006).

The quantification of parasite egg and oocysts is generally performed in order to determine the extent of pasture contamination by the infected animals while grazing, to assess the efficacy of drug treatment and for examination of drug resistance (Zajac and Conboy 2012). Realizing the same fundamental principle, we also quantified the protozoan oocysts and helminth eggs per gram of faecal sample. Leon et al. (2019) documented similar range of OPG for *Eimeria* (820-1270) which was more or less closer to our findings. Wadhwa et al. (2011) reported EPG for strongyle species ranging between 200-1000 with an average of 504 ± 245.41 in cattle which is comparable to our findings. Contrary to our results, Laha et al. (2013) recorded higher the mean EPG in nematodes (50-4000) and OPG for coccidian parasites (50-1400). In relation to the mild climate of Kathmandu valley, absence of parasite control programs in the stray cattle, continuous movement of cattle and taking food whatever available and grazing in the open places, the mean EPG in case of *Fasciola* (408.33 ± 62.51) and *Paramphistomum* (379.16 ± 7.21) compared to nematodes was recorded to be lower than expected. It might be attributed to the type of floatation solution as the trematode eggs are heavier than that of nematodes. The reliability and efficiency of the quantification of parasite eggs depends on the amount of faeces examined (Mes et al. 2001), the floatation solution used, dilution ratio and the McMaster slide area (volume) examined (Cringoli et al. 2004), yet our method (i.e., 5 gram sample with concentration method) should not allow such situation.

Conclusion

This study revealed relatively high (compared to domesticated) but moderate prevalence of gastrointestinal protozoan and helminth parasites in the stray cattle with low to moderate level of infection intensity. Each sampling site represented at least one or more types of GIPs. The quantified EPG/OPG also suggests that there is higher chance of contamination of soil which may result in the transmission of zoonotically important parasites. Therefore, to mitigate the cattle borne parasite burden, public awareness, proper management of the street cattle, administration of anthelmintics and other relevant control measures need to be underlined. Further studies are relevant at the molecular level which will provide obvious evidences of the zoonotic potential of the parasite and therefore, it would provide way forward to take necessary steps for preventive measures.

Declarations

Ethical Consideration

No animals were harmed during this research work.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

BT: Conceptualization, Methodology, Investigation, Software, and Writing-Original draft preparation. **RPP:** Conceptualization, Methodology, Writing-Reviewing. **PD:** Conceptualization, Methodology, Supervision, Investigation and Writing-Reviewing and Resources.

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Tables

Table 1 Overall prevalence of protozoan and helminth parasites in abandoned cattle (n=100) and intensity of infection in terms of mean eggs/oocysts per gram (EPG/OPG) of feces

Identified parasites		Prevalence (%)	Eggs/oocysts per gram of feces		Intensity of infection in terms of egg/oocyst per gram (EPG/OPG) of faeces		
			Range	Mean $\pm$ SD	Light (50-799) (%)	Moderate (800-1200) (%)	Higher (>1200) (%)
Protozoa	<i>Eimeria</i>	27	450-1500	858.02 $\pm$ 63.46 ^a	14 (19.44)	7 (9.72)	6 (8.33)
Nematodes	<i>Ostertagia</i>	9	100-850	422.60 $\pm$ 17.85 ^b	6 (8.33)	3 (4.16)	-
	<i>Haemonchus</i>	7	200-950	616 $\pm$ 71.54 ^c	3 (4.16)	4 (5.55)	-
	<i>Trichostrongylus</i>	5	150-900	500 $\pm$ 60.82 ^c	2 (2.77)	3 (4.16)	-
	<i>Capillaria</i>	3	300-900	544.44 $\pm$ 19.24 ^c	3 (4.16)	-	-
	Strongyle-type*	3	-	-	-	-	-
	<i>Trichuris</i>	2	200-1050	616.67 $\pm$ 104.08 ^c	1 (1.38)	1 (1.38)	-
	<i>Toxocara</i>	1	150-200	183.33 $\pm$ 28.86 ^d	1 (1.38)	-	-
Trematodes	<i>Fasciola</i>	11	250-550	408.33 $\pm$ 62.51 ^b	11 (9.72)	-	-
	<i>Paramphistomum</i>	4	200-500	379.16 $\pm$ 7.21 ^e	4 (5.55)	-	-
Total		72			42 (58.33)	24 (33.33)	6 (8.33)

*EPG not counted. Same superscript within the column are not significantly different (P>0.05) in DMRT. Percentage of light, moderate and severe infection has been calculated out of total 72 infected abandoned cattle.

Table 2 Prevalence of GIPs based on the gender and breed of abandoned cattle

Cattle group		No. of samples	Prevalence	χ^2 /p-value
Gender	Male	57	42 (73.68)	χ^2 =0.0003 p-value = 0.986
	Female	43	30 (69.76)	
	Total	100	72 (72)	
Breed	Local breed	37	24 (64.86)	χ^2 =0.1112 p-value = 0.738
	Cross breed	63	48 (76.19)	
	Total	100	72 (72)	

Table 3 Prevalence of GIPs within different age groups of abandoned cattle

Cattle type	Total	Female	Positive (%)	Male	Positive (%)	Total Positive (%)	χ^2 /p-value
Calf (0-1 Year)	14	2	1 (50.00%)	12	5 (41.67%)	6 (42.86%)	χ^2 = 12.562 p-value = 0.02499<0.05
Heifer (1-3 Year)	17	0	0	17	13 (76.47%)	13 (76.47%)	
Adult (>3 Years)	69	41	29 (70.73%)	28	24 (85.71%)	53 (76.81%)	
Total	100	43	30 (69.77%)	57	42 (73.68%)	72 (72.00%)	

Table 4 Prevalence of GIPs in abandoned cattle based on the sampling sites

Types of parasites		Positive cases (Prevalence %)							
		Gaushala (n=30)	Balkhu (n=20)	Budhanilkantha (n=7)	Kirtipur (n=12)	Naksal (n=4)	Kalimati (n=18)	Jorpati (n=4)	Kalanki (n=5)
Protozoa	<i>Eimeria</i>	8 (26.67)	4 (20)	3 (42.85)	2 (16.67)	2 (50)	6 (33.33)	1 (25)	1 (20)
Nematodes	<i>Ostertagia</i>	3 (10)	2 (10)	1 (14.28)	1 (8.33)	0	2 (22.22)	0	0
	<i>Haemonchus</i>	2 (6.67)	1 (5)	0	1 (8.33)	0	2 (22.22)	0	1 (20)
	<i>Trichostrongylus</i>	1 (3.33)	1 (5)	0	1 (8.33)	1 (25)	0	1 (25)	0
	<i>Capillaria</i>	1 (3.33)	0	0	1 (8.33)	0	0	0	1 (20)
	Strongyle-type	1 (3.33)	0	1 (14.28)	1 (8.33)	0	0	0	0
	<i>Trichuris</i>	0	1 (5)	0	0	0	1 (11.11)	0	0
	<i>Toxocara</i>	0	1 (5)	0	0	0	0	0	0
Trematodes	<i>Fasciola</i>	4 (13.33)	1 (5)	0	2 (16.67)	0	2 (22.22)	1 (25)	1 (20)
	<i>Paramphistomum</i>	2 (6.67)	0	0	1 (8.33)	0	1 (11.11)	0	0
Total		22 (73.33)	11 (55)	5 (71.78)	10 (83.33)	3 (75)	14 (77.78)	3 (75)	4 (80)

Table 5 Pattern of mixed infection of GIPs in abandoned cattle

Infection type	Combination of parasites	Number of cases (%)
Duplet	<i>Eimeria</i> + <i>Fasciola</i>	11 (23.91)
	<i>Eimeria</i> + <i>Paramphistomum</i>	6 (13.04)
	<i>Eimeria</i> + <i>Ostertagia</i>	9 (19.57)
	<i>Eimeria</i> + <i>Trichostrongylus</i>	4 (8.70)
	<i>Eimeria</i> + <i>Haemonchus</i>	2 (4.34)
	<i>Fasciola</i> + <i>Haemonchus</i>	3 (6.52)
	<i>Fasciola</i> + <i>Ostertagia</i>	2 (4.34)
	<i>Fasciola</i> + Strongyle-type eggs	2 (4.34)
	<i>Paramphistomum</i> + <i>Haemonchus</i>	1 (2.17)
	Total	40 (86.95)
Triplet	<i>Eimeria</i> + <i>Fasciola</i> + <i>Ostertagia</i>	2 (4.34)
	<i>Fasciola</i> + <i>Haemonchus</i> + <i>Trichostrongylus</i>	1 (2.17)
	<i>Trichostrongylus</i> + <i>Haemonchus</i> + <i>Capillaria</i>	2 (4.34)
	<i>Trichostrongylus</i> + <i>Trichuris</i> . + <i>Ostertagia</i>	1 (2.17)
	Total	6 (13.04)
Grand total		46 (63.89)

Figures

Figure 1

Map showing sampling sites.

Figure 2

Gastrointestinal parasites identified from abandoned cattle. **A** and **B**: *Eimeria* spp. (17.92-22.4µm), **C**: *Ostertagia* spp. (64.96 × 35.84µm), **D**: *Haemonchus* spp. (73.92 × 38.23µm); **E**: *Trichostrongylus* spp. (53.76 × 39.21µm), **F**: *Capillaria* spp. (58.24 × 24.7µm), **G** and **H**: Strongyle-type (58.24 × 41.20µm), **I**: *Trichuris* spp. (67.2 × 37.13µm), **J**: *Toxocara* spp. (56.0 × 54.21µm), **K**: *Fasciola* spp. (156.8 × 72.15µm) and **L**: *Paramphistomum* spp. (145.6 × 71.68µm).