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Risk factor analysis for the prevalence of gastrointestinal parasites found in large ruminants in Lower Dir Khyber Pakhtunkhwa Pakistan

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ABSTRACT

The present study was designed to investigate the prevalence of gastrointestinal (GI) parasites in cattle and buffaloes of Lower Dir Khyber Pakhtunkhwa, Pakistan. The presence of the eggs, cysts, and oocysts of GI parasites in fecal samples were detected using direct smear methods and concentration techniques including floatation, centrifugation, and sedimentation. Identification of recovered fecal stages were determined by morphology using size and appearance of the recovered eggs, cysts, and oocysts. A total of 314 fecal samples were collected from the different Tehsils (Administrative Districts) and analyzed through microscopy. A higher prevalence was observed in the buffalo than the cow population. A total of 184 samples were positive for GI parasites of which 109/196 (55.61%) were from cattle, whereas 75/118 (63.55%) were from buffaloes. The minimum number of strongyle eggs detected in all the samples were 136.39 eggs/g (EPG). The mean EPG in cattle was 143.30 and 122.56 in buffaloes. The open-source water prevalence of GI parasites was higher than the other sources in cattle and the second highest after tap water in buffaloes. The seasonal prevalence of GI parasites ranged from 32.39% (23/71), in spring to 68.8% (86/125) in summer in cattle. In was For buffaloes the infection prevalence was 52.94% (27/51) and 71.64% (48/67) in spring and summer, respectively. Gastrointestinal parasites are a serious problem in cattle and buffaloes in the lower district of Dir Khyber Pakhtunkhwa Pakistan. In general, the burden of parasitic infection was low in most animals that received previous anti-parasitic treatment.

1. Introduction

The presence of gastrointestinal parasites (GI) is considered among the most critical health problems in pasture-raised cattle. Infections with parasites is one of the main causes of economic losses in the dairy industry due to stunting, low productivity, and the increased susceptibility to other infections (Kamal et al., 2021; Wadhwa et al., 2011). Nematode parasitism can cause changes in milk's nutritional characteristics, such as decreased fat, protein, and lactose content (Rinaldi et al., 2007). Domestic animals can become infected with different parasites and spread them to other animals in their environment (Boomker et al., 1989).

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Several helminths are commonly found in cattle, buffaloes, sheep, goats, horses, donkeys, rabbits, wild ruminants, and humans (Isah, 2019; Mehmood et al., 2017).

Research conducted in Punjab, Pakistan, has shown that the combined rearing of small and large ruminants can result in higher parasitic infections (Khan et al., 2009). It has been observed that in the co-grazing of sheep and goats, both species are exposed and can be infested with *Fasciola* metacercariae (Soulsby, 1987). These small ruminants become the means of transmission to larger ruminants such as buffaloes and cattle through shared pasturage where they are raised. *Haemonchus contortus* is a blood-feeding parasite in the abomasum of ruminants. It is recognized as one of the greatest health problems in ruminants, leading to reduced production and loss of income for farmers throughout the world (Sutherland & Scott, 2010).

A few decades ago, a study revealed that fascioliasis caused a 15% drop in milk production. However, after treatment, the production was recovered by 10%, increasing by 0.63 L per day per animal (Ross, 1970). Moreover, other factors such as stress caused by helminthiasis also significantly affect animal productivity (Hawkins & Morris, 1978; Ross, 1970).

The main reason may be a decrease in the food conversion ratio and a reduction in the food intake by parasitized animals, which reduces the energy constituents absorbed by the small and large intestine (Oakley, 1982). However, according to other criteria, the effect of GI parasite infections on the large ruminant's milk production is minimal or null (Dargie, 1987). Gastrointestinal parasites are often transmitted orally through the ingestion of fecal matter in water, soil, and food acquired during grazing. Ruminants of different groups may come into contact if feeding together at the same areas and sharing food or water (Nunn et al., 2011).

Some of these risk factors include grazing or feeding habits, nutritional deficiency, pasture management, immune status, presence of intermediate hosts and vectors, number of infectious larvae and eggs released into the environment, and favorable climatic conditions for the development of eggs of helminths to infectious stages (Odoi et al., 2007). In many countries, the importance of providing clean drinking water, good quality food, and a decent living environments for their livestock is frequently underestimated (Tikyaa et al., 2019).

In Pakistan, grazing for cattle breeding is carried out without the exclusion of private property. There are areas where several small herds coexist, of which about two million are landless. Small ruminants are raised mainly to generate income from their meat, milk, and wool.

In recent years, the small ruminant population has increased exponentially (Ishaq and ul Haq, 2007), reaching 52.7 million goats and 25.4 million lambs. Currently, the annual growth rate is 4.46 for goats and 4.47 for lambs. Khyber Pakhtunkhwa is a prosperous province of Pakistan in which there is a population of 3.4 million lambs and 9 million goats, according to a survey conducted in Khyber Pakhtunkhawa Kohat district (Pakistan Bureau of Statistics, 2006).

In the Punjab province, gastrointestinal parasite infections were identified in 51% in cattle, 62% in sheep and 47% in buffalo, and 52% in goats. The prevalence rate was higher in small ruminants and lowered in larger ruminants (Raza et al., 2007). Only a few studies have been conducted on helminths in small ruminants and even less on GI infection in large ruminants in Khyber Pakhtunkhwa (Ijaz et al., 2009).

The present study was conducted to investigate the GI profile in cows and buffaloes in Lower Dir district Khyber Pakhtunkhawa Pakistan. The frequency of parasitosis in both cows and buffaloes was compared and classified according to the number of parasitized animals and the different sources of water provided during the spring and summer.

2. Materials and methods

2.1. Study area

The study was conducted at District Dir lower Khyber Pukhtunkhawa (Fig. 1). The district lies in the northern highlands of KPK at a latitude of 34.35, a longitude of 71.85, and an altitude range between 1200 m and 2800 m above sea level. The average annual temperature was 16 °C, and the average annual rainfall recorded was 1186 mm. The relative humidity that occurred varies from 70 to 81% to 40 to 50% in the rainy and dry seasons, respectively.

2.2. Collection of samples and laboratory procedures

Fecal samples were collected at random from all buffaloes and cows in the study area. Fecal material was obtained directly from the rectum using surgical gloves and the appropriate veterinary techniques and immediately transferred to clean sampling plastic bottles containing 10% formalin solution and a sampling number with a unique ID. Microscopic examination of parasites by identifying of GI cysts or eggs based on morphological characteristic was carried out at the Laboratory of Parasitology in the Department of Zoology, University of Malakand, Lower Dir, Pakistan. Peanut agglutinin PNA was also used for the identification of *Haemonchus* egg.

2.3. Direct smear methods (Wet mount Techniques)

Fecal samples were examined in a normal saline solution wet mount. For this purpose, 3 gm of feces were mixed with saline in a glass and then taken an aliquot for examination the slide. A compound microscope was used for the smear examination. Furthermore, concentration techniques, floatation, and centrifugation were used for spotting eggs of nematodes and cestodes. As eggs are lighter and small, they float in the flotation liquid. To prepare the sample for examination, approximately 3 gm of stool were mixed with 42 ml of distilled water in a beaker. The fecal solution was mixed, ground lightly with a mortar and a pestle, and clarified with a tea filter. Subsequently, the fecal solution was poured into a 15 ml Falcon plastic tube and centrifuged at 1000 rpm for 5 min. The upper part of the supernatant was collected and discarded using a pipette, the tube again filled with NaCl solution, and

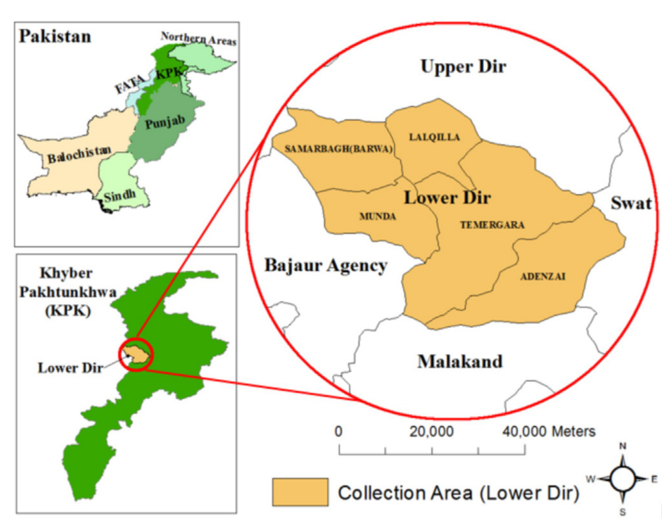

Fig 1. Map of the study area in District Lower Dir KPK Pakistan.

centrifuged at 1000 rpm for 5–7 min. NaCl solution was added to fill the tube and a coverslip was placed on top so that the solution touched the coverslip. Finally, the coverslip was placed over a glass slide and examined at 10X following standardized procedures (Hayat et al., 1996). Finally, a sedimentation technique was used for spotting trematode eggs. These are heavier and denser than other parasite eggs, and thus with centrifugation, the eggs settle down at the bottom of the test tube. With the help of a Pasteur pipette, a drop of the deposited material was transferred to a glass slide. After adding a drop saline solution, the sample was examined under a microscope at 4X and 10X following previously described laboratory procedures (Soulsby, 1982).

2.4. Survey

A questioner was also designed based on a literature review and administered to farm and animal owners with the help of a local survey supervisor and field personnel. The following parameters were included in the survey: Animal category, gender, area, season, age, health status, grazing system, treatment history, condition of farm/household, source of drinking water, and socio-economic status of the farmer.

2.5. Source of water

For drinking water, the following sources were considered for the survey: (1) Open source water that included water from permanent ponds but mainly from temporary rainfall puddles, (2) bore-well water (typically, from a well, with a 6–10 in. diameter and a 200–300 ft of depth) that is obtained through an electric pump, (3) stream water that is provided directly from the irrigation system which is used mainly for agricultural purposes and, (4) tap water, stored for animal drinking purpose, which came from in a pipeline from a water tank that was filled from various sources including hill springs or a well.

2.6. Statistical analysis

The information was compiled and tabulated in the Microsoft Excel (2016). The data were analyzed using the RSTUDIO program, using the EpiR package. The prevalence values by species, water source, and season were analyzed through the *Chi square* test at 95% confidence intervals. The Odds Ratio values were calculated to establish whether the season influences the risk of infestation by species. The probability of parasitosis occurring linked to the water source was analyzed by calculating the Odds Ratio through a generalized linear GLM model for dichotomous variables, in this case, the logit function: $\eta = \log(p/1 - p)$ was applied. The coefficients obtained were adjusted using the model: odds = eβ0·eβ1x1·eβ2x2.

The prevalence of parasites was measured in eggs per gram (EPG) with a mean calculation which showed the overall prevalence of the total samples collected.

3. Results

3.1. Prevalence

Samples were obtained from cattle (n = 196) and buffaloes (n = 118), out of which 109 (55.61%) and 75 (63.55%), were found positive for GI parasites respectively (Table 1). The minimum and maximum number of eggs on one slide were 1 and 5, respectively. By multiplying with 1 and 5, positive samples indicated an overall minimum of 136.39 EPG in which cow and buffalo EPG mean were 143.30 and 122.56, respectively. The EPG means for *Entamoeba*, *Moniezia* spp., *Haemonchus* spp. and for *Coccidia* spp. in cattle and buffaloes can be seen in Table 1.

3.2. Sources of drinking water

The open-source water prevalence of GI parasites was higher than the other sources in cattle and the second highest after tap water in buffaloes. The presence of GI parasites in bore water presented the lowest prevalence. When comparing the prevalence of GI parasites in different water sources (open source, bore, stream, tap) for each species.

The values obtained indicated that cattle would be 32% less likely (OR = 0.68) to contract parasites than buffaloes. Compared with the bore water (which has the lowest prevalence), the probability of becoming infected was 5.32 times higher (OR = 6.34) in the animals that went to the open water source, 3.24 times higher if they went to the stream (OR = 4.24) and 2.87 times higher if they used the Tap.

3.3. Seasonality

The prevalence of GI parasites was higher in the summer season (68.8% cattle; 71.6% buffaloes) than in the spring (32.4 % cattle; 52.9 % buffaloes). In both species, the Odds ratio values appear higher (2.2 cattle: 2.53 buffaloes) in the summer than in the spring.

4. Discussion

The prevalence of GI parasite infection was measured by examining eggs/cyst/oocyst in fecal samples. Mean calculation showed overall prevalence from 314 samples collected, including cattle (n = 196) with 109 samples found positive and buffaloes (n = 118) with 75 positive samples. The minimum numbers of eggs in one slide were one and the maximum number of eggs in one slide was 5. By multiplying by 1 and 5, positive samples indicated an overall minimum of 136.39 EGP in which cattle EPG mean was 143.30 with buffalo overall EPG mean of 122.56. On the other hand, the mean EPG of *Entamoeba* in cows and buffaloes was found to be 123.68 and 168.42, respectively. The EPG values for other GI parasites in cattle and buffaloes were: *Moniezia* 177.77 and 81.81, *Haemonchus* 145.45 and 140, and Coccidia 126.31 and 100, respectively. The current results are highly similar to other findings (Nath et al., 2016).

Table 1
Prevalence of parasites Egg/gram with mean in District Lower Dir Pakistan.

Parasites	Over All positive Samples (N = 314)	EPG Mean	Cattle (n = 196)		Buffaloes (n = 118)	
			Positive Samples	EPG Mean	Positive Samples	EPG Mean
Entamoeba spp.	38	134.615	19	123.68	19	168.42
Moniezia spp.	38	132.051	27	177.77	11	81.81
Haemonchus spp.	84	120.93	44	145.45	40	140
Coccidia spp.	24	158	19	126.31	5	100
Total Parasites	**184**	**136.39**	**109**	**143.30**	**75**	**122.56**

Table 2
Sources of drinking water-wise prevalence of GI parasites in cattle and buffaloes in District Lower Dir. Pakistan.

Animal Category	Sources of drinking water	Examined (n)	Positive Samples (n)	Infection (%)
Cattle	Open	77	52	67.53
	Bore (well)	23	6	26.08
	Stream	40	24	60
	Tap	56	27	48.21
Overall	**Total**	**196**	**109**	**55.61**
Buffalo	Open	45	32	71.11
	Bore (well)	15	4	26.66
	Stream	19	11	57.89
	Tap	39	28	71.79
Overall	**Total**	**118**	**75**	**63.55**
Overall Animals	**Total Sources of drinking water**	**314**	**184**	**58.59**

Table 3
Seasonal prevalence of GI parasites and Odds Ratio values in cow and buffaloes in District Lower Dir.

Category	Season	Positive	Negative	Total	Prevalence	Odds
Cattle	Spring	23	48	71	32.4	0.48
	Summer	86	39	125	68.8	2.2
	Total	109	87	196	55.6	1.25
Buffaloes	Spring	27	24	51	52.9	1.12
	Summer	48	19	67	71.6	2.53
	Total	75	43	118	63.6	1.74

The prevalence of GI parasites in open-source of drinking cow was 67.53% whereas for bore source of drinking water was 26.08%, for stream source of drinking 60% and for tap source of drinking 48.21%, as well as prevalence in open-source of drinking buffaloes were 71.11%, bore source of drinking 26.66%, stream source of drinking 57.89% and tap source of drinking 71.79%. The high occurrence in open water sources may be due to more open water use in animals during grazing and feeding hours. Open water sources are highly contaminated due to exposure to every kind of animal and different savage water sources, which may cause more infection rates in cattle and buffaloes. While Stream water also has different contaminate due to connection with rainwater and other sewage water. More farmers give water to their animals from open water sources and stream water sources in this study area, which may spread GI parasites in these animals. No literature is being available.

In the examination of egg presence versus season, the prevalence in cattle in spring was 32.39%, and summer was 68.8%. For buffalos, the occurrence was 52.94% in spring and 71.64% in summer. Our findings are similar to those of Akanda et al., 2014, where gastrointestinal infections was more common in the rainy season than in the summer or the winter seasons. Prevalence was significantly higher for *Paramphistomum spp* (21.53%) was found in a rainy season whereas *Haemonchus spp* (5.46%), and infections with *Moniezia spp* (4.18%) were higher in summer ($P < 0.05$). For both groups of animals, there was a higher occurrence in summer than in spring. The high occurrence in the summer season may be due to the higher temperatures required for larval development on pastures. In summer, rainfall occurred, which is a main source for spreading GI parasites.

Raza et al. (2007) found that sheep are highly vulnerable to gastrointestinal parasites compared to rams, which are less susceptible to gastrointestinal parasites. The study also revealed that the gender-specific prevalence of gastrointestinal parasites is lower in females than in males. This result was also supported by Kanyari et al. (2009), who found that after puberty, females are more resistant to infection than males, but there is no difference before puberty

5. Conclusion

Gastrointestinal parasite infection is very common in cattle and buffaloes in the district of lower Dir Khyber Pakhtunkhwa Pakistan and likely effects the productivity of the livestock in these areas. The highest mean EPG was found with *Entamoeba spp* in cattle and *Haemonchus* spp in buffaloes. Open sources water is more infectious as compared to other sources of water. The prevalence rate of GI parasites was higher during the summer than in the spring. Treated animals showed less prevalence as compared to non-treated animals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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