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Original Article

Intestinal Parasites in the Fecal Samples of Male Buffalo Calves (*Bubalus bubalis*) in Gorkha, Nepal

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Abstract

Introduction: Buffaloes are one of the most valuable animals for humans. They are an important source of milk and meat around the world. This research aimed to evaluate the prevalence of gastrointestinal (GI) parasites in the fecal samples of male calves in the Gandaki Rural Municipality, Gorkha, Nepal. Further, it sought to record different GI parasites and review their zoonotic probabilities in humans.

Methods: Fresh fecal samples (N=50) from 50 buffalo calves were opportunistically collected and stored at 2.5% potassium dichromate. Then, they were processed via direct mount and acid-fast techniques and observed on a compound microscope.

Results: The prevalence rates of 100% intestinal parasites were present, with 11 species of GI parasites. Zoonotically possible species were *Cryptosporidium, Balantidium coli, Entamoeba coli*, Ascarid, and *Fasciola hepatica*.

Conclusion: The findings revealed that the male calves were heavily parasitized by diverse intestinal parasitic species, indicating the necessity of quick and effective antiparasitic treatment in the future. In addition, the treatment will be necessary to prevent zoonotic parasites that can impact public health, especially nearby environment and humans.

Keywords: Buffalo calves, Zoonosis, Intestinal parasites, Public health, Sustainable development

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Introduction

The water buffalo or Asian buffalo (*Bubalus bubalis*) has been one of the vital livestock of farmers around the globe for many years. In Nepal, buffalo is famous for enhancing the sustainable development of small- and large-scale farmers. Its quality in the context of meat and milk has encouraged people toward its domestication. Previously, people used to throw or ignore male calves (1); however, due to increasing demands of delicious meat or worship of male calves, they are being domesticated in mass or single or by captive or semi-captive methods.

Gastrointestinal (GI) parasites such as protozoa and helminths are critical to buffaloes and their calves worldwide, including Nepal. In a previous case study, concomitant infections of many GI parasites resulted in robust pathology in a male calf (1). Even some of them are important to public health because their parasites have the zoonotic possibility of being transmitted to the nearby humans. They principally include *Cryptosporidium*, *Entamoeba*, *Balantidium coli*, and ascarids. As the intestinal parasites of calves have significance for public or veterinary health, their studies are vital. However, this type of study needed to be included in the current buffalo calves that had been raised mainly for breeding and meat purposes. Therefore, the objective of the research was to investigate the prevalence of GI parasites in the fecal samples of the male calves in the Gandaki Rural Municipality, Gorkha, Nepal. In addition, the study aimed to record various GI parasites and review their zoonotic probabilities in humans.

Materials and Methods

Study Area

The study was conducted in Gandaki Rural Municipality Ward No. 6. The site was Bhumlichowk (287 masl - 1652 masl; 27.85^0–84.68^0) which is full of natural resources and social and cultural diversities. Rice, maize, barley, and wheat are popular crops, whereas cattle, buffaloes, goats, dogs, and cats are important domestic animals. Buffalo calves are managed by small- and large-scale domestication (Figure 1). The village has an animal service center and an agricultural service center that work for animal welfare. The total population is 3545 in this ward.

Sample Collection and Preservation

A total of 50 fresh fecal samples from the 50 male calves (2-3 years old) were collected opportunistically. They were immediately put in a 30 mL vial just after defecation on the ground. The fecal morphology was recorded, and each sample was stored in 2.5% potassium dichromate. Then, the samples were transported to the laboratory of Tri-Chandra Multiple Campus, Kathmandu, Nepal.

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Figure 1. Calves of buffaloes and dung at the study site. (A) Calves in traditional shed. (B) A male calf under a shed. (C) Old and fresh dung in the cattle shed.

Sample Processing and Examination

Literature regarding fecal sample processing and examination was used during the study (1-7). One to two grams of fecal samples were put on the slide. Next, the samples were checked with or without one drop of iodine. Each sample was checked thrice consecutively under x100 and x400 total magnifications on a compound microscope. The acid-fast staining technique was processed for *Cryptosporidium* species using a fixed fecal smear in absolute methanol staining for carbol fuchsin, followed by counter-staining with malachite green. Immersion oil was applied to the slide while checking the acid-fast-stained specimen under 1000× magnification of the microscope. Identification was performed by comparing the photographs published in the literature (1,7-9).

Data Analysis

Microsoft Excel 2007 was used to express data on tables. Similarly, Prism 5 for Windows (Version 5.00 and March 7, 2007) was utilized to analyze probability (*P*) values via Fisher's exact and Chi-square tests. In these contexts, *P*-values less than 0.05 (5% significance level) were considered statistically significant while comparing between/among variables/objects.

Results

In this study, 50 fecal samples (100%) were examined, and all samples were positive for one or more parasitic species. Similarly, 11 parasitic species (seven protozoa and four helminths) were recorded. Among them, *Entamoeba* spp. had the highest prevalence (88%), while *Cyclospora* and *Fasciola hepatica* had the lowest prevalence (each 4%). The prevalence of each protozoan species ($P<0.0001$) or each helminth species ($P<0.0001$) was statistically significant. Obviously, parasitic contamination of feces were 100% in different morphologic forms of feces (liquid, semisolid, and solid). In this study, multiple parasitism was statistically higher than single parasitism in the calves (96% vs. 4%, $P<0.0001$, Fisher's exact test, two-sided). Regarding multiparasitism, two to seven different GI parasites were present in the fecal samples. The rates of prevalence in all fecal samples concerning a number of species of GI parasites were significantly different ($P<0.0001$, chi-square tests, Table 1, Figure 2).

Discussion

The current study evaluated the prevalence and diversity of GI parasites in the buffalo calves and their potential roles in zoonosis in the hilly areas of Nepal. The study recorded 100% prevalence rates of GI parasites in the fecal samples. The rate was higher than reported in India (73.6%) (10, 11). Studies conducted in Iran found eight *Eimeria* species, including *E. zuernii, E. auburnensis, E. bovis, E. ellipsoidalis, E. bareillyi, E. bukidnonensis, E. ovoidalis, E. cylindrica, Cryptosporidium* spp., and *Toxocara vitulorum* (12). A previous case study of a calf reported *E. bovis, E. bukidnonensis, E. cylindrica, E. subspherica, E. ellipsoidalis, E. zuernii, Cryptosporidium* sp., *Entamoeba* spp., *B. coli, Toxocara vitulorum*, *Strongyloides* sp., three morphotypes of Strongyle, and *Trichostrongylus* sp. (1). Different prevalence rates and diversities might be due to various environmental landscapes, management practices, fecal collection seasons, techniques, and applied methodology during processing, examination, and identification.

A total of eleven species of parasites were reported in this study. Among them, *Cryptosporidium*, *B. coli*, *Giardia*, *Entamoeba coli*, ascarid, and *F. hepatica* can be zoonotic. *Cryptosporidium* spp. can be critical to humans as they are spread via direct contact with their oocysts and cause acute and chronic diarrhea. Although this study is based on morphological and staining methods, previous molecular techniques found *C. ryanae* in Nepal (13) and *C. parvum, C. bovis, C. suis*-like, and *C. ryanae* in buffaloes globally (14). Although *C. ryanae* is predominant in ruminants and calves (13,15), *C. parvum* is a usual zoonotic coccidian parasite that circulates between humans and ruminants (16,17).

Balantidium coli is an important zoonotic parasite of both humans and pigs that causes ciliary dysentery (18). This ciliate is transmitted between these hosts via direct contact with the infected food, soil, water, and environment. Its presence in the current calves indicates

Table 1. Prevalence of Individual Parasites and Their Concomitance in the Fecal Samples (N = 50) of Male Calves

Parasite	Positive	%	*P* Value[a]
Protozoa			
Eimeria spp.	15	30	
Cryptosporidium	36	72	
Entamoeba spp.	44	88	
Balantidium coli	41	82	<0.0001
Giardia	4	8	
Cyclospora spp.	2	4	
Entamoeba coli	4	8	
Helminths			
Ascarid	22	44	
Capillaria spp.	3	6	
Strongylida	8	16	<0.0001
Fasciola hepatica	2	4	
Grand total samples	50	100	
Concomitance			
One species	2	4	
Two species	2	4	
Three species	12	24	
Four species	14	24	<0.0001
Five species	14	28	
Six species	4	28	
Seven species	2	4	

[a] Chi-square, Fisher's Exact Tests

that *B. coli* may circulate among domestic animals, humans, and pigs and have an impact on zoonosis (19). Similarly, two morphologic forms of *Entamoeba*, namely, *E. bovis*-like and *E. coli*-like, were present. Although *E. coli* is commensal in humans and other organisms, its presence in the calves cannot be ignored because its presence suggests the existing contaminated food or water sources or poor fecal-oral hygiene (20,21).

Another important zoonotic parasite was *Giardia* sp., which may be both asymptomatic and symptomatic, ranging from acute or chronic diarrhea to cholecystopathy. This flagellate has been diagnosed globally in buffaloes via different methods, including microscopy, immunology, and molecular biology (14). This species has two assemblages with the broadest hosts, A and B, which can be zoonotically transmitted, although *G. duodenalis* assemblage B has not been reported in buffaloes (22). In the same way, *G. duodenalis* assemblage E is more prevalent in ruminants and is associated with an emerging anthropozoonotic cycle (23-25), suggesting the significance of *Giardia*-infected calves in public health.

Ascarids were named for human or pig *Ascaris*-like species and *Toxocara vitulorum*-like species representing soil-transmitted helminths. All these *ascarids* are associated with zoonosis. However, consumption of embryonated eggs of *Ascaris* spp. and milk contaminated with larvae of *Toxocara vitulorum* is the route of transmission into humans (26,27). In this context, ascarids are critical for public health as, in humans, they lead to intestinal obstruction, diarrhea, and vitamin A absorption, resulting in night blindness, although the roles of *T. vitulorum* should be assessed further.

F. hepatica is a crucial zoonotic trematode parasite in livestock and several hosts. Its presence in humans is accidental and is transmitted via the consumption of improperly cooked aquatic plants or their nuts or drinks contaminated with metacercaria (28,29). Zoonotic possibility occurs when both calves and humans share the water sources and any swampy areas containing snail intermediate hosts or its infection causes anemia, hepatomegaly, allergy, and intestinal disturbances in humans (28,29).

Multiparasitism has always been an exciting topic in parasitology and disease pathology. In a previous study, a calf was infected with six species of *Eimeria*, three other protozoa, and four species of helminths, resulting in a strong pathology (1), indicating the possible fatal consequences of mixed infection in the calves. As mixed infections of the parasites may result in positive, negative, or null effects on the hosts (1,9,30), it is not easy to evaluate the roles of particular parasites in the host.

The current research has a few limitations. First, the nearby human fecal samples were not studied, which would give us knowledge of whether the parasites shared by both calves and humans are the same or not. Second, the study is strictly based on the morphometry of the parasites. Histology or molecular methods would confirm the parasites at species and strain levels. However, standard protocols for processing, examining, and identifying the parasites have been followed to erase possible study biases.

Conclusion

Intestinal parasitosis and its zoonotic impact are usually neglected issues in tropical areas. Farmers and people who are usually in contact with zoonotic parasites suffer from acute and chronic diarrhea and other extra-intestinal pathologies, depending on the species. However, few of the hosts do not produce symptoms but can transmit their parasites to humans, especially in the case of *B. coli*, *Giardia*, and *Cryptosporidium* spp. In this context, lack of proper hygienic practices, such as safe disposal of stool, , frequent medications, and handwashing after contact with dung, and the presence of asymptomatic hosts, can be underlying factors of zoonotic spillover to nearby humans or farmers (31-33).

This study, first, detailed the high rates of prevalence and concomitance of GI parasites in the hilly regions of Nepal. It also examined diverse parasitic species in

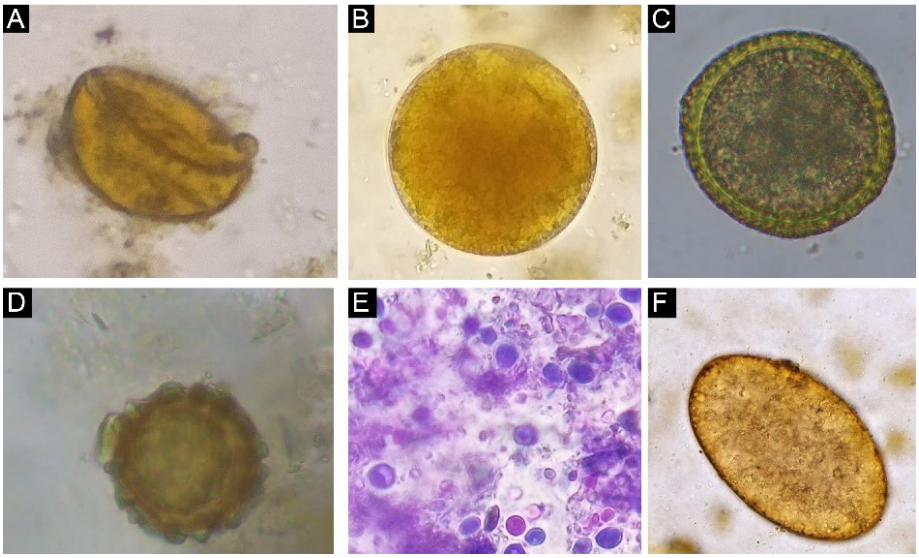

Figure 2. Few Gastrointestinal Parasites of Calves of Buffaloes. (A) *Giardia* sp., x400 (iodine staining). (B) *Balantidium coli*, x100 (iodine staining). (C) *Toxocara vitulorum*, x400 (iodine solution). (D) *Ascaris* sp., x400 (iodine solution). (E) *Cryptosporidium* sp., x1000 (acid-fast staining). (F) *Fasciola hepatica*, x400 (iodine staining)

calves and their possible impacts on public health. As the calves act as reservoirs and natural hosts of many intestinal parasites, ideally, it is not easy to make them parasite-free. Therefore, following integrated prevention methods for GI parasites would be practical in reducing the exposure of parasites from calves to humans and vice versa. Accordingly, calves should be regularly treated with antiparasitic drugs, and preventive measures for zoonotic potentialities in nearby humans should be followed strictly.

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Authors' Contribution

Conceptualization: Kusum Thapa Magar, Tirth Raj Ghimire.
Formal analysis: Tirth Raj Ghimire.
Investigation: Kusum Thapa Magar.
Resources: Tirth Raj Ghimire.
Software: Tirth Raj Ghimire.
Supervision: Tirth Raj Ghimire.
Validation: Tirth Raj Ghimire.
Visualization: Kusum Thapa Magar, Tirth Raj Ghimire.
Writing–original draft: Kusum Thapa Magar.
Writing–review & editing: Tirth Raj Ghimire.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The authors declare that the study was conducted on naturally-infected calves. No experimental infection was established during this research work. The calves were not directly involved in the study. Fecal samples fallen into the ground were collected, and none of the calves were touched during the study. Permission for the research work was obtained from the Gandaki Rural Municipality (62/080/81).

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