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Original paper

Prevalence and diversity of gastrointestinal parasites in domestic buffaloes (*Bubalus bubalis* Linnaeus, 1758) reared under captive and semi-captive conditions in Ratnanagar, Chitwan, Nepal

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ABSTRACT. Buffaloes are one of the most popular domestic ruminants, reared globally for milk and meat. Gastrointestinal (GI) parasitism in these hosts possesses a critical warning factor that severely limits the growth, reproductive performance, and milk production. Thus, the current study aimed to assess the prevalence and diversity of GI parasites in buffaloes in Ratnanagar Chitwan in central Nepal. The fresh faecal samples (n=300) of buffaloes (150 from captive and 150 from semi-captive) were collected and immediately preserved in 2.5% potassium dichromate solution. These samples were processed via direct wet mount, sedimentation, and floatation techniques and examined under a compound microscope at 100×, 400×, and 1000× magnifications. The results showed an overall 90% prevalence of parasites (80% in captive with 22 species and 100% in semi-captive buffaloes with 30 species). *Entamoeba* and *Balantidium coli* were the most prevalent parasites among the captives, whereas *Entamoeba* and *Fasciola* were dominant in semi-captive populations. In conclusions, buffaloes under semi-captive domestication harbor the higher prevalence and greater diversity of GI parasites. GI parasitism in buffaloes varies with captivity and age. Furthermore, awareness programs to the local farmers for healthy husbandry practices and therapeutic and preventive strategies should be conducted to reduce the parasitic loads and cross-transmission of potential parasites from different environments.

Keywords: buffalo, captivity, cross-transmission, *Eimeria*, *Fasciola*, *Schistosoma*

Introduction

Buffaloes (*Bubalus bubalis* Linnaeus, 1758), often considered black gold, are common and the largest ruminants of Bovidae family. They are the prior animal choice among the farmers and are domesticated globally except the USA, Canada, Britain, Scandinavia, and others for their qualitative and quantitative milk and meat values [1], strong musculature, and highly adaptive behavior in different landscapes. In Nepal, buffaloes are reared from the lowlands of tropical Terai belts to the high-altitude temperate Himalayas [2]. The indigenous breeds like Lime, Parkote, and Gaddi represent the major buffalo population in the mid-hills, high hills, and mountains of Nepal [3], whereas lowlands still have a huge undescribed population. However, in recent days, Indian Murrah and their crossbreeds are popularly growing as the major choices among the farmers, especially in the lowlands and mid-hills of the country [4]. The recent data showed a total of 5,177,998 buffalo heads in Nepal [5], and the number is ever increasing. This industry alone adds 6% of the total agricultural contribution [5] and plays a substantial role in achieving the per capita supply of milk and meat within the country [6]. Thus, buffalo industries have been effective in uplifting the economy of the farmers and, ultimately, the country's GDP.

With a recent increment in the demands of the

organic dairy industry, few Nepalese farmers have been attracted towards the mass domestication of buffaloes as commercial husbandry practices. Interestingly, there are mainly two types of domestication of these heads. Many smallholder farmers domesticate the semi-captive buffalo population (SCBP). They normally rear one to three buffaloes, feed them with local feeds and fodders, and allow grazing them in the open areas like crop fields, roadside, nearby forests, and riverside. This type of traditional husbandry practice is prevalent, especially in the rural parts of the country. Secondly, very few smallholder farmers and commercial farmers rear buffaloes in completely captive conditions. This type of population is called captive buffalo population (CBP). In this context, the buffaloes are entirely deprived of open grazing and are provided feed and fodders in their existing places.

While both types of practices have been popularly a great source of income and sustainable development of small- and large-scale farmers, diseases caused by gastrointestinal (GI) parasites might be critical issues in buffaloes for many years. For example, *Cryptosporidium* induces life-threatening diarrhea, retards growth and milk performance, and causes neonatal death [7–9]. *Fasciola* spp. retard milk production [10,11], may lead to apyrexial inappetence, weight loss, icterus of the conjunctiva and vulva, submandibular edema, liver damage, hemorrhage, anemia, infertility, and finally, death [1,12,13]. Similarly, *Eimeria* spp. interfere the nutrient absorption, cause bloody diarrhea and dysentery in calves, and may result in fatal consequences in the immunocompromised aging buffaloes [13,14]. *Balantidium coli* induces bloody diarrheal symptoms, ulcerative colitis, and weight loss in the buffaloes [15,16]. In the same way, *Schistosoma* spp. retard the growth, impair the digestive and reproductive functions, and lead to anemia in these hosts [17,18], indicating the potential roles of the GI parasites in the buffalo industry.

Literatures related to their prevalence rates in Nepalese buffaloes are limited; however, few have recorded or complied amphistomes, *Ascaris*, *Toxocara*, *Buxtonella*, *Capillaria*, *Eimeria*, *Fasciola*, strongylid, and *Trichuri*s in buffaloes from different landscapes [2,19–23]. Although most of the researches have focused on the epidemiology of flukes like *Fasciola* and their management, they have recorded very low diverse species. They have not analyzed the prevalence of GI parasites in buffaloes with different domestication practices. Only determining the prevalence rates and diversities in various rearing systems can be helpful for deworming practices and managing small- and large-scale businesses of buffaloes. Therefore, the study aimed to determine the prevalence and the diversity of the GI parasites in the faecal samples of buffaloes domesticated under captive and semi-captive situations in an agricultural area in central Nepal.

Materials and Methods

Study area

The study was conducted in Ratnanagar Municipality (27°37′N, 84°30′E) in the Chitwan district in central Nepal. Climate is subtropical with an average annual temperature (13.3–23.7°C) and average annual rainfall is 154.5 mm (6–478 mm) (https://www.weather-atlas.com/en/nepal/bharatpur-climate#rainfall, retrieved 4th May 2021). The area lies adjacent to the Chitwan National Park, the oldest national park of Nepal, which is why usual invasion by deer, one-horned rhinos, and the Asian elephants in the study area is common. The site is famous for domestic livestock, poultry, and crops like rice, wheat, maize, mustard, and banana. Local people practice domestication of both CBP and SCBP, which have been essential for the milk and meat values for the district and the capital city of Nepal. Basins of rivers flowing through the study area like Khageri, Panchanadi, Kair, and Rapti, including their small tributaries, provide the buffaloes' major grazing sites. Furthermore, open land nearby the forests and the harvested agricultural fields also aid the grazing pastures.

Sample collection, preservation and examination

The samples were collected from 12th July to 15th October 2019. All buffaloes studied were Murrah and Crossbred buffaloes (mainly Murrah bloodline) of age 1–15 years. Using a purposive sampling technique, 300 fresh faecal samples (150 each from CBP and SCBP) just after defecation were collected non-invasively from the ground in screw-capped 20 ml sterile vials. 2.5% weight/volume (w/v) potassium dichromate solution was used to preserve the samples and then transported to the Research Laboratory for further investigation and microscopic observation. The samples were studied macroscopically for the presence of blood,

mucus, adult nematodes, and detached segments of cestodes. According to the literature previously explained, the laboratory techniques for processing and examining parasites were carried out [24–30]. It involved the following three methods separately: **1.** Direct wet mount technique: the faecal sample at 2.5% potassium dichromate was carefully stirred with the help of a glass rod, and a single drop of the sample was observed under the microscope with or without staining agents like Gram's iodine and methylene blue separately; **2.** Formalin-ethyl acetate (FEA) sedimentation: about 2 grams of faecal sample was mixed with 12 ml of normal saline (0.9% NaCl) and was poured into a conical centrifuge tube via a tea strainer. The mixture was centrifuged (1200 revolution per minute (rpm) at room temperature for 5 minutes, and the supernatant was discarded. Then, 10 ml of 10% formalin and 4 ml of ethyl acetate were added to the tube for subsequent centrifugation (1200 rpm × 5 minutes). Finally, discarding the supernatant, a drop of the sediment was observed under the microscope with Gram's iodine and methylene blue stain; **3.** Saturated salt flotation technique: the centrifuge tube with the sediments was entirely filled with concentrated salt solution (45% w/v NaCl). The mouth of the tube was then covered by a coverslip and left undisturbed for about 10–15 minutes. Finally, the coverslip was carefully removed and observed under the microscope.

Acid-fast staining

The sediment after FEA sedimentation was proceeded for thin faecal smear preparation over a clean glass slide. The smear was then fixed in absolute methanol (2 minutes) and stained with carbol fuchsin (15 minutes). It was subsequently washed with distilled water and acid alcohol, and then the smear was counter-stained with malachite green (1 minute). The smear was then washed gently with distilled water and dried completely at room temperature. Finally, using immersion oil, the smear was observed under 1000× magnification of the microscope.

Parasite identification

Images (1280×720 pixels) of the different stages of the parasites were taken at a total magnification of 100×, 400×, and 1000× using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013–2014 under a compound microscope (Optika Microscopes Italy, B-383PLi). Morphometric analysis was done using ImageJ 1.51 k (National Institute of Health, USA), and identification was performed using the literature previously published [28,29,31–35]. *Fasciola* sp. and *Paramphistomum* sp. were identified using methylene blue stain [32] that produces dark brown color to the former and colorless to the latter.

Data analysis

Data were analyzed using Microsoft Excel 2007, Prism 5 for Windows (Version 5.00, and March 7, 2007). Fisher's exact tests were performed and *P*-values were calculated by comparing any two variables between CBP and SCBP. The *P*-values less than 0.05 (95% confidence level) were considered to be significant.

Ethics approval

The required permission for collecting the faecal samples was issued by Ratnanagar Municipality and Livestock and Veterinary Sector, Ratnanagar, Chitwan (Permission number: 952/2076/2077).

Results

In the current study, a total of 270 (90%) out of 300 faecal samples were found to be infected with GI parasites. The overall prevalence of each reported parasite follows the order: *Entamoeba* spp. (76%), *Fasciola* sp. (38.3%), *Balantidium coli* (36.7%), *Paramphistomum* sp. (30%), strongyle (21.7%), *Eimeria bovis* (21%), *Cryptosporidium* sp. (20.3%), *Eimeria ellipsoidalis* (11%), *E. zuernii* (10%), *E. subspherica* (9.7%), *Giardia* sp. (9.3%), ascarid spp. (7.7%), *Strongyloides* sp. (7.7%), *Eimeria alabamensis* (6.3%), *Trichuris* sp. (5.3%), *Moniezia benedeni* (5%), *Eimeria cylindrica* (4%), *E. bukidnonensis* (3.7%), *E. canadensis* (3.7%), *E. auburnensis* (with non-mammilated wall) (3.7%), *E. bareillyi* (3.3%), *Endolimax nana* (2.7%), *Eimeria auburnensis* (mammilated wall) (2.3%), oxyurid sp. (2%), *Blastocystis* sp. (2%), *Schistosoma bovis* (1%), *S. mansoni* (0.7%), *Capillaria* sp. (0.7%), *Schistosoma indicum* (0.3%), and *S. mekongi* (0.3%) (Tab. 1, 2).

The prevalence of GI parasites among the captive buffaloes was 80%, while the semi-captive showed a cent percent prevalence rate, and the difference was statistically significant ($P<0.05$). Captive buffaloes were infected with 22 varied species of GI parasites, while the semi-captives were infected with 30 species of GI parasites. Both protozoa (100% vs 80%) and helminths (86% vs

Table 1. Parasites detected in the faecal samples of captive buffalo population (CBP) and semi-captive buffalo population (SCBP)

Parasites	CBP (n=150)	SCBP (n=150)	Overall positive (n=300)	*P*-values (two-sided, Fisher's exact test)
Entamoeba spp.	98 (65.3%)	130 (86.7%)	228 (76%)	<0.05
Balantidium coli	41 (27.3%)	69 (46%)	110 (36.7%)	ns
Eimeria subspherica	11 (7.3%)	18 (12%)	29 (9.7%)	ns
E. zuernii	7 (4.7%)	23 (15.3%)	30 (10%)	<0.05
E. ellipsoidalis	11 (7.3%)	22 (14.7%)	33 (11%)	ns
E. cylindrica	5 (3.3%)	7 (4.7%)	12 (4%)	ns
E. alabamensis	7 (4.7%)	12 (8%)	19 (6.3%)	ns
E. bukidnonensis	4 (2.7%)	7 (4.7%)	11 (3.7%)	ns
E. bovis	25 (16.7%)	38 (25.3%)	63 (21%)	ns
E. canadensis	3 (2%)	8 (5.3%)	11 (3.7%)	ns
E. auburnensis (smooth wall)	0 (0.0%)	11 (7.3%)	11 (3.7%)	<0.05
E. auburnensis (mamillated wall)	2 (1.3%)	5 (3.3%)	7 (2.3%)	ns
E. bareillyi	3 (2%)	7 (4.7%)	10 (3.3%)	ns
Cryptosporidium sp.	20 (13.3%)	41 (27.3%)	61 (20.3%)	<0.05
Giardia sp.	15 (10%)	13 (8.7%)	28 (9.3%)	ns
Endolimax nana	4 (2.7%)	4 (2.7%)	8 (2.7%)	ns
Blastocystis sp.	3 (2%)	3 (2%)	6 (2%)	ns
Fasciola sp.	41 (27.3%)	74 (49.3%)	115 (38.3%)	<0.05
Paramphistomum sp.	32 (21.3%)	58 (38.7%)	90 (30%)	<0.05
Strongyle	21 (14%)	44 (29.3%)	65 (21.7%)	<0.05
Ascarid spp.	9 (6%)	14 (9.3%)	23 (7.7%)	ns
Strongyloides sp.	8 (5.3%)	15 (10%)	23 (7.7%)	ns
Moniezia benedeni	0 (0.0%)	15 (10%)	15 (5%)	<0.05
Oxyurid sp.	0 (0.0%)	6 (4%)	6 (2%)	<0.05
Trichuris sp.	5 (3.3%)	11 (7.3%)	16 (5.3%)	ns
Capillaria sp.	0 (0.0%)	2 (1.3%)	2 (0.7%)	ns
Schistosoma bovis	0 (0.0%)	3 (2%)	3 (1%)	ns
S. mansoni	0 (0.0%)	2 (1.3%)	2 (0.7%)	ns
S. indicum	0 (0.0%)	1 (0.7%)	1 (0.3%)	ns
S. mekongi	0 (0.0%)	1 (0.7%)	1 (0.3%)	ns
Total protozoa	120 (80%)	150 (100%)	270 (90%)	<0.05
Total helminths	87 (58%)	129 (86%)	216 (72%)	<0.05
Overall	120 (80%)	150 (100%)	270 (90%)	<0.05
Concurrency of infection				
Single	6 (4%)	0 (0.0%)	6 (2%)	<0.05
Double	38 (25.3%)	10 (6.7%)	48 (16%)	<0.05
Triple	28 (18.7%)	24 (16%)	52 (17.3%)	ns
Quadruple	35 (23.3%)	58 (38.7%)	93 (31%)	<0.05
Pentuple	10 (6.7%)	34 (22.7%)	44 (14.7%)	<0.05
Hexuple	2 (1.3%)	23 (15.3%)	25 (8.3%)	<0.05
Septuple	1 (0.7%)	6 (4%)	7 (2.3%)	ns

Table 2. Characteristics of *Eimeria* oocyst (n=number of oocyst measured)

Eimeria species	Prevalence (%)	Length×width (μm)	Shape index (l/b)	Shape of oocyst
E. bovis (n=108)	20.3	23–32 (27.8) × 17–23 (19.6)	1.4	broadly ovoid and usually blunt at narrow end
E. ellipsoidalis (n=69)	11	14–27 (23.1) × 13–18 (16.3)	1.4	ellipsoidal to slightly ovoid
E. zuernii (n=82)	10.7	15–22 (18.6) × 13–21 (16.7)	1.1	spherical or subspherical
E. subspherica (n=35)	8.7	9–13 (11.7) × 8–13 (10.8)	1.1	spherical or subspherical
E. alabamensis (n=55)	6	13–25 (20.1) × 11–16 (14.3)	1.4	ellipsoidal
E. cylindrica (n=22)	3.7	19–27 (21.8) × 12–15 (13.5)	1.3	cylindrical or narrow cylindrical
E. canadensis (n=46)	3.7	28–37 (30.7) × 20–26 (22.9)	1.3	elliptical and occasionally cylindrical
E. bukidnonensis (n=32)	3.7	38–44 (40.5) × 26–32 (28.3)	1.4	pear-shaped to oval
E. auburnensis (homogenous wall) (n=24)	3.7	33–41 (35.6) × 20–26 (22.7)	1.6	narrowly ovoid, narrow at micropylar end
E. auburnensis (mammillated wall) (n=12)	2	35–41 (38.3) × 25–29 (26.7)	1.4	narrowly ovoid, narrow at micropylar end
E. bareillyi (n=58)	2	27–34 (30.4) × 20–24 (21.1)	1.4	pyriform

58%) were higher in SCBP compared to CBP (P<0.05). Further, the prevalence of protozoa was higher than the helminths in both populations; captive (80% vs 58%) and semi-captive (100% vs 86%). In the context of the helminths, captive buffaloes were infected with trematodes (38.7%; 2 species) and nematodes parasites (27.3%; 4 species) only, while semi-captives were infected with trematodes (64.7%; 6 species), cestodes (10%; 1 species), and nematodes (48%; 6 species). This indicates the greater diversity of GI parasites in semi-captive buffaloes (Tab. 1).

Age-wise parasitic infection was also analyzed, for example, young/yearling (1–3 years) and adult/elderly (>3 years). Among the CBP, young buffaloes (90%; 36/40) had a higher prevalence rate than the adults (76.3%; 84/110). However, all the SCBP showed a 100% prevalence rate irrespective of their ages. Out of the overall sampling population, young buffaloes had a higher prevalence rate (94.7%; 71/75) than those of adult buffaloes (88.4%; 199/225) (Tab. 3). Besides the common *Entamoeba,* and ascarid spp. were the most prevalent parasites among the young, while these nematodes were totally absent in the adults. In contrast, adult populations were mostly infected with *Fasciola* sp. and *B. coli*.

Regarding the concurrency of GI infection, rather than infection with single GI species (2%), remaining 98% prevalence was recorded for the faecal samples with multiple species. Maximum CBP were co-infected with 2–4 parasites at a time, while most SCBP were co-infected with 3–5 species of parasites at a time. In addition, maximum concurrency of up to seven species of parasites was reported in both buffaloes' populations. Interestingly, statistical significant differences were observed in single (P<0.05), double (P<0.05), quadruple (P<0.05), pentuple (P<0.05), and sextuple (P<0.05) infections between SCBP and CBP (Tab. 1).

Similarly, six morphotypes of strongylid eggs were reported (67–142×41–72 μm). In the absence of a larval culture that gives the appropriate diagnosis, the eggs resembling *Bunostomum*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, *Ostertagia*, *Teladorsagia*, and *Trichostrongylus* were considered as strongyle in the current study. In the same way, two morphotypes of ascarid spp. were recorded, one resembling *Toxocara* sp. and the other similar to *Ascaris* sp.

Discussion

The current study is the first in Nepal to compare and contrast the diversity, patterns, and prevalence of GI parasites between CBP and SCBP. The prevalence rates of GI parasites (80%) in the CBP was higher than reported from India (54.12–70.45%) [36,37], Italy (5.4–33.1%) [38,39], and

Table 3. Parasites detected in the faecal samples of captive buffalo population (CBP) and semi-captive buffalo population (SCBP) according to age, (young/yearling: 1–3 years, adult/elderly: >3 years)

Parasites	CBP (n=150)			SCBP (n=150)			Overall positive (n=300)
	Young (n=40)	Adults (n=110)	Positive (Prevalence)	Young (n=35)	Adults (n=115)	Positive (Prevalence)	
Protozoa							
Entamoeba spp.	26 (65%)	72 (65.5%)	98 (65.3%)	30 (85.7%)	100 (87%	130 (86.7%)	228 (76%)
Balantidium coli	13 (32.5%)	28 (25.5%)	41 (27.3%)	12 (34.3%)	57 (49.6%)	69 (46%)	110 (36.7%)
Eimeria subspherica	4 (10%)	7 (6.4%)	11 (7.3%)	7 (20%)	11 (9.6%)	18 (12%)	29 (9.7%)
E. zuernii	4 (10%)	3 (2.7%)	7 (4.7%)	7 (20%)	16 (13.9%)	23 (15.3%)	30 (10%)
E. ellipsoidalis	4 (10%)	7 (6.4%)	11 (7.3%)	7 (20%)	15 (13%)	22 (14.7%)	33 (11%)
E. cylindrica	4 (10%)	1 (0.9%)	5 (3.3%)	1 (2.9%)	6 (5.2%)	7 (4.7%)	12 (4%)
E. alabamensis	2 (5%)	5 (4.5%)	7 (4.7%)	3 (8.6%)	9 (7.8%)	12 (8%)	19 (6.3%)
E. bukidnonensis	4 (10%)	0 (0.0%)	4 (2.7%)	2 (5.7%)	5 (4.3%)	7 (4.7%)	11 (3.7%)
E. bovis	11 (27.5%)	14 (12.7%)	25 (16.7%)	9 (25.7%)	29 (25.2%)	38 (25.3%)	63 (21%)
E. canadensis	1 (2.5%)	2 (1.8%)	3 (2%)	2 (5.7%)	6 (5.2%)	8 (5.3%)	11 (3.7%)
E. auburnensis (smooth wall)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.7%)	9 (7.8%)	11 (7.3%)	11 (3.7%)
E. auburnensis (mamillated wall)	1 (2.5%)	1 (0.9%)	2 (1.3%)	2 (5.7%)	3 (2.6%)	5 (3.3%)	7 (2.3%)
E. bareillyi	1 (2.5%)	2 (1.8%)	3 (2%)	5 (14.3%)	2 (1.7%)	7 (4.7%)	10 (3.3%)
Cryptosporidium sp.	5 (12.5%)	15 (13.7%)	20 (13.3%)	5 (14.3%)	36 (31.3%)	41 (27.3%)	61 (20.3%)
Giardia sp.	5 (12.5%)	10 (9.1%)	15 (10%)	4 (11.4%)	9 (7.8%)	13 (8.7%)	28 (9.3%)
Endolimax nana	1 (2.5%)	3 (2.7%)	4 (2.7%)	0 (0.0%)	4 (3.5%)	4 (2.7%)	8 (2.7%)
Blastocystis sp.	0 (0.0%)	3 (2.7%)	3 (2%)	0 (0.0%)	3 (2.6%)	3 (2%)	6 (2%)
Helminths							
Fasciola sp.	4 (10%)	37 (33.6%)	41 (27.3%)	10 (28.6%)	64 (55.7%)	74 (49.3%)	115 (38.3%)
Paramphistomum sp.	5 (12.5%)	27 (24.5%)	32 (21.3%)	6 (17.1%)	52 (45.2%)	58 (38.7%)	90 (30%)
Strongyle	2 (5%)	19 (17.3%)	21 (14%)	9 (25.7%)	35 (30.4%)	44 (29.3%)	65 (21.7%)
Ascarid spp.	9 (22.5%)	0 (0.0%)	9 (6%)	14 (40%)	0 (0.0%)	14 (9.3%)	23 (7.7%)

Table 3

Parasites	CBP (n=150)			SCBP (n=150)			Overall positive (n=300)
	Young (n=40)	Adults (n=110)	Positive (Prevalence)	Young (n=35)	Adults (n=115)	Positive (Prevalence)	
Strongyloides sp.	2 (5%)	6 (5.5%)	8 (5.3%)	5 (14.3%)	10 (8.7%)	15 (10%)	23 (7.7%)
Moniezia benedeni	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.7%)	13 (11.3%)	15 (10%)	15 (5%)
Oxyurid sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.7%)	4 (3.5%)	6 (4%)	6 (2%)
Trichuris sp.	0 (0%)	5 (4.5%)	5 (3.3%)	2 (5.7%)	9 (7.8%)	11 (7.3%)	16 (5.3%)
Capillaria sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.7%)	2 (1.3%)	2 (0.7%)
Schistosoma bovis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (2.6%)	3 (2%)	3 (1%)
S. mansoni	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.7%)	2 (1.3%)	2 (0.7%)
S. indicum	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.9%)	0 (0.0%)	1 (0.7%)	1 (0.3%)
S. mekongi	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)	1 (0.7%)	1 (0.3%)
Total protozoa	36 (90%)	84 (76.4%)	120 (80%)	35 (100%)	115 (100%)	150 (100%)	270 (90%)
Total helminths	19 (47.5%)	68 (61.8%)	87 (58%)	27 (77.1%)	102 (88.7%)	129 (86%)	216 (72%)
Overall	36 (90%)	84 (76.4%)	120 (80%)	35 (100%)	115 (100%)	150 (100%)	270 (90%)
Concurrency of infection							
Single	3 (7.5%)	3 (2.7%)	6 (4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (2%)
Double	10 (25%)	28 (25.5%)	38 (25.3%)	3 (8.6%)	7 (6.1%)	10 (6.7%)	48 (16%)
Triplet	8 (20%)	20 (18.2%)	28 (18.7%)	7 (20%)	17 (14.8%)	24 (16%)	52 (17.3%)
Quadruplet	10 (25%)	25 (22.7%	35 (23.3%)	11 (31.4%)	47 (40.9%)	58 (38.7%)	93 (31%)
Pentuplet	3 (7.5%)	7 (6.3%)	10 (6.7%)	8 (22.9%)	26 (22.6%)	34 (22.7%)	44 (14.7%)
Hexuplet	1 (2.5%)	1 (0.9%)	2 (1.3%)	4 (11.4%)	19 (16.5%)	23 (15.3%)	25 (8.3%)
Septuplet	0 (0%)	1 (0.9%)	1 (0.7%)	2 (5.7%)	4 (3.5%)	6 (4%)	7 (2.3%)

Pakistan (29.04%) [40]. Compared to this, 100% prevalence rate among SCBP in the current study area was in concordant with the findings from Bangladesh (100%) [41], and higher than reported from Greece (92.73%) [42], Nepal (34.4–86%) [20,21], Poland (44%) [43], and Mexico (32.6–54.6%) [44]. The current study was conducted in the monsoon periods, the time favorable for the development and transmission of the GI parasites [45,46]. Both direct wet mount and concentration techniques have produced high detection rates in the current study. Although the variations in the prevalence rates in the global buffalo populations might be due to different landscapes, seasons, breeds, gender, and therapeutic strategies, further studies should confirm this hypothesis.

It was interesting that the diversity of parasitic species was lower in CBP compared to SCBP (22 species versus 30 species). In both populations, protozoa were the most dominant parasites that included *Balantidium coli*, *Blastocystis* sp., *Eimeria* spp., *Endolimax nana*, *Entamoeba* sp., and *Giardia* sp. and the helminths; ascarid spp., Capillariia sp., *Fasciola* sp., *Moniezia benedeni*, oxyurid sp., *Paramphistomum* sp., *Schistosoma* spp., strongyle, *Strongyloides* sp., and *Trichuris* sp. The current prevalence rate of protozoa (80%) in the CBP was higher than those reported from Egypt (28%) [35] and India (35%) [49]. Compared to this, the prevalence rate (100%) in the SCBP was higher than those reported from Bangladesh (80.28%) [41], Turkey (75%) [47], and Brazil (66.11%) [48]. *Entamoeba* spp. were predominant in both populations indicating they were naturally present in buffaloes. Compared to ten different species of *Eimeria* from Egyptian buffaloes [35], 11 species with *E. bukidnonensis* from SCBP and ten species from CBP were detected. *E. bovis* and *E. zuernii*, which cause severe pathologic effects [26,50], have been detected in both populations indicating a critical role of these coccidia in buffalo health in Nepal.

Regarding helminths, trematodes were the most dominant groups in both populations. Their prevalence rate (38.7%) in CBP was higher than reported from Italy (2.1%) [39]. In the same way, (64.7%) prevalence rate in SCBP was higher than reported from Bangladesh (60.75%) [51] and lower than reported previously from Nepal (86%) [20] and China (87%) [52]. Interestingly, four different blood fluke species like *Schistosoma bovis*, *S. mansoni*, *S. indicum*, and *S. mekongi* were reported only from the SCBP. Although their prevalence rates are meager, it is the first report of *Schistosoma* diversity in buffaloes from Nepal. The current prevalence rate of nematodes (27.3%) in CBP was lower than the findings from India (47.73%) [36]. Their rates (48.7%) in SCBP was higher than reported from Mexico (47.2%) [44], the Philippines (28%) [53], and Australia (5%) [54], but was lower than reported from Nepal (86%) [20] and Brazil (77%) [55].

Notably, only one cestode species, *Moniezia benedeni*, has been reported in SCBP in the current study. The prevalence rate (5%) of this tapeworm was lower than the findings from Mexico (18.1%) [44] while higher than that from Malaysia (1.10%) [56].

In the current study, compared to adults, the young had higher prevalence rates of protozoa and helminths in both domestications. This finding is concordant with the results from Sri Lanka [57] and Australia [54], while it is in contrast with the results from Bangladesh [58] and Pakistan [59], where adult buffaloes had a higher prevalence rate than the young. It is not easy to explain the age-wise predilection. However, few generalizations can be made. First, a field survey found that farmers would allow their young buffaloes to graze only after completing the late weaning periods. As a result, newer/recent exposure to the pastures, water bodies, and contact with other animals might have contributed to the acquisition of diverse parasites in them. In addition, local farmers completely neglect the medication of their young buffaloes and deworm their adults, 2–1.5-month before their parturition period only; this might also create a difference in the prevalence rate. Furthermore, enhanced immunity with age decreases the susceptibility of adult buffaloes to parasitic infection [60].

Considering the concurrent infection, most of the CBP showed double infection followed by quadruplet infection, while most of the SCBP showed quadruplet infection followed by pentuplet infection. In general, concurrent infection is commonly a natural phenomenon; however, it may alter the infection risk [61], the intensity of infection [62], and the fitness of the host [63]. This is because polyparasitism results not only in positive or neutral but also in negative consequences [64]. In a positive case, suppression of the host immune response by one parasite can increase the likelihood or severity of infection with another co-infecting parasite [65].

In contrast, the competition between the co-infecting parasites may decline the infection severity in negative cases [66]. In our previous case study, concomitant parasites in the faecal samples of a 1.5-month buffalo calf were associated with its robust pathology [26]. In the same way, concomitant infections of nematodes and *Mycobacterium bovis* resulted in accelerated mortality in the African buffaloes (*Syncerus caffer*) [67]. However, due to the lack of detailed pathology in the current study, how the interactions between two protozoa, two helminths, or protozoa and helminths might result in positive or negative, or null consequences are elusive.

While the current study found higher diversity and prevalence rates of GI parasitism in SCBP than CBP, it is not easy to explain the preference. In this context, several hypotheses may determine the composition of parasites. Thus, in SCBP, host movement, the exposure of the animals to diverse environmental conditions and the host contact with other livestock and wildlife either might solely or jointly contribute to GI parasitism. Host movement results in the spread of the parasitic bodies because the grazing animals usually defecate elsewhere on the pasture or nearby water bodies and contaminate them with parasitic eggs, cysts, oocysts, or larvae. As a result of grazing [68] and drinking contaminated water of river or pond, or other sources [69,70], the diversity of parasites and their transmission rates get magnified. For example, *Fasciola* and *Paramphistomum* is related to grazing on wetland and drinking water contaminated by the infective metacercariae [71]. Transmission of *Schistosoma* spp. is associated with the exposure of animals in both running and stagnant water bodies contaminated with infective furcocercus cercaria larva during swimming and wallowing or cooling, which we have observed in this study. Similarly, ingestion of contaminated oribatid mites during grazing leads to the acquisition of *Moniezia* spp. [72,73] indicating how semicaptivity plays a role in enhancing parasite transmission.

Sharing of the same pastureland and water bodies by wild and domestic animals significantly increases the prevalence of GI infection [74] via interspecific transmission. In our study, the grazing buffaloes would usually share overlapping niches with other herbivores like domestic goats, sheep, and cattle, as well as wild herbivores. Previously, we have identified *Entamoeba* sp., strongyle, *Strongyloides* sp., *Fasciola* sp. in Chital (*Axis axis*) in Barandabhar Corridor Forest of Chitwan [75], the forest adjacent to the study site. In this context, cross-transmission of the parasites from wild herbivores to grazing buffaloes might be possible. In addition, similar parasites like strongyle and *Strongyloides* spp. are diagnosed in the faecal samples of goats and sheep [24,76]. However, further detailed epidemiologic evidence should be required to prove cross-transmission.

It was also critical that despite the lack of movement in the open environment, the confinement in a small space, and the lack of grazing, swimming, and wallowing, CBP had a higher prevalence rate than other studies around the world [35,49]. This might be because of poor indoor management, overstocking, fodder supplement, and unregulated deworming practice. A well-ventilated and lighted shed maintains essential humidity and air and thus reduces the growth of parasites [77,78]; however, most of the smallholder farms in the study area were built in the backyard of houses with poor air and sunlight passage. Our field survey also found that the floors were uneven, either built with wood, stone, brick, or mud securing higher moisture content inside the shed. Besides, the heap of manure was deposited nearby the shed. These factors would be favorable for the reinfection of the CBP by the parasites.

Overstocking of the animals in the shed may lead to the transmission of many parasites among livestock at a time [78]. The existing practice of rearing kid and adult buffaloes and other livestock within the same shed can transmit the parasites in the CBP. In addition, the absence of regular deworming practices and regular supplementation of kitchen wastes, fresh grasses, and rice straw too possess infection risk as they may contain infective parasitic stages in them. For example, rice straw contained infective metacercaria larvae and had already been proven as a major source of fasciolosis and paramphistomosis in the domestic buffaloes [2,79].

In conclusion, buffaloes domesticated under semi-captive conditions with open grazing in the ground, nearby ponds, and crop fields possess a higher prevalence and greater diversity of GI parasites than those reared under captive situations. Contact with other buffaloes, other domestic animals, consumption of parasite-contaminated soil, water, and grasses enhance the acquisition, diversity, and frequency of the parasites. However, as both CBP and SCBP contain a massive GI

parasitic species, control and preventive strategies should include usual sanitation practices, farm and pastureland management, and awareness programs regarding regular deworming practices. These strategies will be critical for the healthy husbandry practices, sustainability, and profitability of the buffalo industry.

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