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Original paper

Prevalence of gastrointestinal helminth parasites of buffaloes brought to slaughterhouse in Bhaktapur, Nepal

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ABSTRACT. Livestock farming has significant economic importance to the Nepalese society. Gastrointestinal helminth parasitism in buffaloes possess a warning for poor growth, milk production and, development. Thus this study aimed to study the prevalence of gastrointestinal helminth parasites in slaughtered buffaloes in Bhaktapur Municipality. Faecal samples (100) and visceral organs (100) i.e. small intestine, large intestine, rumen, and abomasums of 100 slaughtered buffaloes were collected and preserved in 2.5% potassium dichromate and 70% alcohol, respectively. Faecal samples were processed via direct and concentration techniques whereas organs were examined macroscopically for adults. The result showed an overall prevalence of 51% of helminth parasites. *Paramphistomum* sp. from the rumen and *Oesophagostomum* sp. from the large intestine were collected from the macroscopic examination. Altogether ten genera of helminth parasites were reported and among them, *Paramphistomum* sp. (18%) was found to be the dominant parasite in faecal examination followed by *Fasciola* sp. (16%), *Oesophagostomum* sp. (11%), *Strongyloides* sp. (7%), *Haemonchus* sp. (5%), *Schistosoma* sp. (3%), *Capillaria* sp. (2%), *Cooperia* sp. (2%), *Trichostrongylus* sp. (1%) and *Syngamus* sp. (1%). Single infection was prevalent in comparison to multiple infections in both examinations. Buffaloes were imported from Nepalgunj, Birgunj, and Jitpur whereas the location-wise prevalence showed no significant difference. Furthermore, awareness programs among butchers and farmers of farming places from where buffaloes were brought were involved to reduce parasitic loads among slaughtered buffaloes.

Keywords: buffalo, parasite, slaughterhouse, helminth, Bhaktapur

Introduction

Livestock farming specially buffalo farming is a major part of our country. Raising different animals is the important parts of the farming system and buffalo farming is one. Human societies have highly relied on animal products for food, fertilizers, employment, and survival. There is the significant importance of livestock farming to the Nepalese economy. More than 90% of the farmers from rural areas of Nepal have income from the sale of different products from domestic animals [1]. In the case of Nepal, most of the marginalized farmers relied on animal farming. Animal products act as indemnity for their survival during crop loss [2]. In 2013, about 1,72,414 metric tons of beef and 11,53,838 metric tons of milk were produced in Nepal whereas the demand has been increased in 2014. About, 1,75,232 metric tons of beef and 11,88,433 metric tons of milk from buffaloes were produced in 2014. According to the Veterinary Epidemiology Center, 2014, the numbers of milking buffaloes were 51,33,139, and 52,41,873 in 2013 and 2014, respectively. Population of the livestock for the last ten decade showed higher units of cattle followed by buffaloes i.e. 5,159,931 in the year 2020/2021. The livestock population (buffaloes) in Bhaktapur was 9,334 in the year 2021 [3].

Buffalo (*Bubalus Bubalis*) (Linnaeus,1758) is the major livestock that belongs to the order Artiodactyla and family Bovidae. Buffalo is mostly used in farming animals for their products and about two-thirds of consumption of meat is buffalo meat. Buffaloes are important multipurpose and economically important livestock species [1]. It is most important as a source of meat, dairy, dung, and manure. Besides animal proteins, products such as bones, skin, and different goods are also of great

importance to people [4]. The highest consumption of meat is buffaloes i.e. 64% followed by meat of goats, pigs, chickens, and sheep in Nepal [5]. Slaughtering animals in huge numbers causes numerous problems and public health concerns [6].

Buffalo is susceptible to different helminth parasites and diseases. The meat of such infected buffalo causes different diseases and infections in humans. Helminthiosis among slaughtered buffaloes is caused by different parasites such as nematodes, trematodes, and cestodes that inhabit inside host body like the gut, gastrointestinal tract, body cavity, lung, liver, blood, and tissues [7]. The most prevalent nematodes in buffaloes are *Oesophagostomum* sp., *Bunostomum* sp., and strongyle group (*Ostertagia* sp., *Nematodirus* sp., *Cooperia* sp.). These nematodes cause damage the small intestinal mucosal layer. Parasites such as *Haemonchus* sp. and *Bunostomum* sp. cause loss of blood and protein in abomasums and intestines due to parasitic damage and heavy infection cause anemia. Trematodes are parasitic in buffaloes that belong to the Digenea. Disease caused by liver fluke is the most common one caused by *Fasciola* sp. in ruminants [1]. Trematodes mostly inhabit the liver small intestine and sometimes the lung also. Trematodes mostly include *Schistosoma* sp., *Fasciola* sp., and *Paramphistomum* sp. Cestode majorly includes *Taenia* sp. and *Monezia* sp. which causes poor growth and diarrhea in buffaloes [7,8]. *Taenia* sp can be diagnosed with the cysticercus stage in muscles of buffalo whereas *Echinococcus granulosus* can be diagnosed with the hydatid cyst in different organ such as lung and liver.

Intestinal helminth parasites are very common in buffaloes which causes considerable economic loss to the domestic livestock industry and farming sector due to mortality and less production [9]. Gastrointestinal parasites in buffaloes bring problems and economic loss from millions to rupees [10]. Amphistomes are usually more prevalent among buffaloes but this parasite is neglected during treatment and control [11,12].

Buffalo slaughtering practices are done for meat consumption as well as for religious and traditional ceremonies. In Nepal, there are no proper slaughtering techniques and facilities which cause unnecessary contamination and loss of meat and its

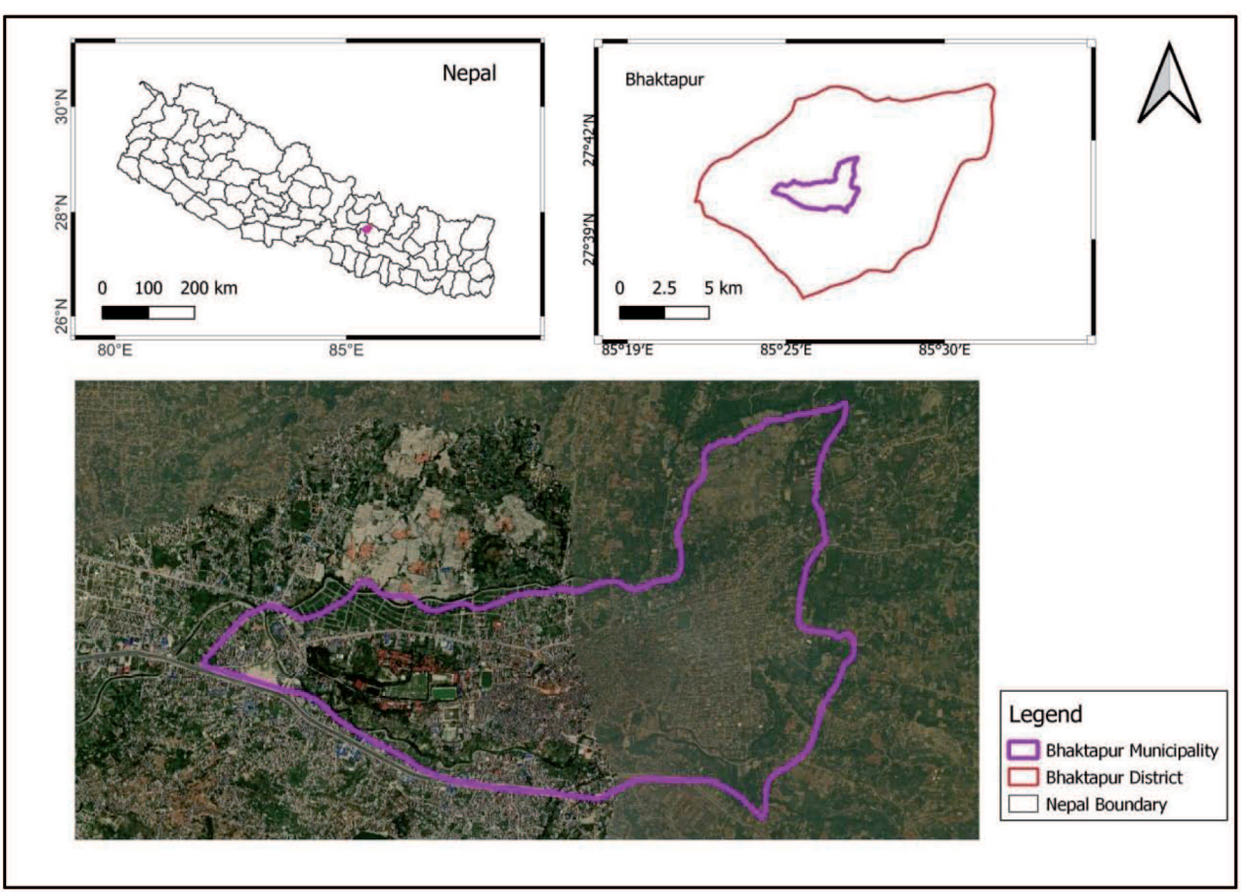


Figure 1. Map showing study area

products. Slaughtering places in and around Kathmandu are usually contaminated with excreta, street dust, and animal blood which have low quality due to infections with bacteria. These have negative impacts on meat consumers causing food poisoning. Lacking of meat inspection program in slaughtering places is the major reason of helminth infection to humans and animals. In the current study the authors have assessed the prevalence of GI helminth parasites and their diversity in the fecal and organ samples.

Materials and Methods

Sample collection, preservation and transportation

The present study was carried out on slaughtered buffaloes of Bhaktapur Municipality which lies at the east corner of Kathmandu Valley (Fig. 1). Altogether 30 slaughterhouses were present in 10 wards of Bhaktapur Municipality. One slaughter-house from each ward was included by lottery method. Ten buffaloes from each slaughterhouse were included. Three slaughterhouses each day alternately were visited early morning during the slaughtering process for sample collection. After buffaloes were slaughtered, the visceral organ was examined and certain parts of it were collected in ziplock bags and fresh faecal samples in sterile vials.

A total of 200 samples from 100 slaughtered buffaloes including faecal samples (100) as well as visceral organs (100) i.e. rumen, abomasums, large intestine, and small intestine were collected from June 2023 to Jan 2024 from the study area, preserved in 70% alcohol (organ) and potassium dichromate (faecal) and transported to the laboratory of the Central Department of Zoology, Kirtipur, Nepal.

Laboratory processing and examination

The laboratory methods of examining parasites were done by direct smear, sedimentation and floatation method given previously [13]. A drop of 1% Lugol's iodine solution was kept on a clean microscopic slide and a mixed sample was placed using a toothpick. Then a coverslip was kept on the slide and a smear was observed under a microscope with 10× and 40×.

Floatation method was specially used for the nematode eggs. In floatation method, first of all 2 gm of faecal sample was mixed with 12ml of 0.9% normal saline solution in a beaker and was mixed. It was grinded and filtered using a tea strainer or muslin cloth. The filtrate was transferred in a 15 ml centrifuge tube and was allowed to centrifugation for 5 minutes at 1200 rpm using a Remi R-303 centrifuge machine. The supernatant solution in the test tube was discarded and saturated NaCl solution (45% w/v) was added and again centrifuged (1200 rpm × 5 minutes). After centrifugation, saturated NaCl solution was added up to the brim of the test tube and coverslip was kept there and was allowed to left for 10 minutes. Then, the coverslip was kept on a slide and observed under 10× and 40× magnification for examination.

Sedimentation method was specially used for the identification of trematodes and cestodes egg. For sedimentation technique, 2 gm of faecal sample was mixed with 12 ml of 0.9% normal saline solution and was filtered using a tea strainer. The filtrate was kept in a centrifuge tube and allowed to centrifugation (1200 rpm × 5 minutes) using a Remi R-303 centrifuge machine. The supernatant was discarded and then, 10 ml of 10% formalin and 4 ml of ether were added and centrifuged (1200 rpm × 5 minutes) again. The solution except sediment was discarded. The sediment was mixed with one drop of iodine and examined under a microscope 10× and 40× for examination.

Measurement and identification of parasites

The measurement and photograph of the egg were done by ImageView software using a microscope (Olympus CX43) The software of Olympus CX43 was standardized with the measurement at 10×, 40×, and 100× magnification The size was measured by using scale in software and the photograph was snapped and saved. The identification of the egg was done by comparing the size, morphology, and internal content of the egg using various references [14,15]. Species such as *Cooperia* sp., *Trichostrongylus* sp., and *Haemonchus* sp., belongs to Strongyle group and were differentiated according to the size and internal contents such as *Haemonchus* sp., has oval, embryo contain 16–32 cells and oval, thin transparent oval layer, multisegmented and grape-like embryonic mass for *Trichostrongylus* sp. The size of Strongyle egg includes *Cooperia* sp (75.4×39 μm), *Haemonchus* sp. (71×46.4 μm) and *Trichostrongylus* sp. (92×39 μm).

Statistical analysis

Data were tabulated into an Excel worksheet and

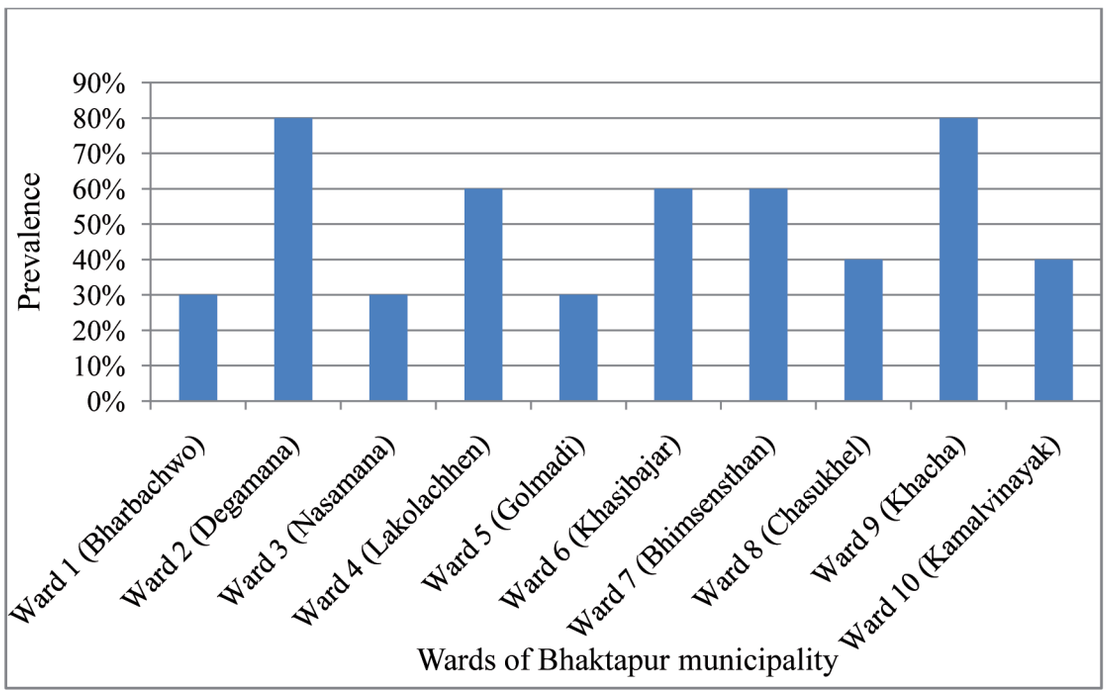


Figure 2. Bar graph showing ward-wise prevalence of gastrointestinal helminth parasite

R Studio was used for the statistical analysis. The Chi-square ($\chi 2$) and Fisher Exact test was used for the analysis of data. Chi-square test was done to find out the association between variables whereas fisher exact test was done in software having low expected values. 95% confidence interval (CI) and $P \leq 0.05$ were always taken into consideration for statistically significant differences.

Table 1. Prevalence (%) and concurrency (%) of helminth parasites of slaughtered buffaloes

Species	Number of positive faecal samples	Prevalence	$\chi 2$ (df)	P-value
Paramphistomum sp.	18	18%	54.303(9)	<0.001
Fasciola sp.	16	16%		
Oesophagostomum sp.	11	11%		
Strongyloides sp.	7	7%		
Haemonchus sp.	5	5%		
Schistosoma sp.	3	3%		
Capillaria sp.	2	2%		
Cooperia sp.	2	2%		
Trichostrongylus sp.	1	1%		
Syngamus sp.	1	1%		
Concurrency				
Single	31	31%	49.596(3)	<0.001
Double	13	13%		
Triplet	2	2%		
Quadruplet	1	1%		

Table 2. Prevalence in relation to where buffaloes were brought

Location	Infected sample	Prevalence	Chi-square (df)	*P*-value
Jitpur	20	66.67%	5.929(2)	0.052
Birgunj	16	53.33%		
Nepalgunj	15	37.50%		

Ehical approval

Ethical approval was obtained from the Nepal Veterinary Council (NVC), Tripureshwor, Kathmandu (Ref No. 104/2080/81).

Results

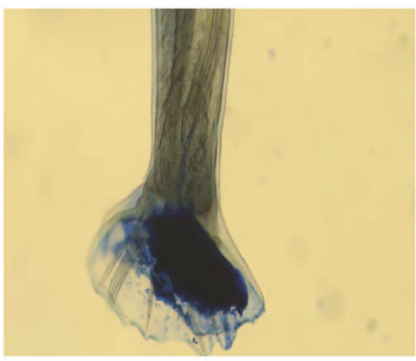
Macroscopic examination of visceral organs (100) and microscopic examination of faecal samples (100) of 100 slaughtered buffaloes showed prevalence of 51% for one or more gastrointestinal helminth parasites (Fig. 1 and 2). Two adult helminth parasites were detected with *Paramphistomum* sp. (adult) in 10 cases (10%) and *Oesophagostomum* sp. (adult) was detected in 7 cases (7%). *Paramphistomum* sp. was collected from the rumen and that of *Oesophagostomum* sp. from large intestine. There is no significant difference between the prevalence of helminth parasites among *Paramphistomum* sp. and *Oesophagostomum* sp. (*P*-value = 0.613). Faecal examination by three different methods revealed the most dominant parasite was trematode i.e. *Paramphistomum* sp. (18%) followed by *Fasciola* sp. (16%) whereas the prevalent nematode parasite was *Oesophagostomum* sp. (11%) and the difference was highly significant between different helminth parasite ($\chi2$=54.303, df=9, *P*-value <0.001). The macroscopic postmortem examination of organs showed two types of infection single infection (11%) and of double infection (3%) with statistical significant difference (*P*-value=0.049) (Table 1).

Regarding the concurrency of helminth parasites during faecal examination single infection (31%) showed higher prevalence than double (13%) followed by triple (2%) and quadruplet infection (1%) (Table 1). Maximum concurrency of four species was reported. There is a highly statistically significant difference in different types of infection ($\chi2$=49.596, df=3 *P*-value<0.001). Helminth infection from slaughterhouses located in ward 2 and ward 9 showed a highest prevalence (80%) which is followed by Ward 4,6 and 7 (60%). The buffaloes for slaughtering were all brought from outside the valley. They were imported from Jitpur, Nepalgunj, and Birgunj and the butchers bought them. Among 100 buffaloes, 40 buffaloes were from Nepalgunj, 30 buffaloes were from Jitpur and 30 were from Birgunj. The location from where animal were brought showed prevalence of 66.67% (Jitpur) followed by 53.33% (Birgunj) and 37.50% (Nepalgunj) (Table 2). There is no significant difference between infection status and location ($\chi2$=5.929, df=2, *P*-value=0.052).

Discussion

This study indicated that the prevalence of gastrointestinal helminth parasites in slaughtered buffaloes was 51% which was higher than the findings of previous studies conducted by [14] with a prevalence of 12.42%. The study was conducted in the monsoon and post-monsoon periods, the prevalence of helminth infection might be due to the favorable period for the transmission and development of different stages of gastrointestinal helminth parasites. The prevalence of helminths in buffaloes might be due to the exposure of buffaloes to contaminated food (grass, hay, straw) with the infective larval stages of the parasite. The prevalence of helminth parasites was supported by the studies [15,16,19] which reported 51.45%, 40.92%, and 54.83%, respectively. On the other hand, [17] and [7] observed a higher prevalence of gastrointestinal helminth parasites compared to our findings. The difference in the prevalence of intestinal helminth parasites in different regions might be due to changes in climatic conditions [15].

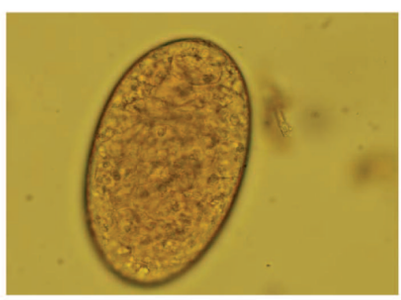
Different genera of gastrointestinal helminth parasites were found to be infected. Two genera of trematode and eight genera of nematode were found during examination but in this study, cestodes were not found which is supported by Mamun et al. [18] and Saha et al. [11] who reported no cestode parasite in their studies.



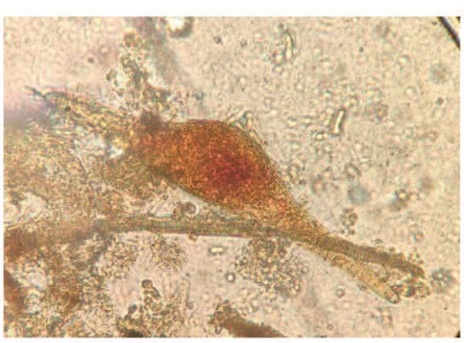
Oesophagostomum male posterior

Oesophagostomum male anterior

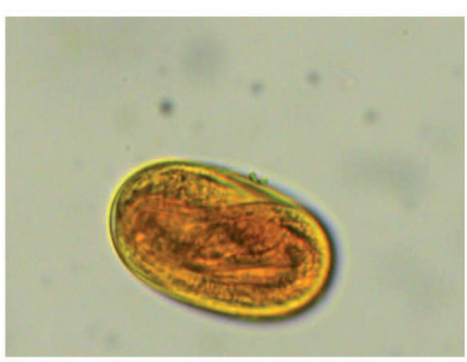
Paramphistomum sp. (adult)



Paramphistomum sp. (162×81.2 µm)



Schistosoma sp. (289×73.05 µm)



Strongyloides sp. (50×35 µm)

Figure 3. Eggs and adult stages of selected parasites detected in buffaloes (part 1)

The findings of this research align with the findings of prior studies done by [11,19,26,27). The higher case of *Paramphistomum* sp. might be due to ingestion of contaminated grass/straw/hay near the water sources which increases the risk of infection with the parasite due to the presence of an intermediate host i.e. snail. In contrast among nematodes *Oesophagostomum* sp. showed the highest prevalence followed by *Strongyoides* sp., *Haemonchus* sp. which was supported by the study [16]. Geographic and climatic condition of the slaughterhouse as well as in farming places from where animals were brought supports the growth and propagation of the infective larval stage of these nematodes [10].

The current study highlights that slaughtered buffaloes suffer from multiple parasitic infections with mixed infections of up to four different genera.

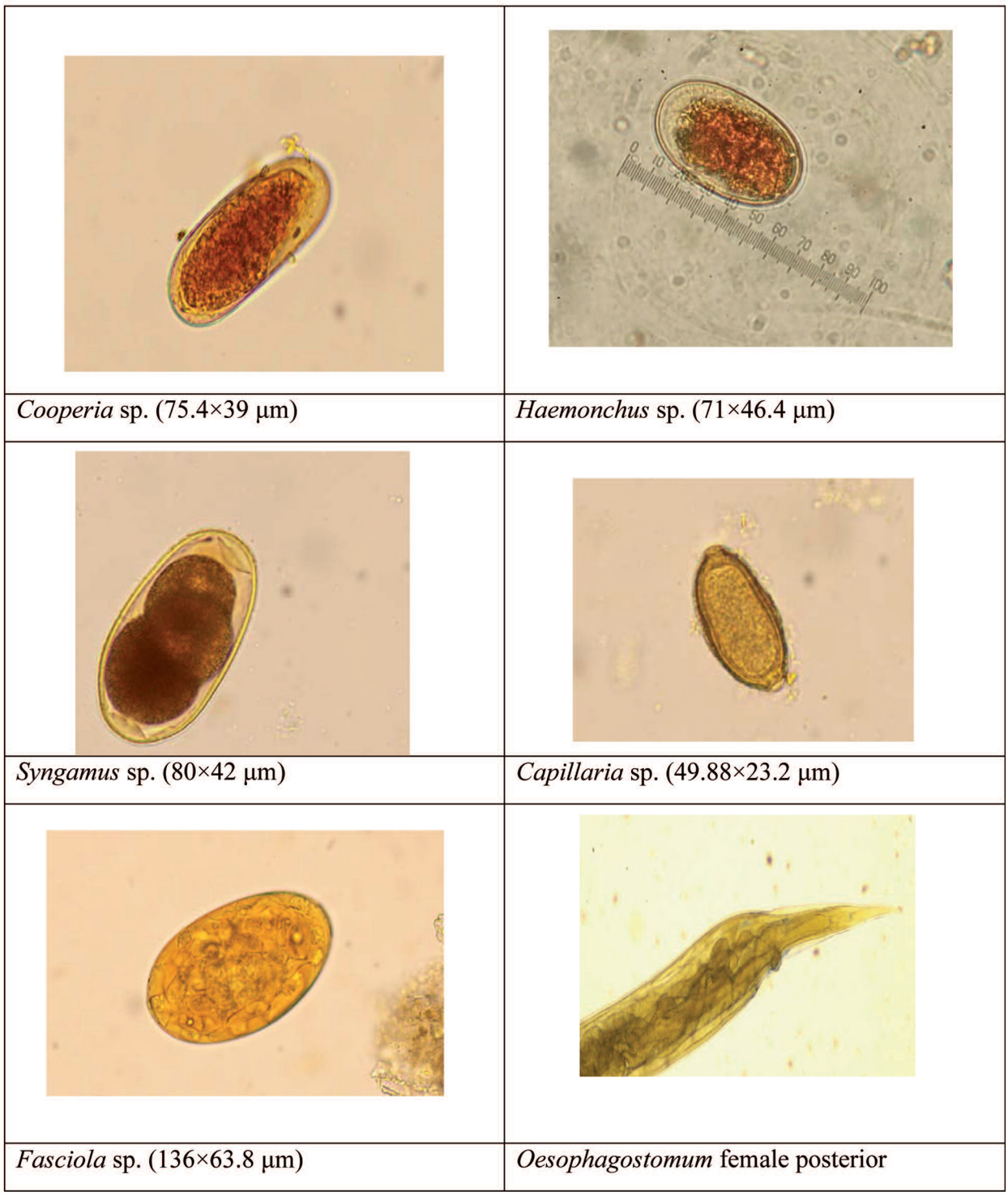


Figure 4. Eggs and adult stages of selected parasites detected in buffaloes (part 2)

The macroscopic examination of visceral organs showed single and double infection with 11% and 3% respectively whereas that of microscopic faecal examination showed the prevalence of single infection (31%) while double, triple, and quadruplet with 13%, 2% and 1% of cases, respectively. The findings of the current study were different from the other findings done by [8,14,17,25] reported a higher prevalence of mixed infection rather than single infection. The higher prevalence of mixed infection in this study was described with the grazing of buffaloes with the pasture lands contaminated with other livestock. The higher prevalence was also described with the suppression

of the host immune which increases the severity of infection with more than one parasite. The effect of these parasites is strongly related with the number of parasites and the nutrition of the animals [20].

Majority of the slaughtered buffaloes showed infection with single infection which can be described by the fact that buffaloes to be slaughtered were restricted to a defined shed as well as they are bound to certain areas in farming places rather than grazing on the pasture land with other livestock.

Macroscopic postmortem examination showed helminthic infection with 14% prevalence and that of faecal examination with 47%. A comparative study of faecal and organ examination in this study revealed that four samples that got infected during macroscopic examination were not positive during microscopic examination. This focuses on the combined examination of samples including organs as well as the total burden of parasites. The *post mortem* examination showed helminth infection with different genera such as *Fasciola* sp. in the liver, *Paramphistomum* sp. in the rumen, and *Ascaris* sp. [21]. The findings of organ examination were supported by [22] who reported five genera of nematode in the abomasum and intestines of ruminants with the highest prevalence of *Oesophagostomum* sp. The organ examination of buffaloes such as the rumen was agreed with the study by [12] who reported 20% infection of *Paramphistomum cervi* by examining the rumen of 10 buffaloes.

The buffaloes for slaughtering were all brought from outside the valley such as from Jitpur, Nepagunj, and Birgunj and the butchers bought them. The location from where animal were brought showed the prevalence of 66.67% (Jitpur) followed by 53.33% (Birgunj) and 37.50% (Nepalgunj) but there is no significant difference. A study done by [23] also found that slaughtered buffaloes in Kirtipur Municipality were also imported from Birgunj, Nepalgunj and from India.

In conclusions, GI parasites in the study area are critical in the slaughtered buffaloes so periodic examination at slaughterhouse to break the lifecycle of parasites if butchers were allowed to discard the contaminated carcasses and management should be done for better livestock production. Similarly, butchers must be known about parasites, proper management of slaughterhouse and awareness on GI parasitic diseases, and their control can livestock industry.

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