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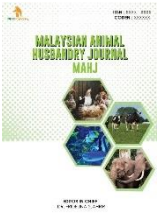

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RESEARCH ARTICLE

A STUDY ON THE PREVALENCE OF PARAMPHISTOMUM IN BUFFALOES OF SIDDHARTHANAGAR MUNICIPALITY, RUPANDEHI, NEPAL

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ABSTRACT

Paramphistomosis is one of the most commonly described serious gastrointestinal parasitic infections in buffaloes, is distributed all over the world, especially tropical and subtropical climatic conditions. This disease causes considerable economic losses to the farmers. This study was designed to determine the prevalence of Paramphistomum in buffaloes of Siddharthanagar municipality, Rupandehi, Nepal. This cross-sectional study was carried out during July 2021 to September 2021. A total of 244 fecal samples were examined using direct smear method and simple sedimentation method as described by Soulsby. Prevalence association with sex, breed, age, feeding method, deworming status and interval were analyzed using SPSS version_ 25 at 5% significance and 95% confidence level. Overall 31.56% prevalence recorded. Prevalence according to age, deworming status and deworming interval wise was recorded statistically significant ($p < .05$). Age wise prevalence was 10%, 21.74%, 33.85% and 45.83% in buffaloes of less than one year, 1-2-year, 2-4 year and more than 4 years respectively. Sex wise a greater number of females (33.85%) were found positive than males (22.45%) but the relation was statistically non-significant ($p > .05$). Breed and feeding method wise prevalence was observed non-significant ($p > .05$). The study suggested that there is high prevalence of Paramphistomosis in buffaloes of Siddharthanagar municipality, Rupandehi. Non-dewormed, occasionally dewormed and productive buffaloes are found more susceptible than the young and calves.

KEYWORDS

Paramphistomum, Buffaloes, Siddharthanagar, Rupandehi, Nepal.

1. INTRODUCTION

1.1 Background

Nepal is an agriculture based country, with more than 66 percent of people engaged in agriculture, either directly or indirectly (Poudel et al., 2020). Livestock farming represents for over 11% of national GDP and more than a third of overall employment of entire agriculture gross domestic output (Lamsal et al., 2020). Livestock, one of agriculture's most viable sub-sectors, is vital to human health and the state's economic prosperity (Pandey and Subedi, 2020). Among domesticated animals, buffalo husbandry is by far the most important for milk and meat production. In Nepal buffaloes are farmed in mixed farming system that ranges from the tropical plains of Terai to the temperate climate of the high altitude Himalayas based on the country's agro-eco situation (Joship and Mahato, 2013; Lamsal et al., 2020). Gaddi, Parkote, and Lime as well as Indian Murrah and Nili Ravi are recorded breeds of buffalo in Nepal (Lamsal et al., 2020). The overall number of stages buffalo in the country is 52, 57,591, with 16, 35,492 of them being nursing buffalo. Buffalo headcount was 49, 93,650 in 2010/11 (B.S.) and buffalo production have increased by 5.29 percent since then (Krishi Diary, 2078). The total milk and meat production in Nepal is 23,01,000 metric tons and 5,52,156 metric tons of which buffalo contribute around 60% and 34.3% respectively (Krishi Diary, 2078). Livestock sector, one of agriculture's most hopeful sub-sectors, is critical for human health and, indeed, the country's economy (Pandey and Subedi, 2020).

In South Asia, gastrointestinal helminth infections are common in bovines mainly due to contaminated grasses and water, and climate conditions, bad management practices (Thapa Shrestha et al., 2020). Buffalo gastrointestinal parasite infections are frequent, causing huge financial losses to the buffalo industry and rural communities around the world (Chauhan et al., 2015). Some researchers described paramphistomosis as one of the most common and dangerous gastrointestinal parasite illnesses in ruminants (Swarnakar et al., 2014; Yadav, 2014). Paramphistomiasis is distinguished by sporadic outbreaks of acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young stock (Horak, 1971). Paramphistomosis is found all over the world, but it is most common in the tropics and subtropics of Asia, Africa, Australia, Russia, and Eastern Europe (Chauhan et al., 2015; Debbra et al., 2018; Osman, 2017). The disease is caused by a digenean trematode parasite of the genus Paramphistomum. The trematode belongs to the Paramphistomatidae family, and the parasites in this family are also known as 'amphistome fluke,' 'conical fluke,' or 'rumen fluke' (Bhatia et al., 2010). There are numerous species of this trematode parasite, but the most common are Paramphistomum cervi, Paramphistomum explanatum, Gastrothylax crumeniferand, Fischoederius elongates (Info, 2014). Environmental factors such as temperature, humidity, rainfall, and the ecology and infection of the snail intermediate host, Lymnaea spp. are linked to the disease's epidemiology (Acharya et al., 2020).

According to the Veterinary Epidemiological Center's report, 65,468 buffaloes were affected and 24 died as a result of Paramphistomosis, with a case fatality rate of 0.04 percent (Veterinary Epidemiological Center's, 2015). In Nepal, the point prevalence of Fasciolosis and Paramphistomosis is high (more than 50-60 percent) throughout the country's plains and mid-hill regions, as well as at higher altitudes. Paramphistomiasis alone has not been explored in isolation; however, in the field, Paramphistomiasis infection is most often mixed with Fasciola infection; thus, all illnesses are credited to Fasciola infection (Joship and Mahato,

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2013). Thus, this prevalence study aims to determine the incidence of Paramphistomosis in buffaloes of Siddharthnagar Municipality, Rupandehi district, Nepal.

1.2 Statement of the Problem

The parasitic disease, Paramphistomosis is one of the important serious diseases in buffalo farming which causes heavy economic losses to the livestock industry annually in many countries (Osman, 2017; Syed Shabih and D, 2006). The most common and serious parasitic diseases of buffaloes in Nepal under the sedentary management system are fascioliasis, paramphistomosis, and ascariasis in calves (Joship and Mahato, 2013). It is one of the neglected tropical diseases in which immature flukes infection is commonly associated with anemia, hypoproteinemia, submandibular edema, enteritis, and emaciation of the host (Shaheen et al., 2013). It causes significant economic losses due to poor and rough hair lowered milk, weight, and feed utilization efficiency, growth, and meat output (Muhammad et al., 2017; Acharya et al., 2020; Shabih, 2006; Shaheen et al., 2013). Paramphistomosis leads to severe neutrophilia, eosinophilia, and anemia of normocytic normochromic type (Chauhan et al., 2015). So, this study is designed to determine the incidence of Paramphistomosis in buffaloes of Bhairahawa, Indo Nepalese border, situated in Rupandehi district.

1.3 Rationale of the Study

The total population of buffalo in Rupandehi is 1,56,336 and 52,562 are milking ones (Cooperation et al., 2021). The total milk production in this district is 63,470 metric tons of which 47,306 metric tons (75%) is contributed by buffalo farming only. Also, in Rupandehi buffalo farming supplies 46% of total meat 12,174 metric tons (Cooperation et al., 2021). The population of buffalo in Siddharthanagar municipality is 1556 (MOLD, 2017). The hot weather of the Rupandehi district is ideal for parasites like Paramphistomum to thrive (Lamichhane et al., 2020). Siddharthanagar municipality is situated at the southern border of the Rupandehi district.

Previous studies by some researchers have revealed a high prevalence of Paramphistomum in the Terai region of Nepal (Lamichhane et al., 2020; Bista et al., 2018; Regmi et al., 2020; Mukhial et al., 2007). However, regarding the prevalence of Paramphistomum prevalence in buffaloes, there are scanty researches in the Rupandehi district and no baseline information available for Siddharthanagar municipality. This study will be fruitful as it will give information regarding the prevalence of Paramphistomum in buffaloes of Siddharthanagar Municipality, Rupandehi. This study will also be for future investigators, those investigating the disease in buffaloes.

1.4 Objectives

1.4.1 General Objective

To determine the prevalence of Paramphistomum in buffaloes of Siddharthanagar Municipality, Rupandehi.

1.4.1 Specific Objectives

To know about the qualitative examination.

To determine the overall prevalence of Paramphistomosis in buffaloes of Siddharthanagar Municipality, Rupandehi.

To determine the association with factors such as; age, sex, feeding method, deworming status, deworming interval.

2. Literature Review

2.1 Classification and Description

Paramphistomum is a genus of digenetic trematode parasites which mostly parasitizes the domesticated ruminants and causes a disease condition known as paramphistomiasis (Europe, 1901). The genus Paramphistomum is a member of the Paramphistomatidae family. These have been noted in ruminants throughout Asia and Europe. The most commonly reported species in Asia are Paramphistomum cervi and Paramphistomum epiclitum (Ali et al., 2018). Paramphistomes, also widely recognized as rumen fluke, seems to be pear-shaped with a slightly concave ventral and convex dorsal shape. The parasite is about 5–13 mm long and 2–5 mm wide across the midsection (Panyarachun et al., 2010). To complete their lifecycle, Paramphistomum parasites involve an intermediate snail host and a final ruminant host. Freshwater snails (Indoplanorbis exustus, Lymnaea luteola, Gyraulus convexiusculus) serve as the intermediate hosts (Bhatia et al., 2010; Soulsby, 1982). Paramphistomes have three larval stages (sporocysts, rediae, and cercariae) and reproduce asexually within the intermediate host before emerging as metacercariae from the snail and encyst. The infection of the definitive host is caused by the ingestion of vegetation containing dormant encysted metacercariae (Huson et al., 2017). Metacercariae excyst in the duodenum penetrates the mucosa and migrates anteriorly through the tissues after being consumed with vegetation. They return to the lumen and creep further forward to the rumen once they reach the abomasum or true stomach. They attach among the villi and reach maturity in two to four months (Fredericksen, 1983). Adult Paramphistomes primarily inhabit the rumen and reticulum (Hajipour et al., 2021). Although stomach fluke commonly parasitizes the rumen epithelial cells, Paramphistomum cervi (stomach fluke) is also reported to infect and damage buffalo liver (Debbra et al., 2018). The severity of the disease varies according to the age of the host, parasitic load, and the stage of the parasite. In general, the young immature flukes inhabit the duodenum and are more pathogenic than adult flukes (Ates and Umur, 2020). Watery diarrhea, dehydration, rapid weakening, and sometimes inter- mandibular edema, hypoproteinaemia, anemia, hemorrhagic enteritis symptoms are observed during the high burden of immature rumen flukes (Ates and Umur, 2020; Huson et al., 2017). Ruminal papillae atrophy, ulceration at point of attachment, and negative impact on milk yield feed conversion performance and growth rate are associated with adult fluke infections (Huson et al., 2017).

2.2 Global Scenario

In an epidemiological study at Punjab of India, a researcher reported a prevalence rate of 7.59% and 3.8% in buffalo and cattle (Syed, 2006). A total of 1608 buffalo and 651 cattle fecal samples were examined using sedimentation technique and 122 and 22 samples respectively were observed positive.

A group researchers did a prevalence study at Kafr El-Sheikh, Egypt Governorate to determine the epidemiological and clinical features concerning paramphistomiasis in ruminants and found 38.92% and 41.74% prevalence rate in cattle and buffalo respectively (Osman et al., 2017). They observed that the result was significantly ($p< 0.05$) associated with the sex and age of the animals.

During one year study period from 2003 to 2004 in Pakistan, a group researchers examined 9600 cattle samples (2400 slaughterhouses + 2400 each from farms, veterinary hospitals, and households) and found an overall 7.89% prevalence rate (Khan et al., 2008).

A study at Zabol, southeast of Iran, reported 19.5% positive cases during fecal inspection for the presence of amphistomum eggs (Hajipour et al., 2021). They identified amphistome species and their prevalence were Paramphistomum cervi (13.3%), Cotylophoron cotylophorum (19.5%), Gastrothylax crumenifer (5.9%), and Carmyerius spatiosus (2.7%). The association of prevalence with age, breed, and grazing system was significant ($p < .001$).

In another coprological examination conducted by some researchers reported 13.74% prevalence in buffaloes as compared to cow (10.25%) but the gap was statistically not significant (Bhanot and Gupta, 2019). Also, they found a significant difference between diarrheic and non-diarrheic animals concerning the presence of paramphistome eggs in the feces.

2.3 Important findings in Nepal

During a study in western Chitwan, Bharatpur Metropolitan City, ward 26 & 16, out of 43 buffalo and 57 cattle fecal samples, fluke infestation was found in 18.6% and 28.1% of the sample respectively (Bista et al., 2018).

During a Comparative study of gastrointestinal helminth parasites of buffalo in Syangja, out of 50 buffalo fecal samples, 4 (8%) samples were found positive (Pandey and Subedi, 2020).

A group researcher in their qualitative fecal study for gastro-intestinal parasites, at AFU livestock farm Rampur, Chitwan described an overall 24% prevalence of Paramphistomum Spp (Regmi et al., 2018). Out of 30 random fecal samples from buffalo and 20 random samples from cattle, 20% and 30% prevalence were observed in buffalo and cattle respectively.

In a retrospective study from the record book of the Veterinary Teaching Hospital, Agriculture and Forestry University from July 2012 to July 2017, a total of 712 buffaloes helminth cases were studied and 35% cases were found positive for Paramphistomosis (Regmi et al., 2020).

A group researches reported a 15.64% prevalence of Paramphistomum in buffaloes brought to Kathmandu for slaughtering. The major species reported was Paramphistomum cervi (Mukhial et al., 2007).

A study the prevalence of Paramphistomum infestations in buffaloes in a mid-hill village of Surkhet district was studied (Parajuli, 1993). Out of 37 buffaloes examined, the prevalence of Paramphistomiasis was 35.1%. Parajuli, B.

3. Methods and Methodology

3.1 Site of Study

This study was conducted from July 2021 to September 2021 at Siddharthanagar municipality of Rupandehi district of Nepal. Rupandehi district, a part of Lumbini Province, is located in the southern and western parts of Nepal. It covers an area of 1,360 square kilometers, with 16.1% located in the Churia Range and the remainder in the Terai region. The district's elevation ranges from 100 meters to 1,229 meters above sea level. It encompasses three distinct climatic zones determined by elevation: 89.3% falls within the lower tropical zone (below 300 meters), 10.5% within the upper tropical zone (300 meters to 1,000 meters), and 0.2% within the subtropical zone (1,000 meters to 2,000 meters). Siddharthanagar municipality is located at geographic coordinate's 27o 30' 0 North latitude and 83o 27' 0 East longitude with a total area of 36.03 km2. It is situated at an altitude of 110m from sea level. The average annual rainfall is 1436.5 mm with temperatures ranging from a maximum of 45.2 oC and a minimum of 2.4 oC. The climate of the municipality is mostly tropical. The municipality has 13 wards.

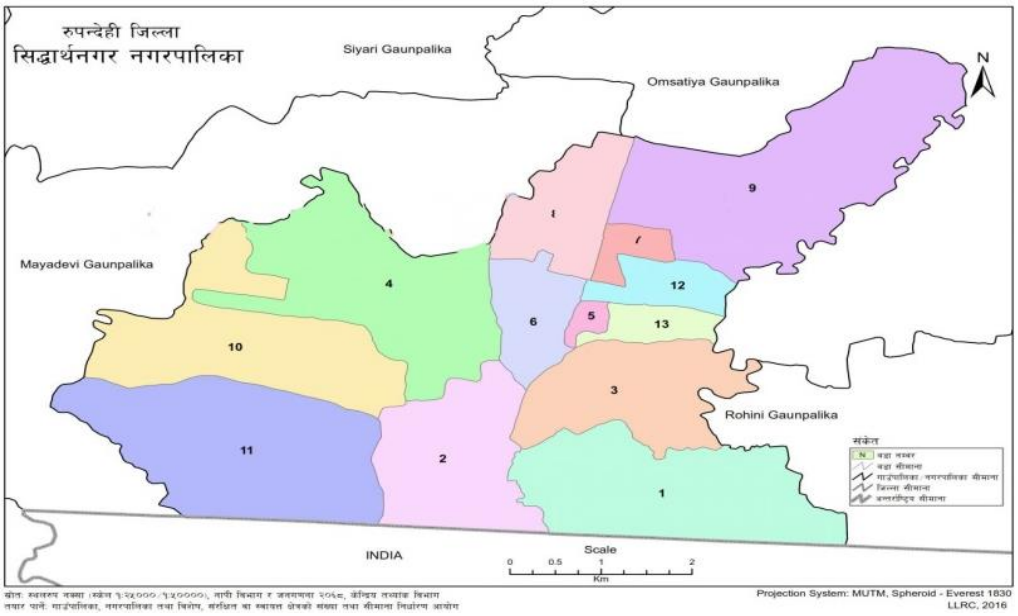

Figure 1: Map of Siddharthanagar municipality, Rupandehi Source: https://www.nepalarchives.com/content/siddharthanagar-municipality-Rupandehi-profile/

3.2 Study Design

A cross-sectional descriptive study was performed from July 2021 to September 2021.

3.3 Sample Size Calculation

Sample size was calculated using Daniel equation (1999),

Sample size (n) = Z2 * p (1-p)/ d2

Where,

Z= Z- score value at 95% level of confidence (1.96)

p= estimated prevalence based on previous study

d= level of precision 5% (0.05)

From the above formula, obtained sample size (n) is 245 at 20% (S. Regmi et al., 2018) estimated prevalence.

3.4 Sampling Collection and Preservation

20-30-gm fecal samples were collected directly from the rectum and as well as recently excreted fresh samples. Samples were kept in a zip-lock plastic bag with a label and in case of dry feces moistened by normal saline. The sample was immediately kept in an icebox and taken to the lab within 3-4 hours after collection. Then samples were examined fresh or stored at 4oC for later examination. Information such as age, sex, management practices, etc. was recorded in a closed type questionnaire.

3.5 Parasitological Techniques

The collected fecal samples were examined using qualitative and quantitative techniques.

3.5.1 Qualitative Examination

The direct smear method and simple sedimentation method as described by Soulsby was used to detect Paramphistomum eggs (Soulsby, 1982).

3.5.1.1 Direct Smear Method

A small quantity of fecal sample was taken on a clean glass slide and mixed with 1-2 drops of tap water. The sample was spread out to form a translucent film and covered with a micro-coverslip (18mm x 22mm) and then examined under a microscope at 10X magnification. At least two slides were prepared from different parts for each of the samples.

3.5.1.2 Sedimentation method

Sedimentation technique is used for the detection of trematode eggs. It provides good results as the eggs of trematode are a bit heavier than the other eggs and deposited at the bottom.

- 3 gm of fecal sample homogenized with tap-water in mortar and pestle and strained through a tea strainer. Then placed in a plastic cup or test tube and left for sedimentation for 20-30 minutes.
- The supernatant was discarded; again water was added to the sediment till the supernatant was clear.
- Finally, 1-2 drop of sediment was taken in a slide with the help of transfer pipette and covered with micro-coverslip (18mm X 22mm) and observed in the microscope in 10X.

3.6 Statistical Analysis

All of the data was first entered into Microsoft Excel 2010. Then analyzed by using SPSS version 25 software. The prevalence was analyzed with different factors such as age, sex, feeding practices, grazing, deworming status, etc. using the Chi-Square test at the confidence level of 95% and 5% ($p < 0.05$) level of significance.

4. Results

4.1 Overall Prevalence

Out of 244 fecal samples examined 77 (31.56%) samples were positive for Paramphistomum spp.

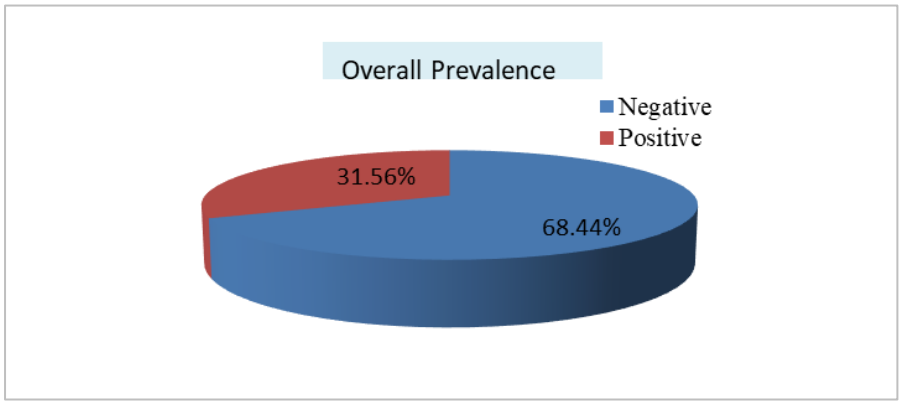

Figure 2: Overall Prevalence of Paramphistomum

4.2 Sex Wise Prevalence

Out of 244 samples, 195 samples were from female buffaloes and 49 were from male buffaloes. Among 195 female samples, 66 (33.85%) and among 49 male samples, 11(22.45%) were observed to be positive.

Null hypothesis: there is no association between Paramphistomum prevalence and the sex of buffalo.

To test the hypothesis of experiment chi-square test was followed. The test showed that the prevalence was not significantly [$\chi 2$ (1, N=244) = 2.355, p = .125] associated with the sex. The odds of positive cases in females were 1.767 times the odds of positive cases in males at 95 % confidence interval [0.8486 to 3.6812] but statistically, a non-significant relationship was observed.

4.3 Breed Wise Prevalence

Out of 244 buffalo fecal samples, 145 samples were from local indigenous breeds and 99 were from improved crossbred buffaloes. Among them, 40 (27.59%) local and 37 (37.37%) improved breeds were found to be positive.

Null hypothesis: there is no association between Paramphistomum prevalence and breed of buffalo.

To test the hypothesis chi-square test was performed which yielded non-significant [$\chi 2$ (1, N=244) = 2.609, p= .106] association, and thus, the null hypothesis was accepted. The odds of positive cases in the improved breed were 1.567 at 95% C.I. [0.9072 to 2.7051] times the odds of positive cases in local breed buffaloes.

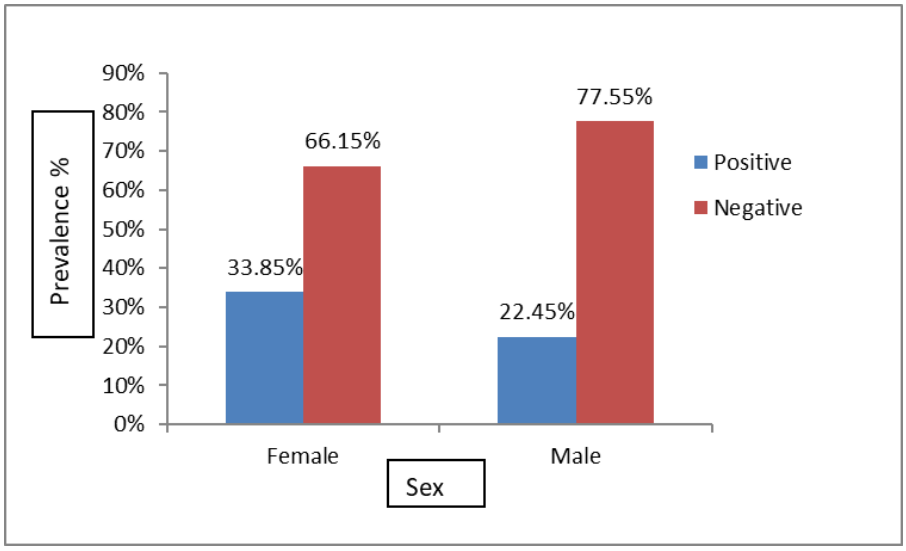

Figure 3: Sex wise Prevalence of Paramphistomum

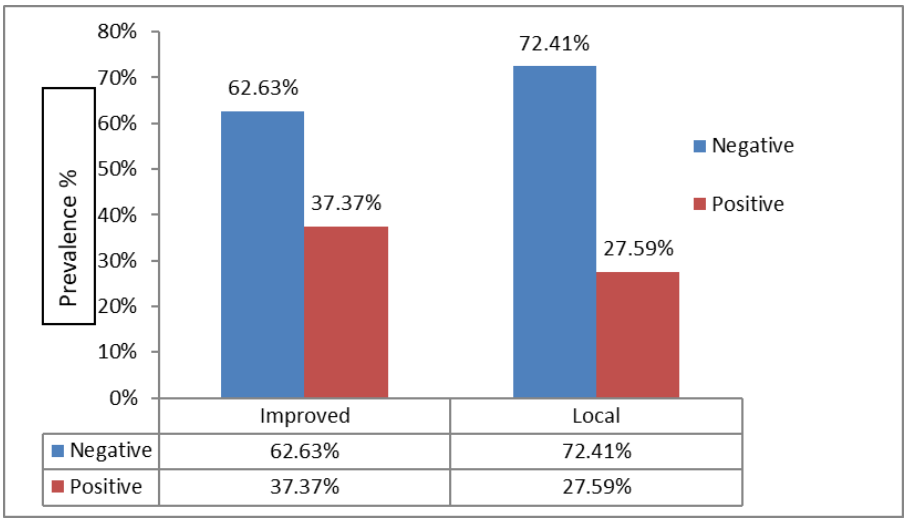

Figure 4: Breed wise prevalence of Paramphistomum

4.4 Age-Wise Prevalence

Out of 244 fecal samples, 60 samples were from less than one year, 23 samples from 1-2-year age group, 65 samples from 2-4-year age group, and 96 samples were from buffaloes more than 4 years. 6, 5, 22, and 44 samples were observed to be positive in less than one year, 1-2 years, 2-4 years, and more than four years respectively.

Null hypothesis: there is no association between the prevalence of Paramphistomum and the age of buffaloes.

To test the hypothesis chi-square test was performed and the result showed that the prevalence of Paramphistomum was significantly [$\chi2$ (3, N=244) =23.152, p < .001] associated with the age of buffaloes. The Cramer's V was 0.308 which indicates a moderate strength effect between the prevalence of Paramphistomum and different age groups of buffaloes.

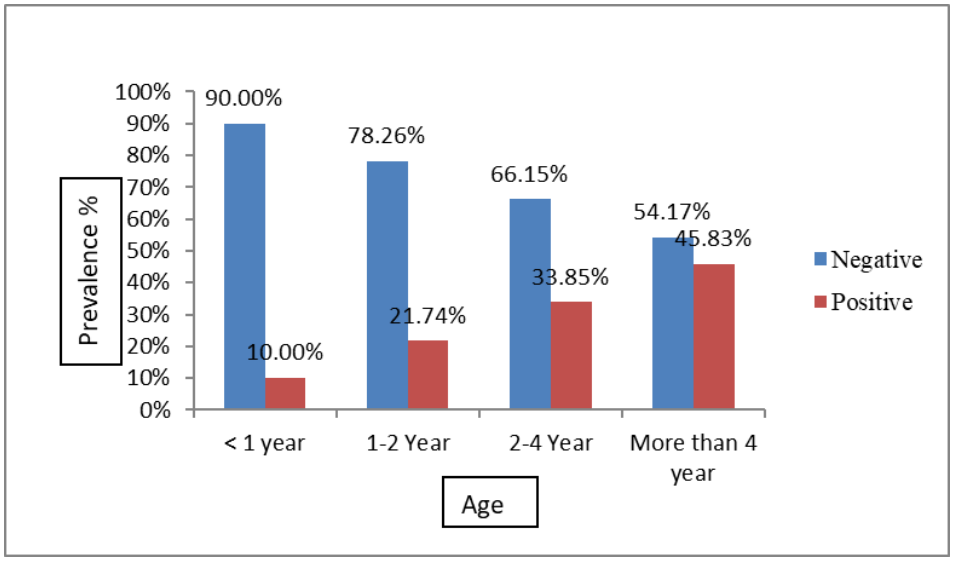

Figure 5: Age-wise prevalence of Paramphistomum

Table 1: Age wise p value and odds ratio

S.N.	Age Group	d.f.	$\chi2$ value	P-value	Cramer's V	Odds ratio	95% C.I.
a)	≥ 1 year and < 1 year	1	17.119	< 0.01*	0.265	5.656	2.3126-13.8277
b)	≥ 2 year and < 2 year	1	19.513	<0.01*	0.283	4.547	2.2402-9.2306

*means significant at p<0.05 significance level
d.f.= degree of freedom

The relationship was further analyzed between buffaloes having age; a) less than one year and more than or equal to one-year b) less than two years and more than or equal to two years.

This shows that there is a significant relationship between the prevalence of Paramphistomum and the age group of buffaloes. The odds of positive cases of Paramphistomum in buffaloes of age more than or equal to one year is 5.656 times the odds of positive cases in buffaloes having age less than one year. Similarly, the odds of positive cases of Paramphistomum in buffaloes of age more than or equal to two years is 4.547 times the odds of positive cases in buffaloes having age less than two years. In both, the scenario Cramer's value was 0.265 and 0.283 which shows a moderate strength association between age groups and prevalence of Paramphistomum.

4.5 Feeding System-Wise Prevalence

Out of 244 samples, 194 (79.5%) samples were from farmers practicing both grazing and stall feeding and 50 (20.5%) samples were from farmers practicing stall feeding only. Among them, 65 (33.51%) from mixed feeding practice and 12 (24%) from stall feeding only were found positive. The result was statistically non-significant [$\chi2$ (1, N=244) = 1.66, p= .197] with the association between prevalence of Paramphistomum and feeding system of buffaloes. Cramer's V value 0.083 showed very weak strength effects with the occurrence of Paramphistomum and feeding practice.

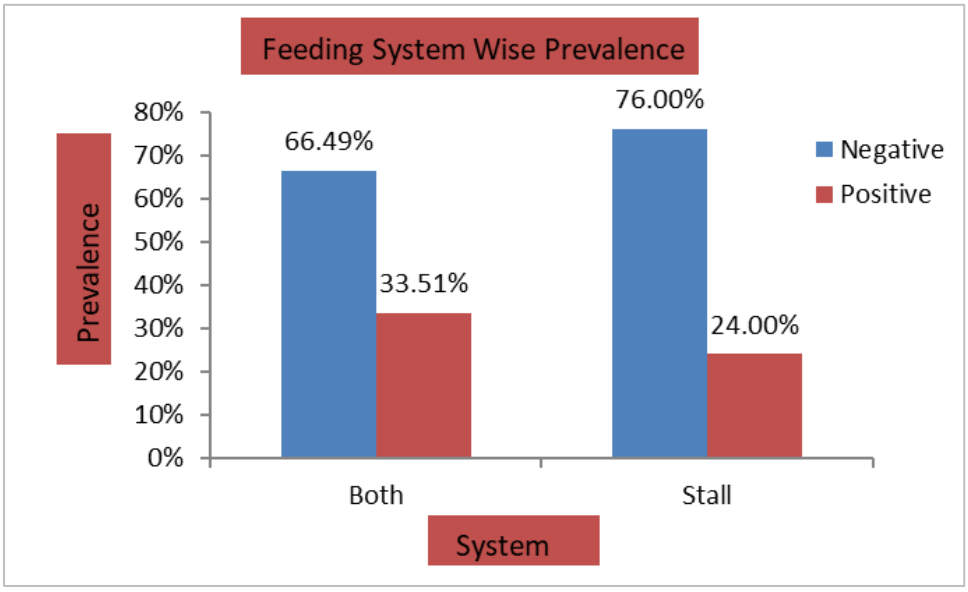

Figure 6: Feeding System Wise Prevalence of Paramphistomum

4.6 Deworming Status Wise Prevalence

Out of 244 samples, 175 (71.72%) samples were from non-dewormed buffaloes and 69 (28.28%) samples were from dewormed buffaloes. Among them, 70 (40%) in the non-dewormed group and 7 (10.14%) in the dewormed group were found to be positive. The result showed that Paramphistomum is significantly, [$\chi2$ (1, N=244) = 20.42, P < .001] associated with the status of deworming. The odds of positive cases in the non-dewormed group were 5.91 times, at 95% confidence interval [2.554 to 13.650] the odds of positive cases in the dewormed group. The Cramer's V value yielded is 0.289 which indicates moderate association strength.

4.7 Deworming Interval Wise Prevalence

Out of 244 samples collected; 27 (11.07%), 22 (9.02%), 93 (38.11%), and 102 (41.80%) were from farmers practicing yearly, half-yearly, never, and occasional deworming interval. Among them, 2 (7.41%), 1(4.55%), 37(39.78%), and 37 (36.27%) samples were found positive in respective groups.

The result was significant, [$\chi2$ (1, N=244) = 18.69, p < .001] with the association between prevalence of Paramphistomum and deworming interval.

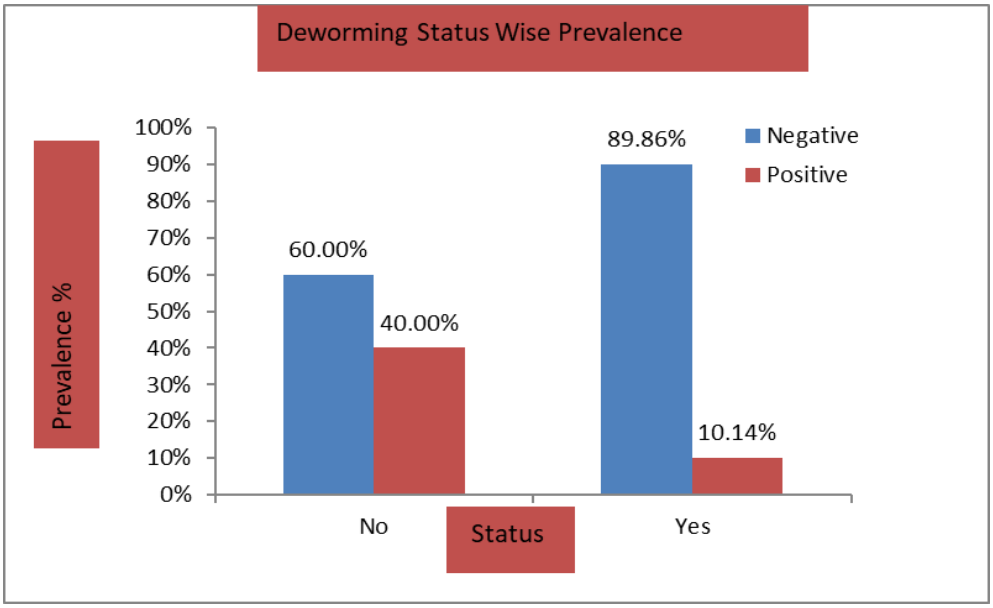

Figure 7: Deworming Status Wise Prevalence of Paramphistomum

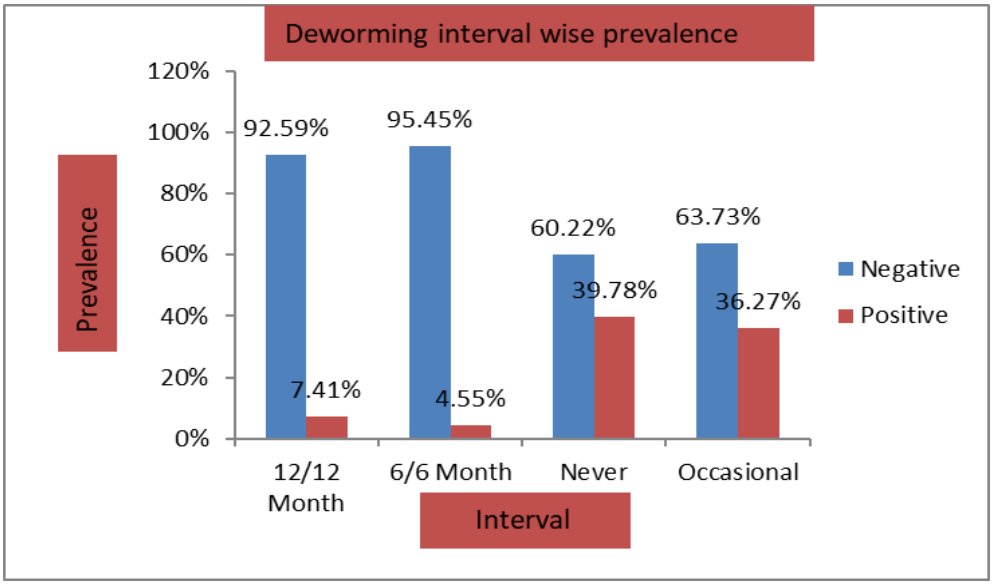

Figure 8: Deworming Interval Wise Prevalence of Paramphistomum

The prevalence association between different deworming intervals was further analyzed at 95% confidence interval. There was no statistically significant difference between 12/12 month and 6/6-month deworming but odds of positive cases in the yearly interval was 1.68 times the odds of cases in the half-yearly deworming interval. There was statistically a significant difference between occasional + never and 6/6-month deworming interval practice and occasional + never and 12/12-month deworming interval practice.

Table 2: Deworming interval wise prevalence association p value and odds ratio

S.N.	Deworming interval	d.f.	$\chi2$ value	P-value	Cramer's V	Odds ratio	95% C.I.
a)	12/12 month and 6/6 month	1	0.17	.6805	0.059	1.68	0.1422 to 19.8541
b)	Occasional+ Never and 6/6 month	1	9.75	.0018*	0.212	12.84	1.6920 to 97.4818
c)	Occasional+ Never and 12/12 month	1	9.83	.0017*	0.21	7.65	1.7594 to 33.2169
d)	Occasional and Never	1	0.26	.6136	0.036	1.16	0.6505 to 2.0712

*Means significant at p<0.05 significant level
d.f.= degree of freedom

5. Discussions

This prevalence study shows overall a high prevalence, (31.56%) of Paramphistomum in buffaloes of Siddharthanagar Municipality, Rupandehi district. This finding is in accordance with who found 32.4% prevalence in buffalo and comparable with the finding of who found 35% prevalence in buffalo (Ates and Umur, 2020; Regmi et al., 2020). This prevalence rate is lower than the findings of who reported 45%, 41.74%, and 75.63% prevalence of Paramphistomosis in buffalo but higher than the findings of who reported 15.64%, 20%, 18.6%, 12.3% and 10.57% prevalence of Paramphistomum (Gupta et al., 2012; Osman, 2017; Swarnakar et al., 2014; Mukhial et al., 2007; Regmi et al., 2018; Bista et al., 2018; Info, 2014; Yadav, 2014). This may be due to variation in sample size, climatic condition, epidemiology of parasite and intermediate host according to geography, management practice of buffaloes, etc.

In this study, the prevalence of Paramphistomum is comparatively high in female buffaloes (33.85%) than male buffaloes (22.45%). However, the prevalence is non-significant ($p > .05$) with the sex of buffalo. A similar finding was also reported by who reported significant ($p < .05$) sex predisposition, female (41.61%) than males (27.45%) (Osman, 2017). But some researchers documented a higher prevalence of Paramphistomosis in male buffaloes as compared to female buffaloes (Khan et al., 2006; Swarnakar et al., 2014). This difference may be due to more female fecal samples (195) compared to male fecal samples (49), stress factors due to lactation and parturition, etc.

Age-wise prevalence study showed a highly significant ($p < .001$) association between prevalence and age of buffaloes. The result was highly significant ($p < .001$) when prevalence between age group; a) ≥ 1 year and < 1 year and b) ≥ 2 year and < 2 years was analyzed. The odds ratio was 5.66 and 4.54 in the group (a) and (b) respectively. The prevalence was; 10% in less than one year, 21.74% in buffaloes of 1-2-year group, 33.85% in 2-4 years, and 45.83% in more than four-year age group buffaloes. This finding is comparable to the findings of who reported 6.25%, 45.71%, 53.33%, and 55% in buffaloes of less than one year, 1-2 years, 2-4 years, and more than four years respectively (Osman, 2017). A group researchers also reported high prevalence (90%) in adult buffaloes than calves (56.83%) in calves (Swarnakar et al., 2014). The possible reasons may be less chance of exposure at the early calve stage to the grazing field and approximately two months large prepatent period of the parasite (Miller, 1997). But this finding varies from the findings of who reported higher prevalence in buffaloes less than two years old than buffaloes above two years of age (Khan et al., 2006). The possible cause of this discrepancy may be the sampling technique adopted by who studied samples from slaughterhouses, livestock farms, and households. Other factors may be variation in management and housing practice (Khan et al., 2006).

Breed-wise, the improved buffaloes (37.37%) were found more susceptible to Paramphistomum than the local breed buffalo (27.59%). However, the result was statistically non-significant ($p > .05$). A similar, result was also reported by who reported 21.4% prevalence in Murrah crossbred buffaloes (within breed group) and 20% in local buffaloes (Bista et al., 2018). The prevalence of Paramphistomum was also found non-significant ($p > .05$) with the feeding practice (i.e. stall feeding only and grazing). However, comparatively more prevalence (33.51%) was found in grazing buffalo than stall-fed buffaloes (24%). The possible reason may be the high population of snails, the intermediate host, feeding of cut grasses from the common field where buffaloes and other ruminants (cattle and goat) graze which contaminate the roughages with their feces.

The prevalence of Paramphistomum was found highly associated with deworming status and deworming interval of the buffaloes. Among the dewormed group, 10.14% buffaloes were observed to be positive whereas 40% positive cases were observed in the non-dewormed group. Similarly, the prevalence with deworming interval; yearly, half-yearly, and occasional wise were 7.41%, 4.55%, and 39.78% within each group. In cases of never dewormed the prevalence was 36.27%. The findings were statistically significant ($p < .001$) deworming status and deworming interval wise. This shows that the prevalence of Paramphistomum is high in the regions where deworming is not practiced at the appropriate deworming interval. In this study, about 41.80% of farmers were found occasional deworming habits and 38.11% of farmers found never dewormed to their buffaloes. This shows that the majority of farmers are unaware of the importance of deworming in livestock.

The present study investigated the overall 31.56% prevalence in buffaloes of Siddharthanagar Municipality, Rupandehi district of Nepal. This study shows that buffaloes of this area are highly infested with this trematode parasite due to the unawareness of farmers about deworming. Factors like sex, breed, feeding system, and pregnancy were found non-significantly associated with the prevalence of Paramphistomum. Age and deworming practices are identified as risk factors. The prevalence was significantly higher in non-dewormed buffaloes, as well as in adult and older productive buffaloes. Paramphistomosis being one of the important reasons behind loss in the buffalo production sector, further epidemiological study is required with strategic control measures to combat the parasitism in buffalo production.

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