Study of Prevalence of *Paramphistomum cervi* in domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

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ABSTRACT

Pramphistomum cervi has been a neglected but very crucial trematode parasite causing huge unseen economic loss in ruminant production globally. It has become an endemic species of fluke predominant in tropics and subtropics of Nepal. This study was aimed to estimate the overall prevalence of Pramphistomum cervi eggs in different domestic ruminant species of Pokhara Lekhnath Metropolitan City of Kaski district and to find the interaction of prevalence with other variables. A cross sectional study was performed to study the prevalence of Paramphistomum cervi eggs identified by Parasitological unit of Veterinary Hospital and Livestock Service Experts Centre, Kaski from January 2021 to April 2022. Out of 900 observations,842 observations with complete information were filtered out and included in our study. Statistical analysis was done with EpiInfo (Version 7.2.4.0) and graphical representation was done with MS Excel 2016. The overall prevalence of Pramphistomum cervi in different species of domestic ruminants was found to be 16.98%(143/842). The highest prevalence was seen in Cattle that was18.21% (45/247), followed by goats 16.66% (5/30) and buffaloes 16.46% (93/565). Seasonwise study of prevalence of Paramphistomum cervi in all the three ruminant species showed the highest overall infectivity during summer i.e 20.42% (88/431) followed by 13.38% (55/411) in winter and this difference was statistically significant (p-value 0.0065 at 95%CI). The higher prevalence of parasite was observed in adult lactating females of improved breeds. Thus, Paramphistomum cervi infection has become an important animal health issue to be addressed for safeguarding animal health and improvement of ruminant production in Pokhara Lekhnath Metropolitan City, Kaski. The biological intermediate host control measures in the pasture and routine screening of the parasite with routine deworming is essential to reduce the fluke burden among ruminants of Pokhara Metropolitan City in the future.

Keywords: *Paramphistomum cervi, Prevalence, Ruminants, Season*

INTRODUCTION

Paramphistomum spp. are gastrointestinal helminths of ruminants belonging to the class trematode and family Pramphistomidae having dorsoventrally flattened and leaf like shape. These are found all over the world tropics and sub-tropics (Hanna,1988). They have indirect type of life cycle like other trematodes involving the freshwater snails (*Bulinus* spp., *Glyptanisus gilberti, Indoplanorbis exustus, Planorbis planorbis* , Stagnicola spp.and *Lymnaea bulimoides*) as intermediate host and ruminants as definitive host. (Chaodhary et al.,2015, Hotessa et al.,2020). Paramphistomum infects cattle, sheep, goats migrating in the small intestine cause more severe damage and other livestock as well as many wild ruminants. (González et al., 2013).

The common rumen fluke, *Paramphistomum cervi* is considered to be one of the most important species of Paramphistomes. (Khan et al.,2008) The mature stages residing on walls of rumen and reticulum rarely produce clinical disease while immature migrating froms can bury into sub mucosa of duodenum, destroy epithelial cells resulting in severe illness and often death due to anaemia. (Dube et al.,2010). The infection with immature *P. cervi* can result in a gastrointestinal disease in ruminants called paramphistomosis, which can have a deleterious impact on livestock production. The prevalence of this disease has been reported higher in tropical and sub-tropical regions of the world. (Hotessa, et al., 2020). Pramphistomosis belongs to the category of food and water borne trematodiasis and considered one of the most significant parasitic disease of domesticated ruminants (Mursyidah et al.,2017).

Kaski district have more than 44 thousand cattle, 112,000 buffalo and 126,000 goat population which serves as an important economic sources for the farmers (MOALD, Gandaki Province, 2076/7). Pramphistomosis, favored by humid and sub-tropical climate, are causing deleterious impact on economy of the ruminant farmers of Kaski district. Pokhara Lekhnath Metropolitan City is the largest municipality in Kaski district and has largest number of domestic ruminants. Paramphistomosis causes huge economic loss due to various unseen but critical loss such as reduced fertility, delayed growth and development, delayed sexual maturity, poor productivity, cull animals, and poor hide quality (Dube et al., 2010). However, the epidemiology of Paramphistomosis in domestic ruminant of Kaski is poorly understood. This study will provide some basic information about the prevalence of the parasite, interaction with other factors for formulating policies about strategic control of this diseases. The objective of this study to find out the prevalence of *Pramphistomum cervi* eggs in Pokhara Lekhnath Metropolitan Municipalty, Kaski and and to study the association between prevalence of *Paramphistomum cervi* eggs and other variables like season, age, breed, location, and type of animal.

MATERIALS AND METHODS

Study site

The study was carried out in largest metropolitan city of Nepal (in terms of area), Pokhara Lekhnath Metropolitan City of Kaski, Nepal. Kaski district lies in the western part of Nepal with co-ordinates 28 °12 '30 "N, 83 °59 '20 "E. The Pokhara Lekhnath Metropolitan city has a humid subtropical climate and receive a high amount of precipitation annually. Faecal samples were brought by the farmers from all the wards of the Metropolitan city for test of helminthes.

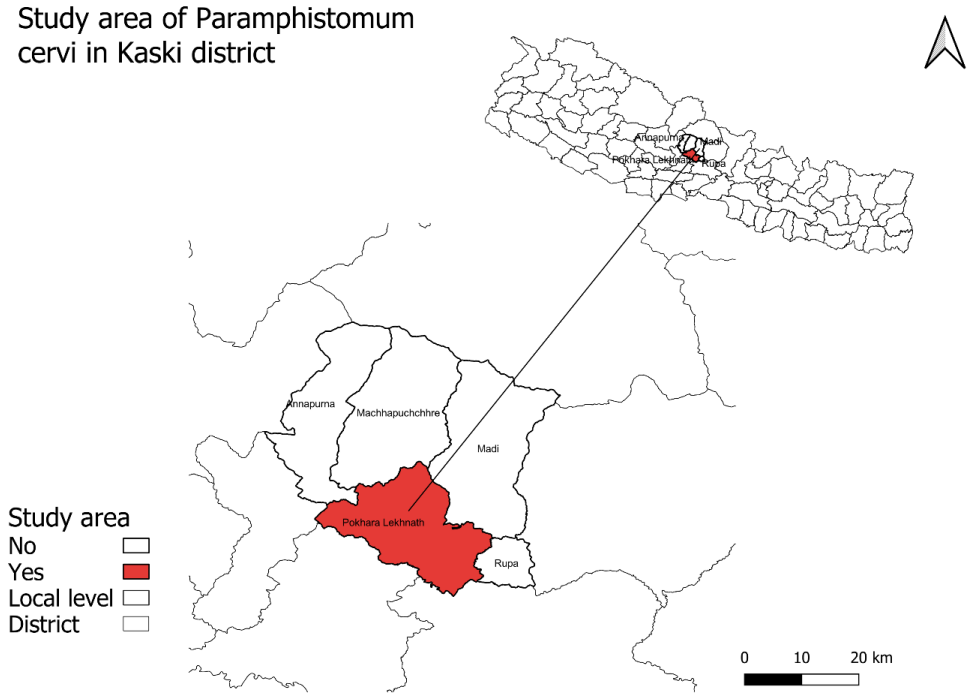

Figure 1.Study site (Pokhara Lekhnath Metropolitan City,Kaski,Nepal)

Study design and sampling

A cross-sectional study was performed to analyze the case data recorded in the case registry book of Parasitological Unit, Veterinary Hospital and Livestock Service Expert Centre, Kaski from January to April 2021(mid Poush 2077-mid Baishakh 2078).The four months study period was divided into two seasons namely Winter(January-February) and Summer (March-April).There were altogether 900 cases recorded in the register along with 7 variables: date, species of animal, breed, age, location of farm, season of the year and test outcome. All of them were entered in MS Excel-2016 and data cleaning was done manually. The incomplete observations and observations from municipality other than

Pokhara Lekhnath Metropolitan City were removed and final number of cases included in the study was 842. The age group of the animals was categorized as: Young kids, calves and Heifers less than 2 years of age, Adult and lactating doe, cattle and buffalo in early parity -2-5years, and Adult and lactating doe, cattle and buffalo in late parity- 5-10 years. The hill cattle, lime and parkote breed of buffalo and khari breed of goat are included under local breeds. While the improved breeds include Jersey cross and Holstein Cross cattle, Murrah cross buffalo, Khari × Jamunapari and Khari × Boer breeds of goat.

Procedure of Fecal Test

Samples were brought to the laboratory in sterile polythene bags by the farmers and were examined immediately in the parasitological unit of the hospital. Sedimentation technique was followed to examine the presence of Pramphistomum eggs in the faecal samples brought. About five grams of faeces was mixed in 30-50 ml of water and strained through a sieve to remove the course material. The mixture was allowed to sediment for half an hour and the supernatant was decanted. Washing was continued until supernatant became clear. Then after, a drop was taken from sediment with a Micropipette on a clean glass slide and was examined under microscope at 10 X for the presence of Paramphistomum eggs (Raza, et al., 2012)**.** The identification of eggs of *Paramphistomum cervi was* done according to (Mukhia, et al.,2007) according to whom eggs are 114-176 µm by 73-100 µm in size, Oval in shape, whitish to transparent in colour, distinct operculum, knob-like thickening at the acetabular end of shell and embryonic cells are distinct.

Statistical analysis

All the data entered in MS Excel were first analyzed by pivot table in MS excel 2016. Descriptive analysis was done with EpiInfo (Version 7.2.4.0) and MS Excel 2016 was used to generate graphical presentation of the data. 2× 2 cross tabulations were performed with EpiInfo and the result were expressed in percentage with P-value and significance was determined when $P<0.05$

RESULTS AND DISCUSSION

Out of the 842 faecal samples collected from different small and large domestic ruminants of Pokhara Lekhnath Metropolitan City,16.98% (143/842) were positive for *Pramphistomum cervi* eggs on Fecal test by Sedimentation technique. The result is similar to the overall prevalence of 15.64% *P. cervi* in buffaloes of Kathmandu as reported by Mukia, et al.,2007. Raza et al.,2012 also observed 15% prevalence of *Paramphistomum cervi* in buffaloes of Punjab, Pakistan by similar method. 10.78% *Paramphistomum sp*. in buffalo of Pokharatok VDC in Arghakhanchi district, Nepal was reported by Devi et al.,2012 adopting the method similar to ours. Likewise, (9.69%) of the total faecal samples of different domestic ruminants (cattle, buffalo, goat and sheep) were found to be positive for paramphistome eggs in India according to Maitra, et al., 2014.

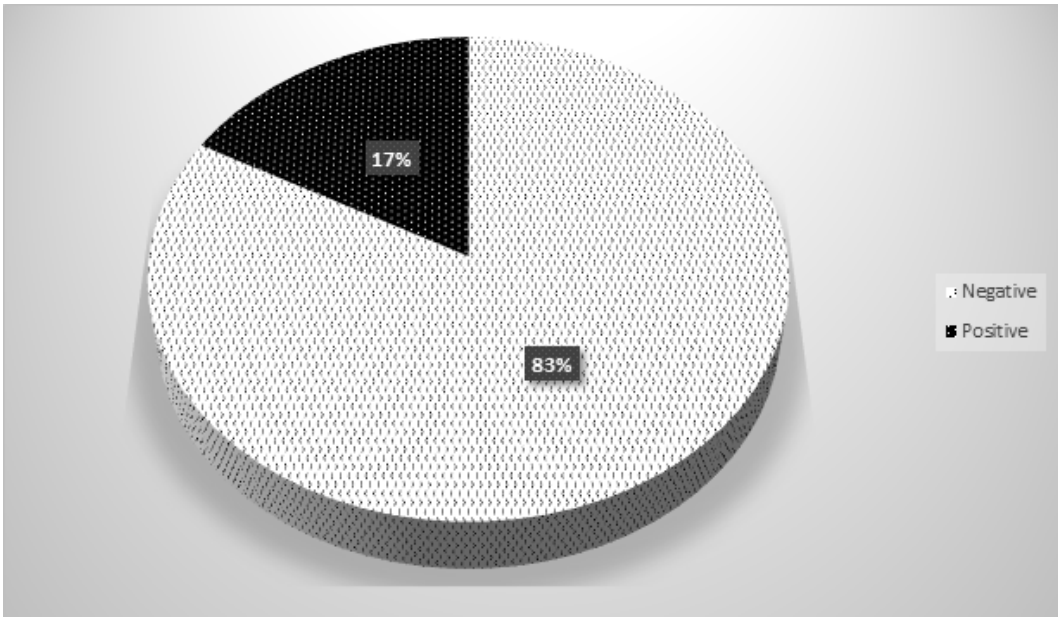

Figure 2:Overall prevalence of Paramphistomum cervi in Pokhara Lekhnath Metropolitan City, Kaski

Host-wise Prevalence

The host-wise prevalence of *Pramphistomum cervi* showed that the prevalence of eggs in cattle was found to be the highest i.e Cattle 18.21% (45/247), followed by goats 16.66% (5/30) and buffaloes 16.46% (93/565). As per the study of Khan, et al., 2009, the incidence of paramphistomes was significantly high in buffaloes than cattle that shows dissimilarity to the trend in our study. In our study, the prevalence in cattle is almost 2.5 times lower than prevalence of *Paramphistomum* sp. in cattle (48%) and that in buffalo is slightly higher than 11% as reported by Adhikari, et al.,2003 in cattle and buffaloes of Kathmandu valley through faecal test by sedimentation method. Our result is a bit higher than the result reported by Maitra, et al., 2014 who reported the overall prevalence of paramphistomes in different hosts as, cattle (12.4%) and buffalo (12.3%) followed by goat (4.9%) in uttarakhand, India. Hanna, et al., 1988 reported that of all buffaloes, 92-94% were found to be infected with paramphistomes in India. Contrasting to our study Purja, et al., 2015 reported 4.09% *Pramphistomum sp*. in goats of Puranchour VDC, Pokhara which is much lower than the prevalence found in our study among goats. This is due to the mixed ruminant husbandry. Our study showed slightly higher prevalence in larger domestic ruminants (cattle and buffalo) 16.99% (138/812) than in small domestic ruminants(goats) 16.66% (5/30). But the observed difference among large and small ruminants wasn't statistically significant. (χ^2 value0.0022, p-value 0.96, OR 1.02,95%CI (0.38-2.72).

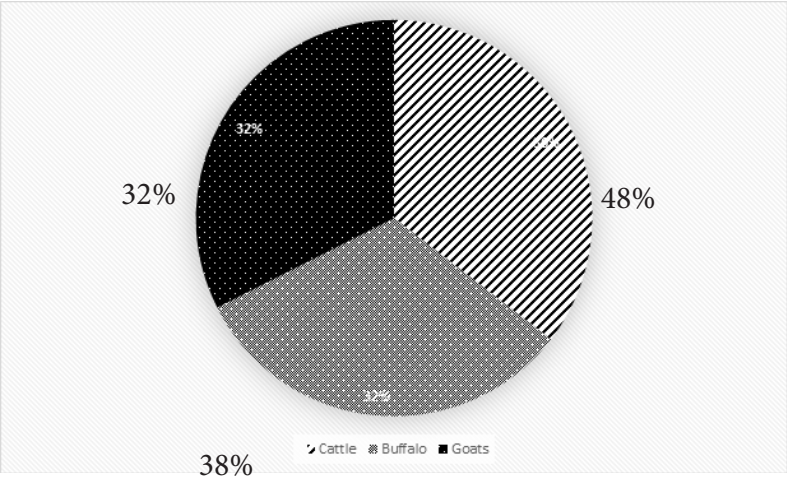

Figure 3: Host-wise prevalence of Pramphistomum cervi in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

Month and Season wise prevalence

The monthwise prevalence study showed the highest prevalence during April (20.08%) (46/229) followed by March (17.98%) (41/228). Jan (14.94%) (29/194) and February (14.13%) (27/191). As a whole the prevalence had increasing trend from January to March. The host species wise monthly prevalence showed that cattle showed the highest infectivity in month of January (23.25%) and lowest was during February (14.51%). Buffalo showed the highest infectivity in April (20.68%) and lowest was observed in January (12.16%). Goat showed maximum infection in January (33.33%)and lowest in February (9.09%).

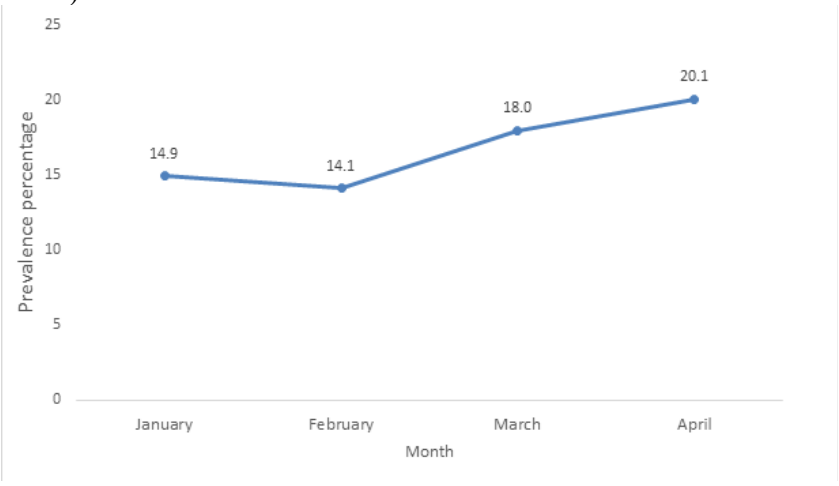

Figure 4: Overall monthly prevalence of Pramphistomum cervi in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

	January	February	March	April
Cattle (%)	23.25	14.51	17.18	19.23
Buffalo(%)	12.16	14.44	18.18	20.68
Goat (%)	33.33	9.09	20	16.66

Figure 5: Month-wise prevalence of Pramphistomum cervi in different domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski

Prevalence of *Paramphistomum cervi* in all the three ruminant species showed the highest infectivity during summer i.e 20.42% (88/431) followed by 13.38% (55/411) in winter. The cross-tabulation of season and overall prevalence showed that the presence of parasite was significantly different in summer and winter (χ^2 value7.38, p-value 0.00657, OR 0.60,95%CI (0.42-0.87). The result is in line with Sardar, et al.,2008 who reported highest prevalence rates of different parasites were observed in the rainy season (July to October). Likewise, Chhabra et al.,1972 reported maximum prevalence of parasite eggs in the faeces of domestic stock in the Punjab during July with lowest prevalence in December and a sudden rise in June. *Paramphistomum* spp infection showed significant ($P<0.05$) seasonal variation in rainy season in a study by Devi et al,2012 in Pokharthok VDC of Arghakhachi district Nepal. According to Maitra et al.,2008, the infectivity was the highest during monsoon and post monsoon (12.77%) followed by summer (9.74%) and winter (6.54%). Shah et al., 2015 also reported that 71 (47.01%) samples were found positive in winter and 80 (53.33%) samples were found positive in summer.

The higher overall prevalence during summer or pre-monsoon in our study compared to winter season is due to the increasing humidity triggers the development of larval stages of parasite and starting of premonsoon precipitation in Kaski district from March creates favourable environment for survival of abundant intermediate hosts(snails) of Pramphistomum .Thus the ruminants ingest the infective stage (metacercaria) of the parasite through grass from damp and water logged areas or while grazing in such areas. (Akanda, et al., 2014).

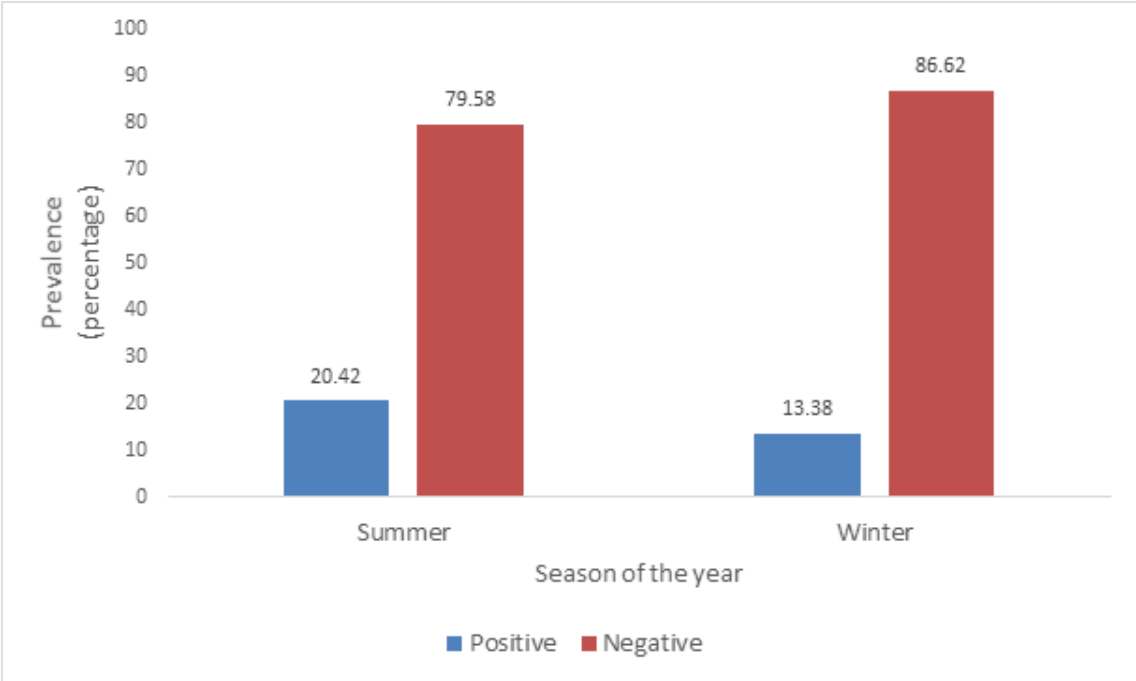

Figure 6: The relationship between season and prevalence of Paramphistomum cervi in Pokhara Lekhnath Metropolitan City, Kaski

Age-wise Prevalence

Faecal tests of different age groups showed that the presence of the parasite was highest in greater than 5 years' age group 18.28%) (64/350) followed by 17.69%(60/339) in 2-5 years' age group and 12.41%(19/153) in less than 2 years' age group. Further categorizing the age groups of the above age groups as adult and lactating females of greater than 2 years' age group than the young ones and heifers of less than 2 years' age group, higher prevalence17.99% (124/689) was seen among adult and lactating females and lower prevalence12.41% (19/153) was seen in young and heifers. But this observed difference wasn't statistically significant (χ^2 value2.76, p-value 0.09, OR 1.54,95%CI (0.92-2.59).

Similar to our study, Akanda et al.,2014 observed that prevalence increased with age in a linear pattern. Sardar et al.,2003 also gave the same line of finding for paramphistomes but the prevalence of *Ascaris, Strongylid* and *Strongyloids* were very high in the young animals of age group of < 12 months and gradually lowered in higher age groups. But Raza et al., 2007 reported higher prevalence of gastrointestinal parasites in younger ruminants in Pakistan. Higher prevalence of Pramphistomum cervi in larger age groups is supported by the reasons that such ruminants have been kept for longer time in the farm and gone through different kinds of stress like production stress, environmental stress, nutritional deficiency and other various kinds of stressful factors that can weaken their

immune system and predispose to parasitic infection (Akanda et al., 2014).

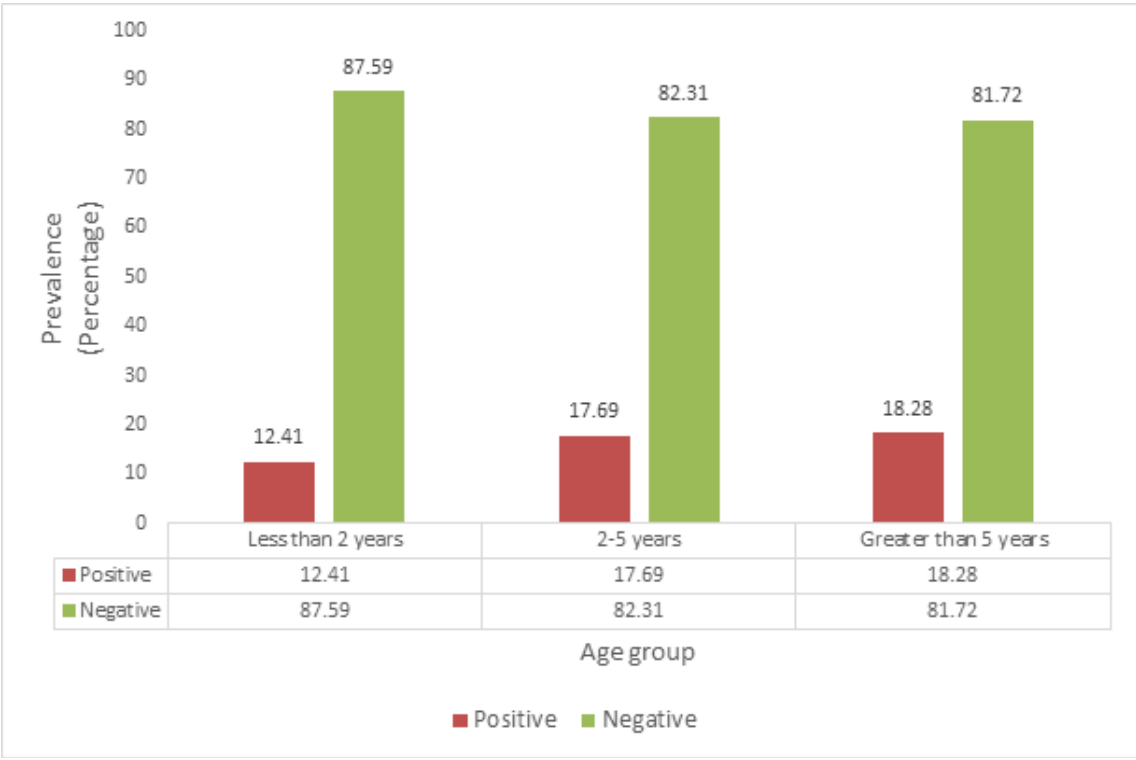

Figure 7: The relationship between the age group and prevalence of *Pramphistomum cervi* in Pokhara Lekhnath Metropolitan City, Kaski

Breed-wise Prevalence

Out of the total observations, 587 were improved breeds of cattle, buffalo and goats while 255 were local breeds. The improved breeds of cattle included 133 Jersey cross and 46 Holstein cross, that of buffalo included 390 Murrah cross and that of goats included 13 Khari*Boer and 5 Jamunapari cross. Likewise, the local breeds included 67 local cattle,176 local buffaloes,12 Khari goats. The prevalence was found to be 17.37% (102/587) in improved breeds and 16.07% (41/255) in local breeds. Although higher prevalence was seen in improved breeds compared to the local breeds, the difference was not statistically significant (χ^2 value0.21, p-value 0.64, OR 1.09,95%CI (0.73-1.63)

In a similar study, Bista, et al., 2018 reported higher prevalence of flukes in Cross breed species as, Jersey Cross (39.4%), and Murrah Cross (21.4%) as compared to the prevalence in local buffalo breed (20.0%) and pure Jersey (12.5%) in Western Chitwan by sedimentation method. Ross *et al*. (1959) reported that the *Bos indicus* are more resistant to parasites than *Bos taurus*. Similar findings as the overall prevalence of gastro-intestinal parasites of crossbred cattle (51.11%) was higher than that of native bred (45.28%) was

reported by Sardar et al.,2003. Haque et al., 1986 also reported that the gastro-intestinal parasitic infestation rate was higher in crossbred cattle. The higher prevalence in cross-breed cattle can be justified due to the acclimatization of local breeds to the agro-climatic condition of the particular location of study and are more resistant to disease and parasites while the cross breeds go through some extent of environmental stress and are less resistant to disease and parasites compared to local breeds.

Location wise prevalence

Prevalence of *Paramphistomum cervi* in the wards of Pokhara Lekhnath Metropolitan City inside the city core area was 18.13% (111/612) and the prevalence in the wards outside the city core area was 13.91% (32/198). There was no significant difference between the location and the prevalence of *Pramphistomum cervi* (χ^2 value 2.11, p-value 0.14, OR 1.37,95%CI (0.89-2.09). The difference in agro climatic zone and type of animal husbandry system inside the city area and outside the city area might be accounted for this difference in prevalence inside and outside the city core area of Pokhara Lekhnath Metropolitan City. The farmers outside the city area have minimum access to laboratory faecal test facility at the Veterinary Hospital lying in the city due to which the sample size from outside the city area is quite small. This might have caused lower prevalence of Pramphistomum eggs in the wards outside the city core area. On the other hand, the city core area is valley while the wards outside the city core area lie towards hilly topography. The valley has more humid climate than the areas outside the valley.

Table 1 Table showing the relationship between prevalence of Pramphistomum cervi and other variables in Pokhara Lekhnath Metropolitan City, Kaski

SN	Variable	X^2 value	(P-value)	Odds Ratio(95%CI)
1	Season	7.38	0.006	0.602
2	Breed	0.21	0.64	1.09
3	Age	2.76	0.09	1.54
4	Location	2.11	0.14	1.37

CONCLUSION AND RECOMMENDATIONS

The study shows the overall prevalence status of *Pramphistomum cervi* eggs in domestic ruminants of Pokhara Lekhnath Metropolitan City, Kaski district was 16.98%. Present study shows that there is relationship between prevalence and other variables like season, breeds, age, location, etc. but only the season is statistically significant. The prevalence of this parasite is seasonal and more common in summer than winter. The data explored in this study helps to understand the current situation of this parasite and forms the base for the farmers and stakeholders to construct further strategies and programs to control this parasite for management of animal health services. The farmers should be made aware about routine parasite control programs in their farm. The biological control methods for

intermediate hosts (snails) should be brought to practice to ensure safe grazing place and breaking the chain of the parasite's lifecycle.

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