Seasonal and Altitudinal Prevalence of Fascioliasis in Buffalo in Eastern Nepal

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ABSTRACT
Buffalo is the most important livestock commodities for milk, meat production and several other multipurpose uses distributed densely from southern tarai to northern mid-hills in Nepal. Among several internal parasitic diseases fascioliasis is highly economic one caused by Fasciola in buffaloes. However, there are only few studies carried on prevalence of fascioliasis emphasizing buffaloes in relation to seasonal (summer and rainy, and winter) and altitudinal variations. Therefore, we examined prevalence of fascioliasis seasonally and vertically. For the purpose, we selected two districts of eastern Nepal and sampled from low altitude area known as Madhesha ranging from 175-200, Dhankuta from 800-1200 m, and Murtidhunga from 1800-2200 m elevation from the sea level, representing tarai, mid hills and high hills, respectively. Altogether from February 2013 to January 2014 at every two months interval we collected 798 fecal samples from buffaloes; 282 from Murtidhunga, 239 from Dhankuta and 277 from Madhesha. The samples were examined microscopically for the presence of Fasciola eggs using sedimentation technique. Results showed that overall prevalence of fascioliasis in buffaloes was 39.9% (319/798), ranging highest 42.6% in Madhesha followed by 39.7% in Murtidhunga and 37.2% in Dhankuta, respectively. The prevalence of fascioliasis was found to be significantly (p <0.05) high in winter (44.9%) comparing to rainy season (34.4%). The prevalence of fascioliasis in buffaloes was relatively higher in low altitude than high altitude, although it was not statistically significant (p <0.05). In our findings the female buffaloes showed higher prevalence for fascioliasis than in male. Since the fascioliasis in buffaloes is highly endemic, thus strategic deworming in high risk period is recommended along with measure to prevent pasture contamination with buffalo feces.

Keywords: Altitude, Buffalo, Season, Fascioliasis, Dhankuta and Sunsari districts

INTRODUCTION
Buffalo (Bubalus bubalis) is one of the important livestock commodities especially in South Asia including Nepal (Mahato and Harrison 2005). According to the MoAD (2016), there were about 6.4 million heads of buffalo in Nepal by the year 2015. Buffaloes are considered as “black gold” among livestock farmers because of its higher value in their livelihood and income generation. The buffalo contributes about 67.7% of the total milk production and 57.4% of the total meat production in Nepal (MoAD 2016). Buffalo suffers from various parasitic, viral, bacterial and fungal diseases. Parasitism reduces animal efficiency and productivity (Harrison
Prevalence of Fascioliasis in buffaloes by Sah et al 2000, Vercruysse and Claerebout 2001, Khan et al 2011). Fascioliasis is one of the important diseases caused by internal parasites (Harrison 2000) which inhabit in liver of host (Nguyen et al 2017).

Fascioliasis, commonly known to cause by liver fluke, is the most important infection in buffalo caused by two species of trematode: Fasciola hepatica and F. gigantica (Nyindo and Lukambagire 2015). The F. hepatica species occurred almost throughout the world, but predominates in temperate zones, while F. gigantica is found in most continents, primarily in tropical regions (Andrews 1999, Bennema et al 2014). Both species are transmitted in livestock by the snails of the family Lymnaeidae with potentiality to acute and chronic infection with expected blood loss of about 0.2–0.5 ml/worm/animal/day (Khan et al 2009, 2011; Nguyen et al 2017). Mahato and Harrison (2005) observed that the prevalence ranged from 30-70% in buffaloes in Nepal. It is an emerging parasitic infection, impacting substantially on both veterinary and human health worldwide (Lazaras et al 2010). Infestations of Fasciola in ruminants cause heavy loss irrespective of geographical boundaries posing serious concern on the socio-economic of livestock farming in terms of milk and meat production (Saleha 1991) as well as human health (Nyindo and Lukambagire 2015). The occurrence of fascioliasis might be influenced by a multiple factors such as host, parasite and environment (Maqbool et al 2002 ). In past, few studies have already reported the prevalence of fascioliasis from many parts of Nepal (Mahato and Harrison 2005, Joshi 1988). However, prevalence of this disease in buffalo in relation to altitudinal variation and seasons has rarely been carried out. Hence, we estimated the prevalence of fascioliasis in buffaloes at various altitudes and seasons in from eastern Nepal.

MATERIALS AND METHODS

Study Area
For the present study, three locations of eastern Nepal belonging to two districts (Figure 1A) of eastern region were selected because these places were known to possess relatively higher density of buffaloes. The study locations were differentiated into three agro ecological zones to know the variation of prevalence representing - high hill, mid hill and lowland or plain (tarai). The highest altitude from where samples were collected located at the range of about 1800-2200 meter above sea level (masl) in Murtidhunga (high hill, 27° 09’ 64.87” N; 87° 36´ 37.94” E); 800-1200 masl in Dhankuta (mid hill, 26° 07’ 09.96” N; 87° 34´ 31.09” E) of Dhankuta District and 175-200 masl in Madhesha (low land, 26° 03´ 43.79” N; 87° 18´ 60.72” E) of Sunsari District. Farmers raised buffaloes as semi-intensive type of management. Majority of farmers offered straw and grasses as feed. Only limited farmers provided commercial feed along with local feed materials to buffaloes.

Figure 1. Map of Nepal showing studied districts depicted with star signs (A), and altitudinal locations of the study area (B).

Study Design
A total of 798 fecal samples were collected randomly from buffaloes aged above 1.5 years in different three sites from February 2013 to January2014 for 12 months. Breeds of buffaloes were local and Murrah cross. The samples were collected from buffalo dairy farms and household animals of three different sites of Dhankuta and Sunsari Districts namely Murtidhunga (n=282), Dhankuta (n=239) and Madhesha (n=277) representing three different altitudes, high hill, mid hill and lowland agro-ecological zones, respectively (Fig 1A, B). Samples were collected in every two months interval representing into three viz. summer (March to June), rainy (July to October) and winter (November to February) from the same locations

Laboratory Examination
Fecal samples were collected per rectum from the buffaloes. Each samples of 5-10 g of fecal material was collected in clean polythene bag containing 10% formalin as preservative. The samples were properly labeled and brought to the laboratory of Agricultural Research Station, Pakhribas, Dhankuta, Nepal. The samples were tested microscopically for the presence of fluke (Fasciola) eggs using sedimentation technique (Soulsby 1983).

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Statistical Analysis
Data were compiled in Microsoft Excel using R 3.4.2 packages for analysis. The relationship of altitude with prevalence of fascioliasis and season were investigated. Spearman’s Chi-square Test was used at the level of P<0.05 to compare the effect of season and altitude on the prevalence of fascioliasis in buffaloes.

RESULTS
Fascioliasis in buffaloes was prevalent in all seasons and in all altitudes. The overall prevalence was found to be 39.9% (319/798) as shown in Table 1. The overall prevalence of fascioliasis was significantly (p <0.05) higher in winter season in comparison to rainy season.

Table 1. Prevalence of fascioliasis in buffaloes in different seasons from the study areas

| Season | Positive/Tested animal | Prevalence % | 95% Confidence interval (CI) | Chi-square test | p-value |
|--------|------------------------|--------------|-----------------------------|----------------|---------|
| Summer | 106/263                | 40.3         | 34.36-46.57                 | 6.2327         | 0.0443  |
| Rainy  | 89/259                 | 34.4         | 28.52-40.49                 |                |         |
| Winter | 124/276                | 44.9         | 38.91-51.0                  |                |         |
| Overall| 319/798                | 39.9         | 36.56-43.48                 |                |         |

The prevalence ranged from 39.3%-41.7% in summer; 28.3%-40% in rainy and 36.5%-57.4% in winter season in buffaloes (Table 2). In low altitude tarai the prevalence of fascioliasis was significantly (P<0.05) higher in winter in comparison to rainy season.

Table 2. Seasonal prevalence of fascioliasis in buffaloes in different altitudes

| Season          | Positive/Tested animal | Prevalence % | 95% CI  | Chi-square test | p-value |
|-----------------|------------------------|--------------|---------|----------------|---------|
| Murtidhunga (1800-2200 masl) |                      |              |         |                |         |
| Summer          | 37/94                  | 39.3         | 29.44-49.98 | 0.01         | 0.9959  |
| Rainy           | 32/80                  | 40.0         | 29.20-51.56 |              |         |
| Winter          | 43/108                 | 39.8         | 30.52-49.68 |              |         |
| Dhankuta (800-1200 masl) |                    |              |         |                |         |
| Summer          | 31/78                  | 39.7         | 25.34-46.14 | 0.32         | 0.8506  |
| Rainy           | 31/87                  | 35.6         | 25.65-46.62 |              |         |
| Winter          | 27/74                  | 36.5         | 20.31-39.76 |              |         |
| Madhesha (175-200 masl) |                    |              |         |                |         |
| Summer          | 38/91                  | 41.7         | 30.08-50.56 | 16.23        | 0.0003  |
| Rainy           | 26/92                  | 28.3         | 19.36-38.61 |              |         |
| Winter          | 54/94                  | 57.4         | 46.82-67.59 |              |         |

The prevalence of fascioliasis in buffaloes was relatively higher in low than higher altitude; however, the prevalence was not significantly different (Table 3). The prevalence of the facioliasis was higher in female comparing to male in all the study sites (Figure 2).

Table 3. Prevalence of fascioliasis in buffaloes in different altitude level from the study area

| Altitude (masl) | Positive/Tested animal | Prevalence % | 95% CI | Chi-square test | p-value |
|-----------------|------------------------|--------------|--------|----------------|---------|
| 1800-2200       | 112/282                | 39.7         | 33.96-45.69 | 1.55         | 0.4609  |
| 800-1200        | 89/239                 | 37.2         | 31.09-43.70 |              |         |
| 175-200         | 118/277                | 42.6         | 36.70-48.66 |              |         |
DISCUSSION

We showed that fascioliasis prevalence in buffaloes persisted almost round the year in the study sites. The overall prevalence of fascioliasis was 39.9% in buffaloes with higher prevalence recorded in winter (44.9%) than in summer and rainy seasons (Table 1 and Table 2). It is possible that the buffaloes were exposed to *Fasciola* infection at least 2–3 months earlier i.e. in the monsoon season as have been indicated elsewhere by Khan et al (2017). These findings indicated that infection might be supported by monsoon seasons when the temperature and moisture levels were the most favorable both for the vector snails and *Fasciola* developmental stages (Khan et al 2017). Our results showed agreement with the findings reported by Singh (2001) in Uttar Pradesh India and Bhutto et al (2012) in Pakistan.

The prevalence of fascioliasis was found to be significantly (P>0.05) associated with season in our study. The *Fasciola* eggs observed in fecal test actually released into the soil and water, where the infection in the form of cyst enters into the animal through grazing along with grass, straw, drinking water into the stomach of buffaloes (Nguyen et al 2017). Moreover, no restriction on animal importation from outside, movement between the infected localities, grazing and feed materials to animals may also responsible for endemic infestation (El-Bahy 1998). Earlier, Mahato and Harrison (2005) reported prevalence of fascioliasis ranging from 30-70% in buffaloes in Nepal. Joshi (1988) found above 50% prevalence of fascioliasis from cattle and buffalo in six villages of mid-western hills of Nepal. Other studies have shown 48.7% (Jithendran and Bhatt 1999), 50% (Molina et al 2005), 44.7% (Liu et al 2009) and 50% (Siddiki et al 2010) prevalence from various countries. Such variations in fascioliasis infection are known to occur due to management practices, presence of intermediate hosts, intermediate hosts in localities, and meteorological differences such as humidity, temperature and rainfall patterns (Bhutto et al 2012).

Our data showed there was no significant difference in the prevalence of the fascioliasis in higher mountain and the tarai lowlands, but more prevalence of 42.6% vs 37.2 in tarai vs 39.7% in high hills occurred in present study (Table 3). This result might be arbitrary because we assumed that intermediate host of the *Fasciola*, the snails probably occur more frequently in tarai, however, infection were seen in high hills as well. Future studies might require examining the prevalence of intermediate host towards higher uplands as well.

The lower altitude was represented by Madhesa situated from 175 to 200 masl, there was significantly (P>0.05) higher percentage of fascioliasis in winter season (Table 2). This was probably due to abundant low sub-tropical irrigated rice cultivated areas as Copenh (1999) had observed higher prevalence of fascioliasis in buffalo in rice cultivated areas in Indonesia. In our study female buffaloes had higher facioliasis prevalence than in the males. It might be due to number of reasons, as buffaloes in winter or dry seasons were fed with rice straw as common practice all over Nepal even in high hills that could be the reasons of prevalence of the fascioliasis in high hills. The other reasons could be because there were higher numbers of female animals in the study areas, mostly kept for milking purpose by feeding the rice straw, in general. It is likely that dried straw fed to buffaloes might play important role to carry the dormant stages of fascioliosis parasites as Mahato et al (2000), Mahato et al (2005), Harrison (2005) and Nguyen et al (2017) described higher disease prevalence in stall fed buffaloes in rice producing areas. Since the male animals were generally sold and slaughtered for meat purpose, therefore disease infection might have been represented poorly in data pool. Since there is limited studies showing the relationship between fasciolosis and nutrition (Harrison 2000) future research should also be focused on such important aspects.
CONCLUSION

Fasciolasis in buffaloes is endemic and wide spread in study areas of Nepal but seasonal and altitudinal variation occurs. Control measures should be taken for destruction of intermediate host (snail population). Periodic anthelmintic treatment should be given for getting maximum productivity from the buffaloes. Strategic deworming in high risk period like winter season in low land (Madhesh) is recommended along with measure to prevent pasture contamination with buffalo feces.

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