Study on ovine fascioliasis: Case study, associated risk factors and economic significance at sheep and goat research program, Guthichaur, Jumla, Nepal

Ramesh Prasad Sah, Mohan P Yadav, Surendra P Kanu and Tirtha Raj Rijal

Abstract
Fascioliasis is an economically important zoonotic disease of domestic livestock, especially cattle, buffalo, sheep, goat, horse, donkey, rabbit, wild ruminant and human. A cross-sectional investigation on ovine fascioliasis was carried out for six months in sheep of Sheep and Goat Research Program, Guthichaur, Jumla to determine prevalence of fascioliasis in sheep as well as to assess the major associated risk factors and its economic significance. Altogether 100 fecal samples (50 in March and 50 in June month) were collected randomly and tested for presence of eggs of Fasciola. Similarly antemortem inspection and slaughtered sheep examination were performed in 30 sheep for abnormality. Case study was conducted for deep study about fascioliasis and postmortem of 17 dead sheep was done to observe presence of liver fluke in liver and gall bladder. Major risk factors were identified and analysis was done from obtained data. Prevalence of ovine fascioliasis was recorded 34% in fecal test, 6.7% in slaughtered sheep and 23.5% in post mortem examinations. Mature fluke was present in liver and gall bladder of inspected sheep. Additionally major risk factors of diseases were identified and economic significance was assessed. This prevalence found in the study area could be due to the marshy and water-logged swampy area which is suitable for the breeding of intermediate host (snail). Integrated approach, which is a combination of selective chemotherapy and selective vector control, should be considered more practically and economically feasible.

Keywords: Ovine fascioliasis, prevalence, case study, risk factors, Jumla

1. Introduction
Fascioliasis is an economically important zoonotic disease of domestic livestock, especially cattle, buffalo, sheep, goat, horse, donkey, rabbit, wild ruminant and also in human. Fascioliasis is one of the important parasitic diseases in tropical and subtropical countries which limit productivity of ruminants [1]. The disease is caused mainly by two species of parasitic Trematodes that affect the liver and other associated organs. The disease is caused by digenean trematodes of the genus Fasciola, commonly referred to as liver flukes. Liver flukes belong to the group of food borne Trematodes infection and are zoonotic [2]. The two species most commonly implicated as the etiological agents of fascioliasis are Fasciola hepatica and Fasciola gigantica (family Fasciolidae), are similar and large enough to be visible to the naked eyes [3]. Both species are transmitted in livestock by the snails of the family Lymnaeidae with potentiality to acute and chronic infection caused expected blood loss of about 0.2-0.5 ml/worm/animal/day [4, 5, 6].

Species of the genus Fasciola cause important economic losses due to liver spoilage, high morbidity rates and reduced production of milk, meat and wool [7]. Infestation with fascioliasis is usually associated with grazing wet land and drinking from the snail infesting watering places [8]. Liver fluke infection in lambs and kids is characterized by anemia, edema, weight loss and death [9, 10]. The fluke life cycle requires intermediate host (snail) to complete transmission to a new ruminant host. The liver is damaged and condemned and the subclinical and chronic disease usually results in decrease production of meat, milk and wool, secondary bacterial infection, fertility problems, and great expenses with anthelmintics [11]. The disease causes considerable economic impact due to mortality, liver condemnation, reduced weight...
With the altitude ranges from 2500-3000 (avg 2700) meter above sea level.

<table>
<thead>
<tr>
<th>Table1: General information about Experimental site</th>
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<tbody>
<tr>
<td>Rainfall</td>
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<tr>
<td>Temperature</td>
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<tr>
<td>Snowfall</td>
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<td>Relative humidity</td>
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<td>Sunshine hour</td>
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<td>Latitude</td>
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<tr>
<td>Longitude</td>
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<td>Elevation</td>
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**Source:** Annual Report, Sheep and Goat Research Program, 2019

Farmers raised sheep, goats, cattle, horses as semi-intensive type of management. Sheep and goats remain as intensive housing system during snowfall (December to March). Both the animals were supplied with hay and concentrates during this period. Majority of the farmers offered leaves of fodder trees and seasonal grasses found in pasture as feed. Sheep were taken to high altitude Majhpatan (3300 masl) and other pasture lands for grazing in summer and rainy seasons as free range system. Only limited farmers provided with local feed materials like maize, crop residues etc to their sheep and goats.

Maize, barley, bean, potato, wheat, buck wheat and millets are the major crops cultivated in upland conditions in Jumla. The growing season of all above mentioned crops has only one season in a year and mixed cropping is followed by majority of the farmers in a crop season. Apple is the popular temperate fruit followed by walnut, peach and plums.

2.2 Study Design and sampling techniques

A cross-sectional investigation on ovine fascioliasis was carried out from mid-February (Falgun), 2020 to mid-August (Shrawan), 2020 for six months in the farm. Animals of farm were not dewormed from four months. They were being fed with hay and concentrate. A total of 100 fecal samples (50 samples in March and 50 samples in June) were collected randomly to examine the internal parasitic eggs especially of Fasciola and nematodes. Altogether 30 sheep (not appropriate for farm due to inbreeding) were culled and were sold to nearby farmers for meat purpose. These sheep were observed closely for ante mortem inspection and slaughter time inspection in farmers’ house. Additionally, 17 sheep died in different time and performed post mortem.

2.3 Fecal sample examination: Fecal samples were collected per rectum from the sheep. 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 Veterinary hospital and animal service office, Jumla as well as the laboratory of SGRP, Guthichaur. The samples were tested microscopically for the presence of fluke (Fasciola) eggs using sedimentation technique [21]. At least three smears were prepared for each sample. Presence of one or more fluke egg in a sample was considered as positive. To differentiate eggs of Paramphistomum species and Fasciola species, a drop of 1% methylene blue solution was added to the sediment. Eggs of Fasciola species show yellowish color, while eggs of Paramphistomum species were grey.

2.4 Ante-mortem examination: Complete ante mortem examination of the animals were carried out prior to slaughter.
Inspection of the animals were made while at rest or in motion for any obvious sign of disease.

2.5 Inspection during slaughter: Farmers nearby office of Guthichaur purchased and slaughtered 30 sheep in different time for meat purpose. Close observations were made visually for abnormalities in visceral organs like liver, gall bladder, lungs, heart etc during slaughter.

2.6 Post mortem examination: During post-mortem inspection, each liver was visually inspected, palpated and incised based on routine meat inspection procedure developed by FAO [22]. All condemned liver due to infestation by Fasciola species was registered and collections of flukes were conducted for the purpose of species identification.

2.7 Situation of pasture and surroundings: In order to know the major risk factors, following data were recorded.

2.8 Data management and analysis: All the obtained data were compiled and enter into Microsoft Excel. Microsoft Excel database system was applied for data analysis. The prevalence of ovine fascioliasis was calculated as the number of infected individuals divided by the number of individuals sampled x 100. Statistical significance was set at P < 0.05 or less to determine whether there are significant differences between the parameters measured between the groups. Risk factors were assessed and described.

3 Results

3.1 Examination of fecal samples: Out of 100 fecal samples examined, an overall prevalence of 34 (34%) was found positive for Fascioliasis in the study area (Table 2).

Table 2: Prevalence of fascioliasis in sheep by fecal examination

<table>
<thead>
<tr>
<th>Month</th>
<th>No of sheep observed</th>
<th>No of sheep positive</th>
<th>χ2 statistic</th>
<th>p &lt; .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>50</td>
<td>56% (28)</td>
<td>23.2527</td>
<td>&lt; .00001</td>
</tr>
<tr>
<td>June</td>
<td>50</td>
<td>12% (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>34% (34)</td>
<td></td>
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3.2 Ante mortem examination: All the 30 sheep were examined before slaughter and found no any clinical signs of fascioliasis like poor body condition, bottle jaw etc.

3.3 Inspection during slaughter: Out of 30 sheep slaughtered by farmers in different days, 2 sheep (6.7%) were found infected with liver fluke. From visual inspection of different viscera, enlarged liver along with fluke was detected in two sheep. Gall bladder of those two sheep were also enlarged.

3.4 Post mortem examination: Post mortem was performed in 17 dead sheep within six months study period. Among them 4 sheep (23.5%) were found positive for liver fluke infestation (Table 3). Infested liver was enlarged and presence of Fasciola in incised liver (Figure 2A, B). Liver fluke looks like leaf shape. Remaining 13 animals died due to other causes like enterotoxaemia, plant poisoning, kid mortality, kid killed by dog, accident and other causes.

Table 3: Presence of Fasciola into Liver and bile duct of sheep by PM

<table>
<thead>
<tr>
<th>Month</th>
<th>No of dead sheep</th>
<th>Presence of fluke into liver</th>
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<tbody>
<tr>
<td>February</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>March</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>April</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>June</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>August</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 2: A. Presence of Fasciola parasite (leaf shape) in liver and bile duct indicated by arrow sign (above); B. Harvested of liver fluke/Fasciola from sheep -leaf shape (below)
3.5 Assessment of risk factors: Observed associated risk factors for ovine fascioliasis in the farm are given below:

- Situation of pasture and surroundings: Lower part of pasture land was marshy and water-logged swampy area. Sometimes sheep run there for grazing. Grasses were cut, prepared hay and stored in hay house for lean or winter season. It was found that feeding and housing of sheep were done in four pens/sheds.

- Medication with anthelmintic drugs: Mass medication of sheep was conducted in April and June with anthelmintic drugs. Dipping and vaccination against FMD and PPR were carried out in all animals in April-May.

3.6 Economic significance: Economic losses observed in sheep by fascioliasis are as follows:

- Loss/death of animals from acute, subacute and chronic form
- Production loss due to anaemia/ blood loss
- Hamper in growth due to indigestion problem created by infected liver
- Rapid loss of body condition/ weight and poor fleece quality despite adequate flock nutrition

4. Discussion

4.1 Occurrence and case study

Fascioliasis is a wide spread ruminant health problem causing huge direct and indirect loss in Nepal. In this study, the overall prevalence of ovine fascioliasis was 34%. This observation was similar to observation 35% in sheep and goat made by Shrestha 1996 [23]. The observed prevalence is relatively low from previous studies conducted by different authors [24, 25, 26, 27, 28] who reported between 39-52.6% in Ethiopia. The prevalence of fascioliasis recorded in the present study was higher than the previous report 13.2% in the middle Awash River basin by an author [29].

The prevalence of the disease in the study area might be attributed to the favorable ecological factors for snails (intermediate host) and the parasite. Prevalence of ovine fascioliasis was found significantly higher in March than June (P<0.0001). This might be due to feeding of hay infested with metacercaria to sheep and sheep were confined in shed during winter season. They were not drenched with dewormer since 4-5 months. Whereas prevalence was low in June because sheep were left in pasture land for grazing especially in upland as well as also performed mass drenching with oxyclozanide.

Different farmers bought 30 culled sheep (not appropriate for breeding in our farm) and slaughtered and found liver fluke positive in two cases. These two sheep might be infested with immature fluke during deworming in farm. All sheep were drenched with oxyclozanide and this drug is generally effective against mature fluke. By post mortem examination, four dead sheep had destroyed and enlarged liver infested with Fasciola and also fasciola was present in gall bladder/bile duct. Those sheep were died due to chronic stage of fasciola infestation. They were not treated with dewormer. The infestation may come from hay feeding or infection remained in them from several months.

4.2 Analysis of major risk factors for ovine fascioliasis:

Lower part of pasture land was marshy and water-logged swampy area. As reported by Heinonen et al. [30] water logged and poorly drained areas with acidic soils in the highlands are often endemic areas for fascioliasis. In summer and rainy season, several animal species like cattle, buffaloes, horses, goats and sheep gather every year from different places of different districts in pasture land for grazing. Bringing animals is tradition here. So these animals also act as major risk factor for contamination of pasture. Such animals do not have any shed and they are kept as free range system. Farmers don’t deworm their animals regularly in this area. Such animals excrete parasitic eggs in pasture and thus pasture or grazing area becomes infested with different parasites. Due to marshy and water-logged swampy area, liver fluke eggs get snail population and cercaria as well as metacercaria developed. The whole pasture becomes infested with metacercaria by movement of animals. The grasses of that pasture is collected and hay is prepared and is stored in hay house for use in winter season. In winter season, surrounding areas are covered with snow for four months. In this way infested hay is used as feed in that period. Sheep are kept whole time in shed except 1-2 hours in a day for feeding concentrate and for sunbath. So confinement of animals in shed is major risk factor for fascioliasis.

Adult flukes can survive for many years in the livers of infected hosts and lay between 20,000 and 50,000 eggs/day. Animals grazing in wet marshy areas, favored the intermediate host, are more likely to become infected. Typically, long and wet seasons are associated with a higher rate of infection. However, sheep are more likely to ingest large numbers of cysts during dry periods following a wet season. This is due to a reduction in available pasture, forcing the animals to graze in swampy areas or in areas where the water has reeded, thus exposing them to vegetation heavily infected with metacercaria [31].

Sheep of our farm goes to whole pasture land for grazing. They consume directly cercaria as well as metacercaria during grazing and drinking. So pasture land is also considered as risk factor. As this place is regarded as tourist place, many people come to visit and entertain in surrounding areas. Their movement also transfer metacercaria from one place to other and our boundary get contaminated. That is why visitors also considered as risk factor.

4.3 Economic significance

Liver fluke causes three types of disease - acute, sub-acute and chronic depending upon the level of challenge and the animals’ resilience. Sudden deaths may affect up to 10 percent of sheep at risk causing grave financial loss. Fascioliasis can have a serious financial impact on a sheep farm with immediate losses of up to 10 percent caused by the acute or subacute form of disease. Chronic disease could halve profits by reducing the lamb crop and increasing ewe mortality. Fluke is the second highest cause for abattoir condemnations. The damage from fluke has a huge effect on rapid loss of growth rate, milk yield, finish weights, poor fleece quality and poor meat quality.

5. Conclusion and Recommendations

The result of the present study by post mortem and fecal examinations, indicated that fascioliasis was a highly prevalent sheep disease in the study area. However, it is increasingly evident that a proper evaluation of the epidemiology of fascioliasis is lacking. The relatively high prevalence reported in this study has clearly indicated lack of strategic control measures against the disease as well as poor awareness knowledge about this disease in the community. This prevalence found in the study area could be also due to the marshy and water-logged swampy area which is suitable for the breeding of intermediate host (snail). Based on current conclusion the following recommendations were forwarded:

- Integrated approach, which is a combination of selective chemotherapy and selective vector control, should be considered more practically and economically feasible.

- Supplementation of important nutrient feed in dry season is important to avoid stress conditions that affect the host resistance and susceptibility to parasitic diseases.
• Awareness creation to livestock owners need to be performed with economic significance and control methods of this disease in surrounding areas.
• In order to design and implement control strategy, detailed study should be conducted on the risk factors of the disease.

6. Acknowledgement
Authors acknowledge staffs of veterinary hospital and animal service center of Jimma and staffs of SGRP, Guthicha due to involvement in fecal sample collection and examination for the present study.

7. References