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Prevalence of *Fasciola* Infection in Slaughtered Animals in Kashmir

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Abstract- Fasciolosis is denoted as a significant veterinary health problem. During current study, a total of 714 cattle slaughtered at different abattoirs of Srinagar city (J&K) were examined for the presence of *Fasciola* sps in the liver from January 2014 to January 2016. There was moderate prevalence of 26.84% in the studied area. Predominance of *Fasciola gigantica* (20.86%) was seen as compared to *Fasciola hepatica* (3.361%) infection with mixed infection of 2.66%. Epidemiological determinants like age, gender, breed and body condition showed statistically significant ($p<0.05$) effect on bovine Fasciolosis. Seasonal data showed highest prevalence in autumn (39.87%) followed by winter (28.84%) with lowest prevalence in spring (16.40%).

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I. Introduction

Ruminant productivity around the world is majorily affected by trematode parasitism (Vercruysse and Claerebout 2001). Among them, Fasciolosis gains public concern not only due to its prevalence and economic significance to animal stock in all continents (Scheweizer *et al*., 2005, Mungube *et al*., 2006) but also to its zoonotic aspect. Bovine Fasciolosis is an impedent in profitable bovine farming and for butchers and consumers too. Parasite of genus *Fasciola* i.e *Fasciola hepatica* and *Fasciola gigantica* is the causative agent of Fasciolosis which occur in a wide range of definitive hosts. Over the last decade there has been a substantial increase in the number of fasciolosis cases recorded. It is spurred on by both environmental changes (warmer, wetter climate) and man-made modifications such as an increase in animal movements and intensification of livestock farming (Mas-Coma *et al*., 2005).

According to Annual Reports of Department of Animal Husbandry, Dairying and Fishries, species -wise incidence of Bovine Fasciolosis in India is tabulated as under:

Year	Outbreaks	Attacks	Deaths
2008-2009	85	391402	2
2009-2010	84	375237	6
2010-2011	105	345108	27
2011-2012	130	316363	74
2012-2013	195	509195	31
2013-2014	137	802698	11
2014-2015	129	3606	4

While comparing the apparent prevalence of liver fluke infection, detected by liver, faeces and bile examination it has been reported that examination of liver or bile samples was more sensitive than faecal examination (Braun *et al*., 1995 and Kumar *et al*., 2002).Thus the abattoir study was carried out to determine the prevalence.

II. Material and Methods

A two-year prospective systematic sampling study was undertaken from January 2014 to January 2016 to determine the relative occurrence of *Fasciola* infection in the livers of cattle presented to six abattoirs across the Kashmir. Samples were taken from the three studied localities i.e., Hazratbal, Parimpoora, and Gouskimber of Srinagar district but sampling effort was more important in Parimpoora locality, where four slaughterhouses were closely located.

The sample size was calculated using the formula given by Thrustfield, M. (2005).

$$n = \frac{1.96^2 . P_{exp} (1 - P_{exp})}{d^2}$$

Where n = required sample size
P_{exp} = expected prevalence= 50%
d = desired absolute precision=5%
Hence, d = 0.05 and p= 0.5 (50%).

The expected prevalence in the study area was 55 % (Akhoun and Peer, 2014). Thus the minimum desired annual sample size was calculated to 381. However, due to drastic floods only 316 cattle were examined in Year 2014 as collection areas were inaccessible and sample size was extended to 396 in Year 2015.

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III. Study of Epidemiological Parametes

a) Antimortem analysis

➢ Age, Gender and breed of animal

The age of each animal was confirmed by looking at the physical appearance of body and examining the dental pad and incisor teeth (Cockrill, 1974). The data was collected according to predesigned proforma: Young (1Yr-3Yrs), adult (3-6Yrs) and aged (Above 6 years). During survey the gender and breed of animals was also recorded.

➢ Assessment of Body condition

Body scoring of the cattle was made based on the method described by Nicholson and Butterworth (1986). Each scoring were given number from 1(L-, very lean) to 9 (F+, very fat) and these scores finally included under three body condition scores, good, medium and poor.

➢ Season

On the basis of temperature and precipitation, four seasons in a year recognized in Kashmir valley are: winter (December to February); spring (March to May); summer (June to August); autumn (September to November) (Dar *et al*., 2002).

b) Postmortem examination

➢ Types of infection

Infection based on causative agent were classified as *Fasciola hepatica*, *Fasciola gigantica*, mixed *Fasciola* species (*Fasciola hepatica*, *Fasciola gigantica*) infection.

c) Postmortem fluke recovery

Worms were recovered from infected livers by squeezing them manually to macerate the parenchyma and the flukes were carefully removed and placed in petridish containing 0.15M Dubecco's PBS buffer (pH 7.3) for initial washing. The flukes were stored in collection vials containing PBS and were transported to the laboratory of Department of Zoology, University of Kashmir, Srinagar. Fasciolids were identified primarily on differences in body shape and size of the adults, with the smaller *F. hepatica* exhibiting wide and defined shoulders compared to the slender *F. gigantica* having less defined shoulders and shorter cephalic cones (Soulsby, 1986). For permanent slide preparation flukes were rapidly killed in 70% ethyl alcohol to avoid shrinkage. The flukes were then transferred to vials containing 6-10% formalin for preservation. Flukes were stained with Borax Carmine, dehydrated in ascending grades of ethanol, cleared in Xylene and mounted in Balsam Canada and viewed under monocular light microscope.

d) Data Analysis

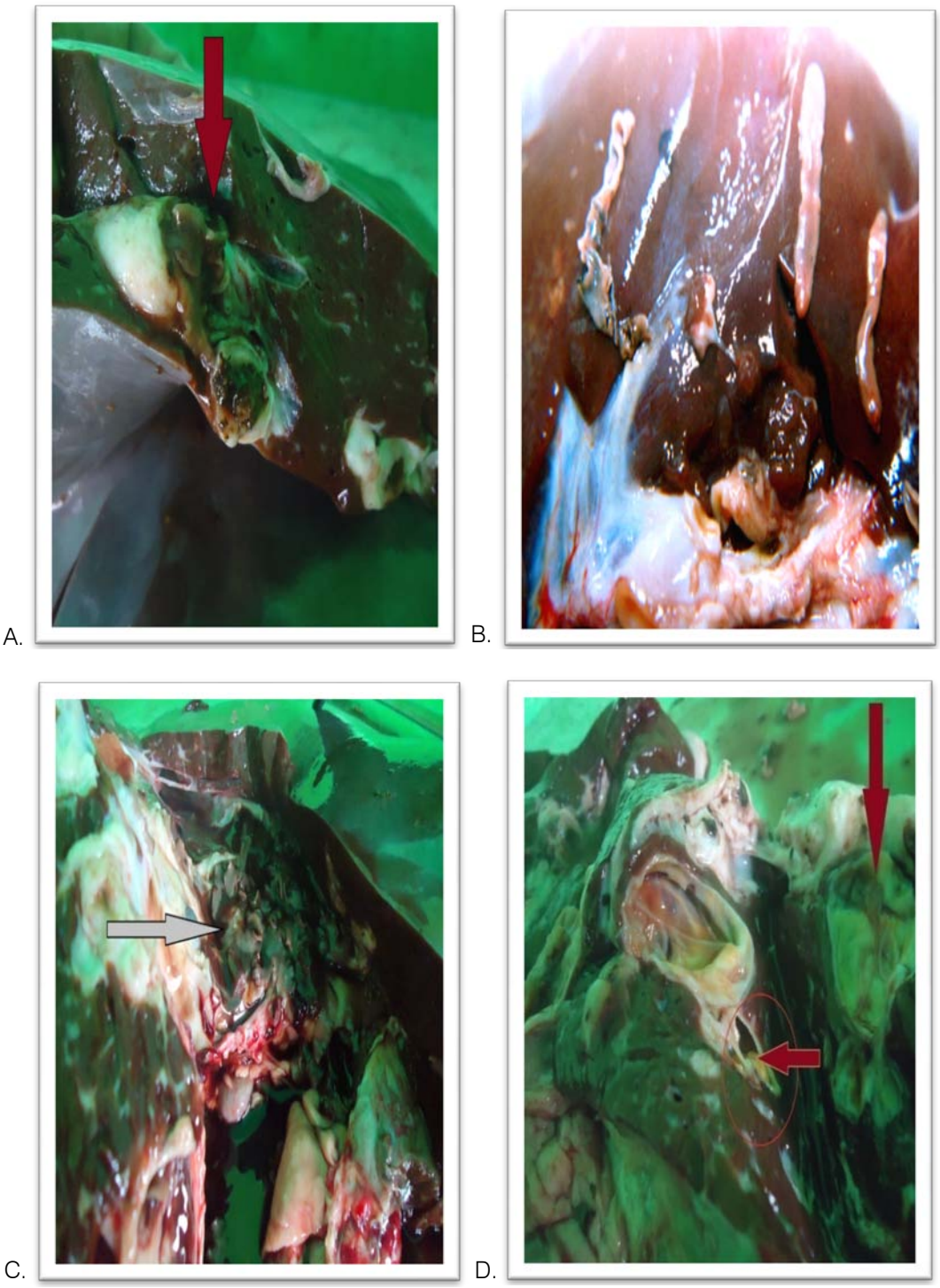
Data was recorded, entered and managed into MS Excel work sheet and analyzed using Minitab Version 13.Prevalence was calculated as percentage of infected among the examined samples. Chi square test was employed to examine the effect of above mentioned epidemiological determinants on the level of parasitism in host. In all statistical analysis, confidence level was held at 95% and P-value is <0.05 (at 5% level of significance) was considered as significant.

IV. Results

Fasciolosis in an area is influenced by a multifactorial system which comprises both definitive and intermediate hosts, parasite and environmental effects. Numerous factors (both intrinsic and extrinsic) form an association posing a potential epidemiological threat and it is important that the existence and localization of such an association should be recognized beforehand so that the situation can be brought under control. Thus in this portion of result, these factors have be assessed and potential reason behind the association have been well documented

Pg 1: Fasciola hepatica

Pg 2: Fasciola gigantica



Pg 1: Infected Liver Samples. (A) Bovine liver showing oozing parasite. (B, C) Parasites lying free and soaked in foul smelling fluid (D) Yellowish tissue

Overall Prevalence (Table 1)

The overall prevalence of Fasciolosis for the period of two years (2014-2015) was found to be 26.84% in the current study areas. In 2015, the percentage prevalence was higher (27.02%) than in 2014 (25.31%). There was an increase of 1.71% in prevalence rate from 2014 to 2015.But difference in prevalence rate was not statistically significant ($p>0.05$) as there was sampling error in year 2014 because of scarcity of data collection for a period of 2 months (September and October) due to Floods that affected the whole valley.

The result of current study indicated that Fasciolosis in cattle is spread relatively with moderate prevalence rate of 26.84% in the study area as compared to high prevalence of 51.42%,42.06% and

43.63% in Ladakh and Srinagar province of Jammu and Kashmir (Kuchai *et al*. 2011; Akhoun and Peer, 2014 respectively).The reported difference may be attributed to different factors like mode of infestation, agroclimatic variations, technique used for data collection and different management conditions under which cattle are reared. However, the result of the present study is in close proximity to the prevalence rates of 29.38% and 25.40% reported earlier by Sheikh *et al*. 2007 and Fatima *et al*. 2012 in neighboring areas of Kashmir using the same abattoir survey. The prevalence rate was also within ranges of findings of other authors like 25.46% by Khan *et al*. 2009 from Pakistan; 27.26% and 25.2% by Kabir *et al*. 2010 and Afroze *et al*. 2013 from different provinces of Bangladesh; 25.9% by Mungube *et al.* 2012 from Kenya; 26.55% by Nega *et al*. 2012 from Ethopia; 23.96% reported by Asressa *et al.* 2012 from Andassa Livestock Research Center in North-West of Ethiopia.

Table 1: Yearwise Prevalence of Fasciolosis

YEAR	EX.	INF.	PREV	χ^2(P-Value)
2014	316	80	25.31%	0.183 0.669
2015	396	107	27.02%	
Total	714	192	26.84%	

The results revealed that the lowest prevalence of Fasciolosis for Year 2014 was in the month of May (14.2%) and highest being in the month of August (35.8%).However in Year 2015, the prevalence rate was highest in the month of September (44.66%) followed by October (39.66%) and lowest in May (9.3%). Moreover, the infection was reported throughout the year due to resistance of metacercariae for desiccation, especially during the dry season and continued presence of the shallow water, enough vegetation and humidity for continued exposure of the animals to encysted metacercariae and no restriction on cattle grazing habits and movement between the infected and treated localities which was also suggested by El Bahy, 1998.

These results are in agreement with Pfukenyi *et al*. 2006 and Faria *et al*. 2005 who reported high intensity in August-September in Zambian cattle and in dairy cattle herd in Minas Gerais, Brazil respectively. Similarly, Qureshi *et al*. 2012 recorded lowest prevalence in the month of May in Buffaloes of Northwestern Punjab, Pakistan which supports the findings of the current study. In both years, the lowest infection in May-June can be related to progression of hot dry weather, as the temperature was high and humidity was low in these months.

Month-wise prevalence (Fig. 1)

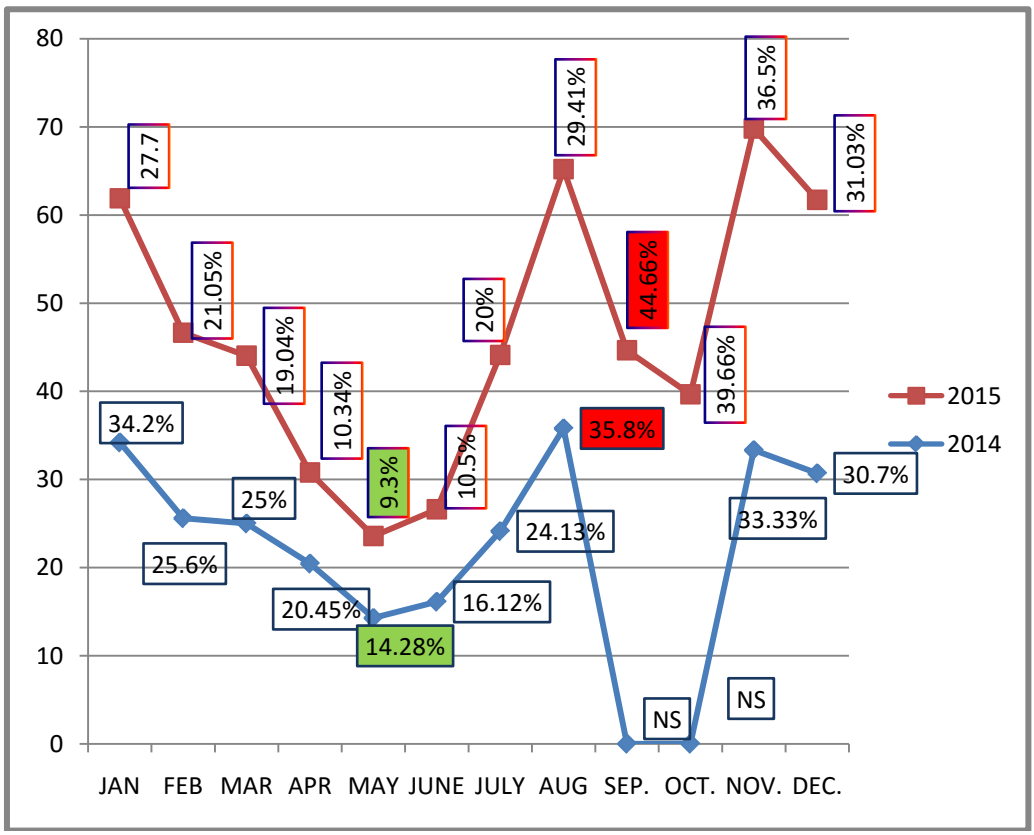


Figure 1: Monthwise prevalence of Fasiolosis (2014-2015)

Season wise Prevalence (Table and Fig 2)

On seasonal basis, the current study showed maximum spread of disease in Autumn Season i.e. 33.33% and 40% in Year 2014 and 2015 respectively. The minimum infection was recorded in spring season showing prevalence of 20% and 12.9% in consecutive studied years. There was no statistically significant difference between seasons in year 2014 which has already been stated could be attributed to skipping the data of two months due to natural disaster Kashmir valley faced. However statistically significant difference was observed between seasons in year 2015.

This difference could be due to a variety of weather condition in each year. The highest prevalence in autumn was also reported by Chaudhri *et al*. 1993; Maqbool *et al*. 1994 and Ghirmire and Karki 1996; Abrous *et al*. 1999; Maqbool *et al*. 2002; Pfukenyi *et al*. 2005 and Haridy *et al*. 2006 who emphasized that the possible reason for the same could be availability of favourable temperature and moisture for the rapid propagation of the parasitic trematode life cycle in this very season.

Table 2: Season wise prevalence of Fasciolosis (2014-2015)

Year	2014			2015		
Season	Ex.	Inf.	Prev.	Ex.	Inf.	Prev.
Spring	115	23	20%	82	10	12.9%
Summer	99	26	26.26%	102	20	19.6%
Autumn	12	4	33.33%	146	59	40%
Winter	90	27	30%	66	18	27.27%
χ^2(p-Value)	3.218(0.486)			25.26(0.000)		

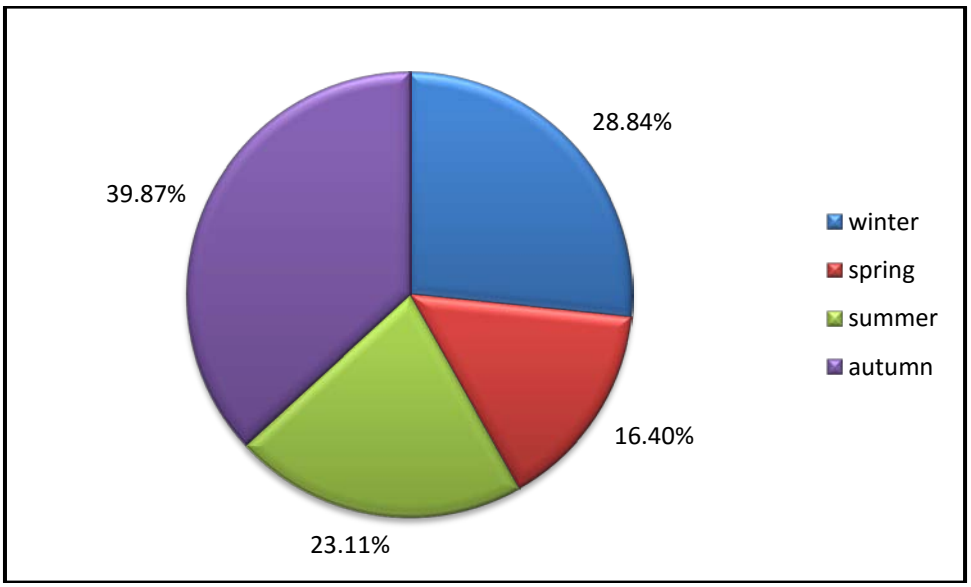


Figure 2: Cumulative Season wise prevalence

Distribution on the basis of infection type (Table 3)

Of the total 192 affected livers by fasciolosis, 149 (77.60%), 24 (12.5%) and 19 (9.89%) respectively showed *Fasciola gigantica, Fasciola hepatica* and mixed infection (*Fasciola hepatica* and *Fasciola gigantica).*

The finding of this study was in consistence with the earlier investigation by Ashrafi *et al*. 2004 from Gilan province; Mir *et al*. 2008 from Kashmir and Khan *et al*. 2009 from Punjab Province (Pakistan) and by Phiri *et al*. 2005; Abunna *et al*. 2009; Fufa *et al*. 2009; Mwabonimana, *et al*. 2009. The predominance of *Fasciola gigantica* could be due to the availability of appropriate environmental conditions and topography (lowland and middle altitude zone) which are favorable habitat to its intermediate host *L. natalensis* (Urquhart *et al*. 1996).However, inverse distribution was reported by Melugeta *et al*. 2011; Belay *et al*. 2012; Chakiso, *et al*. 2014 and Alemu and Abebe 2015. Mixed infection of *F.*

hepatica and *F. gigantica* occurs presumably as a result of the movement of stock between high and low ground or through overlapping of the territories of the snail vector at altitudinal range of 1200-1800 M.a.s.l. (Kendel 1954 and Graber, 1975).

Table 3: Prevalence based on type of infection

Infection Type	Infected	Prev. Among Infected Ones (N=192)	Overall Prevalence (N=714)
F. gigantica	149	77.60%	20.86%
F. hepatica	24	12.5%	3.361%
Mixed	19	9.89%	2.66%
$\chi 2$		254.29(p=0.000)	186.22(p=0.000)

Age-wise distribution (Table4)

Out of 714 cattles, 166 heads were of age group <1-3Years, 396 of age between 3-6 years and 152 having age >6 Years. Among these 3 age categories, prevalence of Fasciolain livers was highest in >3-6 years age group (30.30%) followed by age group >6 years (28.28%) and least infection in bovines of age 1-3Years(17.46%).The results in current study were in consistency with Keyu *et* al. 2005;Rehman *et al*.2015.The sound explanation behind the lower prevalence in age group >6 yrs compared to younger age group(3-6yrs) could be due to self-cure phenomenon (Assanji, 1988) or high acquired immunity which increase with age (Dwinger *et al*. 1982). It has been also reported that *Fasciola* infected hosts may recover from parasitic infection with increasing age and hence become resistant (Yilma and Mesfin, 2000; Shiferaw *et al*.2011; Mufti, 2011.Mulcahy *et al*. 1999 suggested that resistance is not wholly immunologiical based rather resistance to reinfection may be due to hepatic fibrosis resulting from primary infection. Least infection in age group <1-3 years is possibly due to less chances of acquiring infection due to short exposure time as compared to older animals which is in agreement with (Anderson *et al*. 1999)and Teklu *et al*. 2015.

Table 4: Agewise prevalence of fasciolosis

Age	Ex.	Inf.	Prevalence	χ^2 p-Value
1Yr-3Yrs	166	29	17.46%	9.991 0.007
3Yrs-6Yrs	396	120	30.30%	
>6Yrs	152	43	28.28%%	

Genderwise prevalence (Table 5)

Out of 531 males and 183 females slaughtered during the survey period, males won by retaining lesser infection of 19.96% and were par to females who showed higher prevalence of 46.99%. The difference was highly significant and thus revealed sex as determinant influencing the prevalence of Fasciolosis rate. Our findings are in agreement with results of Daniel 1995; Molina *et al*. 2005; Bhutto *et al*. 2012 and Teklu *et al*. 2015 who reported higher prevalence of this parasite in female than male.

In the current studied abattoirs, the number of slaughtered male cattles (531) was far higher than the females (183). The number of positive females was higher in proportion than males even if the number of female cattle that come to abattoir were fewer in number. These results were in consistent to Kara *et al*. 2009. High infection rate in females can be multifactorial like high stress during parturition period (Spithill *et al*. 1999), weak and malnourished making them more susceptible to infection (Blood and Radostits, 2000) or due to the feeding conditions i.e females are generally being let loose to graze freely in pastures. The other possible reason for the same could be that the most of people traditionally feed their lactating cows with grasses during dry season which are grown around rivers and marshy areas for the sake of getting high milk yield as suggested by Gracy *et al*. 1999 and Tilahun *et al*. 2014. However, some authors revealed that male cattle are more prone to Fasciolosis than female counterparts like Khan and Maqbool 2012. But, Rahmeto 1992 and Dagne 1994; Keyyu *et al*. 2005; Phiri *et al*. 2005; Khan *et al*. 2009; Kabir *et al*. 2010; Kanyar *et al*. 2010; Assefa *et al*. 2015 reported no significant difference between the gender of animal and infection rate which could be associated with similar management given to both group of animals or probably due to common grazing pastures on which both are fed together, which expose them to the same risk of infection.

Table 5: Genderwise prevalence of Fasciolosis

	Examined	Infected	Prevalence
Males	531	106	19.96%
Females	183	86	46.99%
$\chi 2$ (p-value)	49.221(0.000)		

Association of body condition with infection (Table 6)

Among all examined animals (n = 714), 30.53% (n = 218) were marked as poor (body score 1-3), 35.05% (n =250) as Medium (4-6) and 34.44% (n = 246) as Good (7-9) body conditions. 42.66% of infection (n_i=93) was recorded in animals with poor body condition, 22.40% of infection (n_i=56) in animals with medium body score and 17.47% of infection (n_i=43) in animals having good body condition. Thus, an inverse association was found between the body condition and infectious rate of Fasciolosis which was statistically significant ($p<0.05$). These findings are in accordance to Mihreteab *et al*. 2010; Tilahun *et al*. 2014 and Teklu *et al*. 2015.

The current findings that lean animals are associated with higher *Fasciola* infection compared to animals with medium and normal body condition could be attributed to emaciation due to lack of nutrients, loss of blood and tissue fluid and demage to liver parenchyma caused by presence of flukes. Similar findings were reported by Beckele *et al*. 2010; Tesfay *et al*.2012 and Assefa *et al*. 2015. Radostits *et al*. 1994 has mentioned that Chronic fasciolosis is the commonest disease in cattle and significantly characterized with weight loss.

Table 6: Effect of body condition on prevalence of Fasciolosis

Body Condition	Ex.	Inf.	Prevalence	χ^2 p-Value
Poor	218	93	42.66%	41.223 0.000
Medium	250	56	22.40%	
Good	246	43	17.47%	

Breedwise prevalence of Fasciolosis (Table7)

Out of the total 71 cattle examined, 213 were reared locally and 501 were imported from other states to the valley for slaughter purpose. The prevalence of fasciolosis was 40.80% and 20.90%for local and nonlocal breed cattle, respectively. There was statistically significant (χ^2 = 29.06, P = 0.000) association of fasciolosis with breeds. Our results are in agreement with study conducted by Teklu *et al*. 2015.

This diference in prevalence based on breed might be due to the management of the animals as most of the local animals were reared in the extensive system of management which makes them easily susceptible to the parasites

Table 7: Breed wise Prevalence of Fasciolosis

Breed	Ex.	Inf.	Prevalence	χ^2 p-Value
Locals	213	87	40.80%	29.06 0.000
Non-locals	501	105	20.90%	

V. Conclusion

Moderate intensity of fasciolosis was recorded in the current study. In line with above findings, it is recommended that strategic application of flukicides should be done and further epidemiological studies on biology and ecology of intermediate host should be carried out so to develop subastantiable planning for considerable success in control of Fasciolosis. There is need to carry out economic analysis so as to give appropriate economic losses directly by liver condemnation.

VI. Acknowledgement

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