Prevalence of *Fasciola* infection in slaughtered Animals in Kashmir

Nazima Gul, Hidayatullah Tak, Khalid M Fazilli and Iram Abdullah and Tanveer A Sofi

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* spp 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%).

Keywords: Epidemiology, *Fasciola*, cattle, Abattoir and Srinagar

Introduction

Ruminant productivity around the world is majorly affected by trematode parasitism (Vercruysse and Claerebout 2001) [63]. 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) [53] but also to its zoonotic aspect. Bovine Fasciolosis is an impediment 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) [36].

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Outbreaks</th>
<th>Attacks</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008-2009</td>
<td>85</td>
<td>391402</td>
<td>2</td>
</tr>
<tr>
<td>2009-2010</td>
<td>84</td>
<td>375237</td>
<td>6</td>
</tr>
<tr>
<td>2010-2011</td>
<td>105</td>
<td>345108</td>
<td>27</td>
</tr>
<tr>
<td>2011-2012</td>
<td>130</td>
<td>316363</td>
<td>74</td>
</tr>
<tr>
<td>2012-2013</td>
<td>195</td>
<td>509195</td>
<td>31</td>
</tr>
<tr>
<td>2013-2014</td>
<td>137</td>
<td>802698</td>
<td>11</td>
</tr>
<tr>
<td>2014-2015</td>
<td>129</td>
<td>3606</td>
<td>4</td>
</tr>
</tbody>
</table>

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) [14, 33]. Thus the abattoir study was carried out to determine the prevalence.
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).

\[
 n = \frac{1.96^2 \cdot P_{\text{exp}}(1 - P_{\text{exp}})}{d^2}
\]

Where \( n \) = required sample size
\( P_{\text{exp}} \) = 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) [4]. 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.

Study of epidemiological parameters
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) [19].

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.

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) [56]. 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.

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.

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 been assessed and potential reason behind the association have been well documented.
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) [32, 4]. 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 [54] and Fatima et al. 2012 [23] 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 [31] from Pakistan; 27.26% and 25.2% by Kabir et al. 2010 [28] and Afroze et al. 2013 [3] from different provinces of Bangladesh; 25.9% by Mungube et al. 2012 [42] from Kenya; 26.55% by Nega et al. 2012 [45] from Ethiopia; 23.96% reported by Asressa et al. 2012 [8] from Andassa Livestock Research Center in North-West of Ethiopia.

### Table 2: Yearwise Prevalence of Fasciolosis

<table>
<thead>
<tr>
<th>Year</th>
<th>EX.</th>
<th>INF.</th>
<th>PREV</th>
<th>χ²(P-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>316</td>
<td>80</td>
<td>25.31%</td>
<td>0.183</td>
</tr>
<tr>
<td>2015</td>
<td>396</td>
<td>107</td>
<td>27.02%</td>
<td>0.669</td>
</tr>
<tr>
<td>Total</td>
<td>714</td>
<td>192</td>
<td>26.84%</td>
<td></td>
</tr>
</tbody>
</table>

Month-wise prevalence (Fig. 1)
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 [21]. These results are in agreement with Pfukienyi et al. 2006 and Faria et al. 2005 [22] 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 [49] 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.

### Fig 4: 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 [60]; Maqbool et al. 1994 [34] and Ghirmire and Karki 1996 [25], Abrous et al. 1999 [1], Maqbool et al. 2002 [33], Pfükenyi et al. 2005 [47] and Hardiy 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 3: Season wise prevalence of Fasciolosis (2014-2015)

<table>
<thead>
<tr>
<th>Year</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>Ex.</td>
<td>Inf</td>
</tr>
<tr>
<td>Spring</td>
<td>115</td>
<td>23</td>
</tr>
<tr>
<td>Summer</td>
<td>99</td>
<td>26</td>
</tr>
<tr>
<td>Autumn</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Winter</td>
<td>90</td>
<td>27</td>
</tr>
<tr>
<td><strong>χ²(p-Value)</strong></td>
<td>3.218(0.486)</td>
<td>25.26(0.000)</td>
</tr>
</tbody>
</table>

**Fig 5:** 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 [7] from Gilan province; Mir et al. 2008 [38] from Kashmir and Khan et al. 2009 [31] from Punjab Province (Pakistan) and by Phiri et al. 2005 [48], Abunna et al. 2009 [3], Fufa et al. 2009 [24], Mwabonimana, et al. 2009 [44]. 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) [62]. However, inverse distribution was reported by Melugeta et al. 2011 [42]; Belay et al. 2012 [12], Chakiso, et al. 2014 [13] and Alemu and Abebe 2015 [5, 58]. 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) [29, 26].

### Table 4: Prevalence based on type of infection

<table>
<thead>
<tr>
<th>Infection Type</th>
<th>Infected</th>
<th>Prev. Among Infected Ones (N=192)</th>
<th>Overall Prevalence (N=714)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. gigantica</td>
<td>149</td>
<td>77.60%</td>
<td>20.86%</td>
</tr>
<tr>
<td>F. hepatica</td>
<td>24</td>
<td>12.5%</td>
<td>3.36%</td>
</tr>
<tr>
<td>Mixed</td>
<td>19</td>
<td>9.89%</td>
<td>2.66%</td>
</tr>
<tr>
<td><strong>χ²</strong></td>
<td>254.29(p&lt;0.000)</td>
<td>186.220(p&lt;0.000)</td>
<td></td>
</tr>
</tbody>
</table>

### Age-wise distribution (Table 4)

Out of 714 cattle, 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 Fasciolai 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 [50]; 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) [9] or high acquired immunity which increase with age (Dwinger et al. 1982) [28], It has been also reported that Fasciola infected hosts may recover from parasitic infection with increasing age and hence become resistant (Yilm and Mesfin, 2000 [64], Shiferaw et al. 2011 [55]; Mufi, 2011 [40]. Mulcahy et al. 1999 [41] suggested that resistance is not wholly immunological 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) [6] and Teklu et al. 2015 [58].

### Table 5: Age wise prevalence of fasciolosis

<table>
<thead>
<tr>
<th>Age</th>
<th>Ex.</th>
<th>Inf.</th>
<th>Prevalence</th>
<th>χ²(p-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Yr-3Yrs</td>
<td>166</td>
<td>29</td>
<td>17.46%</td>
<td>9.991</td>
</tr>
<tr>
<td>3Yrs-6Yrs</td>
<td>396</td>
<td>120</td>
<td>30.30%</td>
<td>0.007</td>
</tr>
<tr>
<td>&gt;6Yrs</td>
<td>152</td>
<td>43</td>
<td>28.28%</td>
<td></td>
</tr>
</tbody>
</table>

### 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 [18], Molina et al. 2005 [49], Bhutto et al. 2012 [13] and Teklu et al. 2015 [58] who reported higher prevalence of this parasite in female than male.

In the current studied abattoirs, the number of slaughtered male cattle (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) [6, 57], weak and maldnourished 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 [27] and Tilahun et al. 2014 [61]. However, some authors revealed that male cattle are more prone to Fasciosiosis than female counterparts like Khan and Maqbool 2012 [49]. But, Rahmeto 1992 [51] and Dagne 1994 [17], Keyyu et al. 2005 [10], Phiri et al. 2005 [48], Khan et al. 2009 [31], Kabir et al. 2010 [28], Kanyar et al. 2010; Assefa et al. 2015 [10] 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 6: Genderwise prevalence of Fasciolosis**

<table>
<thead>
<tr>
<th></th>
<th>Examined</th>
<th>Infected</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>531</td>
<td>106</td>
<td>19.96%</td>
</tr>
<tr>
<td>Females</td>
<td>183</td>
<td>86</td>
<td>46.99%</td>
</tr>
</tbody>
</table>

**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=93) was recorded in animals with poor body condition, 22.40% of infection (n=56) in animals with medium body score and 17.47% of infection (n=43) in animals with 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 with Mihreteab *et al.* 2010 [37]; Tilahun *et al.* 2014 [57] and Teklu *et al.* 2015 [58].

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 Bekele *et al.* 2010 [11]; Tesfay *et al.* 2012 [59] and Assefa *et al.* 2015 [10]. Radostits *et al.* 1994 [50] has mentioned that Chronic fasciolosis is the commonest disease in cattle and significantly characterized with weight loss.

**Table 7: Effect of body condition on prevalence of Fasciolosis**

<table>
<thead>
<tr>
<th>Body Condition</th>
<th>Ex.</th>
<th>Inf.</th>
<th>Prevalence</th>
<th>χ² (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>218</td>
<td>93</td>
<td>42.66%</td>
<td>41.223</td>
</tr>
<tr>
<td>Medium</td>
<td>250</td>
<td>56</td>
<td>22.40%</td>
<td>0.000</td>
</tr>
<tr>
<td>Good</td>
<td>246</td>
<td>43</td>
<td>17.47%</td>
<td></td>
</tr>
</tbody>
</table>

**Breedwise prevalence of Fasciolosis** (Table 7)

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 (χ² = 29.06, P = 0.000) association of fasciolosis with breeds. Our results are in agreement with study conducted by Teklu *et al.* 2015 [58]. This difference 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 8: Breed wise Prevalence of Fasciolosis**

<table>
<thead>
<tr>
<th>Breed</th>
<th>Ex.</th>
<th>Inf.</th>
<th>Prevalence</th>
<th>χ² (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locals</td>
<td>213</td>
<td>87</td>
<td>40.80%</td>
<td></td>
</tr>
<tr>
<td>Non-locals</td>
<td>501</td>
<td>105</td>
<td>20.90%</td>
<td></td>
</tr>
</tbody>
</table>

**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 substantial 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.

**Acknowledgement**

I extend my heartfelt thanks to Dr. Javed, Assistant Professor, Dept. of Statistic, University of Kashmir for proficiency and support regarding Biostatistics computation and analysis. I would like to thank butchers of abattoirs visited during current study for their interest to participate in research work by providing liver samples of cattle which form the basic tool for this piece of research work.

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