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RESEARCH ARTICLE

COMPARATIVE MORPHOMETRIC ASSESMENT AND PROTEIN PROFILING OF *Fasciola hepatica* AND *Fasciola gigantica* COEXISTING IN BOVINES

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ABSTRACT

Fascioliasis is one of the most prevalent helminth infections of ruminants in different parts of the world. The current study highlights the phenotypic differences of aetiological agents of Fascioliasis in bovines i.e., *Fasciola hepatica* and *Fasciola gigantica*. The phenotypic parameters taken into consideration were BL, BW, OL, OW and BL/BW ratios. The data was subjected to one way ANOVA followed by Tuky test by using PRIMER version 4. Morphometrical values of *Fasciola* spp. revealed longer *F. gigantica* (33.66 ±4.42) as compared to *Fasciola hepatica* (25.19±4.22). Moreover, *F. gigantica* had narrower bodies (5.48 ±0.92) compared to *F. hepatica* (5.70 ±1.64). The differences in the mean body length; mean body width; and mean of BL/BW ratios of body were significant ($p<0.05$). The current abattoir study also revealed the predominance of *F. gigantica* (78.16%) to *F. hepatica* (21.39%) in cattle. The electrophoretic pattern under reducing conditions of 12% SDS-PAGE showed some similarities and differences between crude somatic protein extraxt of *Fasciola gigantica* which revealed presence of 11 bands and 14 bands in case of *Fasciola hepatica* coexisting in bovines.

INTRODUCTION

Fasciolosis in domestic ruminants is due to infection with hermaphrodite parasites *Fasciola gigantica* (tropical liver fluke) and *Fasciola hepatica* (temperate liver fluke) which causes significant economic loss. Despite the importance to differentiate between the infection by either fasciolid species, there is neither a direct coprological nor an indirect immunological test available for their diagnosis. (El-Rahimy *et al*., 2012). The specific differentiation can only be made by a morphological study of adult flukes or by molecular tools (Periago *et al*. 2008). Morphology has been the most frequently used criterion for systematic studies on *Fasciola* flukes which had been later invalidated (Periago *et al*., 2006). Moreover, speciation based on morphology and morphometry is not decisive due to overlap in the values of most measurements (Lotfy *et al*., 2002; Ai *et al*., 2011). In recent years, SDS-PAGE and Western blot procedures have created a new era in immunodiagnosis, and greatly reduced cross reactions (Sharma *et al*., 1987). The present work was sought to compare morphometrical parameters and gel electrophoretic patterns between the two forms of fasciolids that coexisted in livers of slaughtered bovines and to compare the current results with those of other studies.

MATERIAL AND METHODS

Isolation of worms

Naturally infected livers were obtained from slaughtered cattle on the day of slaughter from local slaughterhouses past midnight. In order to obtain flukes from liver, gall bladder was incised and then bile ducts were opened, starting from common bile ducts to smaller ones at the periphery of liver. The infected livers were squeezed manually to macerate the parenchyma and the flukes were carefully removed and placed in petridish containing 0.15M PBS (pH 7.3) for initial washing to remove host material and allow regurgitation of gut contents. The flukes were stored in collection vials containing PBS and were transported to the laboratory of Department of Zoology, University of Kashmir, Srinagar. Individual flukes were removed from PBS and spread gently without traction on a slide.

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Morphometric assessment

The general morphological characters were recorded by using a compass. The measurements indicated were taken and assessed against a graduated ruler. Five morphometrical characters of intact worms were measured: a. body length; b. widest body width; c. Cone width d. cone length at proximal end of acetabulum; and e. ratio between body lengths to body width (Periago *et al*., 2006). Moreover, the flukes were kept on cooler with crushed ice throughout the procedure to prevent protein degradation.

Preparation of crude somatic antigens

For preparation of crude somatic antigen (CSAg) flukes of Fasciolid spp were cut into small pieces with the help of fresh surgical blade and then homogenised separately in cooled homogenizing buffer [0.5mM EDTA, 50 mM Tris, 50 mM NaCl containing 0.5% Triton X-100] to which 2mM PMSF was added to prevent proteolytic degradation in tissue homogenizer at 1280 rpm for 3 minutes. The disintegrated parasite extract was then centrifuged at 4^oC at 10000 rpm for 30 minutes and the supernatant was collected as the CSAg. The supernatant obtained was recentrifuged at 14000 rpm 4^oC for 30 minutes so to remove all the cell debris. Then supernatant was stored at -20^0C till use.

RESULTS AND DISCUSSION

Prevalence of Fasciolid spp

An overall of 123 bovine liver samples were checked for presence of *Fasciola* sps, out of which 87 were found infected accounting to overall prevalence of 70.73%. Of the 123 bovine livers 19 livers

(21.39%) harboured *F. hepatica*, 68 livers (78.16%) harboured *F. gigantica* and 9 livers (10.34%) harboured mixed infection. Our finding reported the predominance of *F. gigantica* to *F. hepatica* in cattle which is in consistent with abattoir study carried by Phiri, *et al.* (2005), Abunna, *et al*. (2009), Mwabonimana, *et al.* (2009).

External Morphometric Assesment

Morphometrical data obtained from 54 fully-relaxed whole worms of *F. gigantica* and 47 of *F.hepatica* revealed longer *F. gigantic* (33.66 ±4.42) as compared to *Fasciola hepatica* (25.19 ±4.22). *F. gigantica* had narrower bodies (5.48 ±0.92) compared to *F. hepatica* (5.70 ±1.64). Cone length ranged between 1-4 mm, with overlap at 1-3 mm. However, cone widths in both the species concur. Although differentiation parameters helped in morphological determination of the fluke species, yet some flukes had shared characters. In the current study, morphometrical values of *Fasciola gigantica* individuals in cattle approximates that of *Fasciola gigantica* infecting Pakistan cattle (BL 33.89 ±0.76; BW 6.01±0.17 and BL/BW 5.78 ±0.15). However they were generally larger compared to those obtained from Philippine buffaloes (25-37 mm; χ = 31.2 mm) earlier reported by Kimura *et al.* (1984), Egyptian bovines(19-41; 30 ±6) and Philippine cattle(16-39; 29.3±6.18) by Narva *et al.* (2011) but shorter compared to those of Iranian buffaloes (28.6-48.7 mm; χ = 38.0 ± 0.42 mm) as well as, with those isolated from Iranian (22.7-59.2mm; χ = 37.7 ± 0.27 mm) and African (30.7-52.0 mm; χ = 39.5 ± 0.84 mm) cattle (Ashrafi *et al*., 2006). While the widest body width of *Fasciola gigantica* in the current study (4-9; 5.48 ±0.92) is narrower compared to those infecting Philippine buffaloes (7.1-10.2; χ = 8.5), Egyptian bovines (6-13, χ=8.9 ±1.7), Iranian cattle (3.5-9.8 mm; χ = 6.4 ± 0.04 mm), and African cattle (6.5-11.4 mm; χ = 8.9 ± 0.16) (Kimura *et al.*, 1984; Lofty 2002; Ashrafi *et al*., 2006).

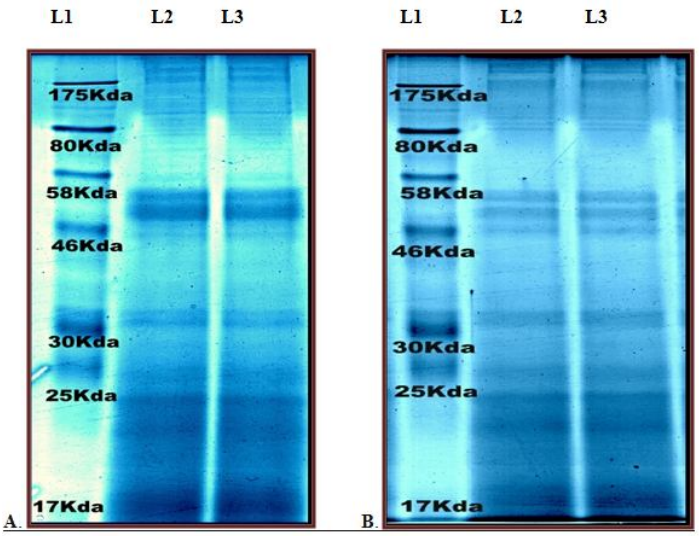
Values within to 0.05 row that do not share the same superscript are significantly different ($^{a-b}P<0.05$). The data was evaluated by one-way ANOVA followed by Tuky test to detect inter morphometric differences. Differences were considered to be statistically significant if $p < 0.05$. In case of *Fasciola hepatica* data on body length (20-32; 25.19 ±4.22) are consistent with the findings of Ghavami *et al*. (2009) at 11.01-48.64 (23.89±0.39) and those infecting Egyptian bovines (23.73±0.33) studied by Periago *et al*. (2006). With respect to body width of *Fasciola hepatica* in current study approximates with those infecting pakistan buffaloes (5.84 ±0.09) but lesser than those infecting Egyptian bovines(5-15; 9±2.2) and Iranian cattle(4.46-15.91; 10.13 ±0.20. (Lofty *et al.*, 2002 and Ghavami *et al.*, 2009).

Characterization of crude adult Fasciola homogenate by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

In the adult soluble protein fraction from *Fasciola gigantica* infecting bovines, protein concentration which was estimated by Lowry method (Lowry *et al*., 1951) came out to be 0.65 mg/ml, whereas in case of *Fasciola hepatica* infecting same host the protein concentration was estimated to be 1.01 mg/ml. Electrophoretic patterns showed some similarities and some differences between the two Fasciolid parasite preparations coexisting in bovines as represented in Pg 1 under reducing conditions in 12% SDS-PAGE where lane -1 represents the marker protein and lane-2 and 3 represents that of parasitic extract. There are 11 bands in soluble protein fraction of *Fasciola gigantica* (bovines) reported in the present study which is in agreement with study carried by Meshgi, *et al*., 2008. However there were 14 bands found in *Fasciola hepatica* (bovines) which is in close association to the results of El-Rahimy *et al*., 2012 who noticed 13 bands.

Table 1. Summary of ranges, mean ±SD of morphometrical values of *Fasciola hepatica* and *Fasciola gigantica* in bovines

Measured Body Part	*F. hepatica*(n=47) Range (mm) Mean ±SD	*F. gigantica*(n=54)Range (mm) Mean ±SD
Body Length(BL)	20-32	27-45
	25.19 ±4.22[a]	33.66 ±4.42[b]
Maximum body	3-10	4-9
width(BW)	6.08 ±1.64 [a]	5.48 ±0.92 [b]
Cone length(OL)	1-3	1-4
	1.63 ±0.67	1.70 ±0.79
Cone width(OW)	1-3	1-3
	1.48 ±0.54	1.62 ±0.59
BL/BW	2.1-9.6	3.7-10.5
	4.86 ±1.47 [a]	6.27 ±1.20 [b]



Pg 1. SDS PAGE profile of soluble proteins of (A) *F. gigantica* (B) *F. hepatica* from bovines (Lane 1 show marker proteins, Lane 2 and 3 show parasite proteins)

The difference in the reported number of bands or molecular weights for *Fasciola hepatica* and *Fasciola gigantica* may be due to the existence of different isolates from different host species or geographic variations (Meshgi *et al*., 2008). Dominant bands for both *Fasciola gigantica* and *Fasciola hepatica* in bovines clustered between 46 and 58 Kda; and also between 17 and 25 Kda. The identified clustered proteins during the current investigation are in accordance to Goreish *et al*. 2008 and Espino *et al.* 1993 respectively. In addition ~24 Kda and ~57 Kda being common protein band between the two species protein extract corresponds to Cathepsin L cystein proteases (Robinson *et al.*, 2008) and leucyl aminopeptidase which are considered to be the relevant candidate for vaccine development against ruminant fascioliasis. (Mc Manus and Dalton 2008; and Acosta, *et al.*, 2008). The electrophoretic scanning also revealed the presence of ~110 Kda protein in *Fasciola gigantica* which was also revealed by Maghraby *et al*., 2007.

Conclusion

In view of current preliminary findings regarding high prevalence of fascioliasis in bovines and dearth of baseline information, it is recommended to carry parasite survelience in different susceptible hosts taking into account wider sampling areas of animal hosts, and jointly profiling of extracts of infected and uninfected liver tissue samples should be done to circumscribe host derived proteins from endogenous components. Moreover, other morphometric parameters should be studied so as to ascertain proper taxanomic status of Fasciolid spp.

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