Serological Prevalence of *Fasciola gigantica* in Mithun (Bos frontalis)

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Abstract
Present study was conducted to determine the prevalence of fasciolosis in Mithun in different rearing conditions. The serum samples were collected from different geographical locations of Nagaland and Mizoram in free range conditions of rearing and from institute farm, serum sample were collected in semi intensive conditions. In the present study, no ova of Fasciola gigantica were recovered on faecal examination but attempts were made for studying the sero-prevalence of this parasitic infection in mithun. Seroprevalence of fasciolosis in mithun was recorded in free range conditions as well as in semi intensive conditions. Out of 156 animals tested, sera of 30 animals were found to be reactive in ELISA which yielded a percentage of 19.23%. Out of this 25.84% were recorded from free range conditions and 10.44% were recorded from semi intensive conditions. The lower prevalence in semi-intensive system may be due to the practice of regular deworming and better system of management. However, in free range condition, there is open access to metacercariae in natural grazing area of forest. The peculiar geography of north eastern hilly region and climatic conditions are mainly responsible for low prevalence of this infection in this region.

Key words: ELISA, Fasciolosis, Mithun, Seroprevalence

Introduction

Mithun (*Bos frontalis*), popularly known as *'the cattle of hills'*, is believed to be a descendant of Gaur (*Bos gaurus*). Mithun plays an important role in the socio-cultural life and economic status of the tribal population of northeast India. Mithun is susceptible to various parasitic diseases as with other ruminants (Rajkhowa and Verma, 1993; Rajkhowa *et al*., 2003). Fasciolosis is one of the most important parasitic diseases of ruminants which results in significant economic loss to the livestock sector. The causative agent of fasciolosis in mithun has been identified as *Fasciola gigantica* (Chamuah, 2005; Chakraborty, 2001). Diagnosis of fasciolosis in mithun by faecal examination has been reported by different authors (Tandon *et al.*, 2005). Gross parasite was recovered from mithun by Chamuah *et al.* (2006) from Arunachal Pradesh. Early diagnosis of fasciolosis is crucial for effective control of this parasite in mithun. Diagnosis by faecal examination is often unsatisfactory because of the long prepatent period of infection. In this circumstance, serological tests are highly promising. Cathepsin L based ELISA has been validated

as a highly sensitive and specific diagnostic assay for the early prepatent detection of fasciolosis in other ruminants in field conditions (Farrell *et al*., 1981; Hillyer *et al*., 1985; Yadav *et al*., 2005; Fagbemi and Obarisiagbon, 1990; Sriveny *et al*., 2006; Raina *et al*., 2006; Varghese *et al.*, 2012). No work has been published on the serodiagnosis of *F. gigantica* infections or fasciolosis in mithun. So, present study was designed to determine the serological prevalence of fasciolosis in mithun in both semi intensive condition and different free ranging areas of Nagaland. In the present study, no ova of parasites were recovered on faecal examination but attempts were made for serodiagnosis of helminth parasites in mithun.

Materials and Methods

Parasite Collection

F. gigantica flukes were collected in physiological saline from the liver of buffaloes, slaughtered at a local abattoir, Bareilly, U.P. The flukes were washed thoroughly with normal saline in order to remove host-origin material.

Purification of Native Cysteine Proteinase

Cysteine proteinases were purified from the *F. gigantica* regurgitant released *in vitro* in the secretory products. Briefly, viable flukes were incubated in RPMI-1640 medium, pH 7.2 (Biological Industries, Israel) with one fluke/ml of the medium, supplemented with 0.2% sodium bicarbonate, 30 mM HEPES and 25 mg/ml gentamycin. After 3-4h incubation at 37^{o}C, the culture medium with *in vitro* released parasite regurgitant was centrifuged at 10,000×g for 20 min. and supernatant was used for precipitation of the cysteine proteinase. Chilled ethanol was added to the culture supernatant, drop by drop, to a final concentration of 60% (v/v) and the suspension incubated at -20^{o}C overnight. Proteins precipitating at 60% (v/v) ethanol concentration were pelleted at 6000×g (20 min, 4^{o}C) and discarded. Ethanol concentration in the supernatant was subsequently raised to 75% (v/v) and supernatant incubated overnight at -20^{o}C. The precipitated proteins were centrifuged at 6000×g (20 min, 4^{o}C). The cysteine proteinases thus precipitated at 75% (v/v) ethanol concentration were rinsed in 70% ethanol, air dried and resusupended in Phosphate Buffer (PBS) pH 7.2. Purified protein was resolved on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) and characterized as cathepsin-L cysteine proteinase (Yadav *et al.*, 2005).

Collection of Serum Samples

Whole blood was collected by puncturing the jugular vein after restraining the animal in a controlling crate. Out of 156 sera samples, 75 samples were collected from different geographical locations of Nagaland in free range condition of rearing and 67 from institute farm maintained in semi intensive

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conditions of rearing. 14 sera samples were collected from Mizoram where free range condition of rearing was practicing. Sera were separated, numbered, aliquoted and stored at -20^{o}C until testing.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed as per the technique described earlier. Optimum concentration of antigen, sera dilution and antiglobulin enzyme conjugate concentration were determined by performing checkerboard titration (Santiago and Hillyer, 1988). Polystyrine microtitre plates (Nunc, Denmark) were sensitized with 100 µl of 0.05 M carbonate–bicarbonate buffer, pH 9.6 containing 2 µg/ml of native cysteine proteinase followed by overnight incubation at 4^{o}C. The wells were washed with PBS containing 0.05% Tween-20 (PBS-T), thrice for 5 min each. Subsequently, blocking was done using skimmed milk (3%) in PBS-T at 37^{o}C for 2 hrs. After three washings with PBS-T, 100 µl of 1% skimmed milk in PBS containing test sera (1:100) was added in each well and incubated at 37^{o}C for 1 hr. Plates were washed thrice with PBS-T and incubated for 1 hr at 37^{o}C after adding 100 µl of 1% skimmed milk in PBS containing 1:8,000 dilution of rabbit anti-bovine IgG–HRP-conjugate (Sigma Chemicals, USA). Finally, after three washings with PBS-T, 100 µl substrate buffer (OPD-8 mg, 10 ml of citrate buffer pH 5.0, 10 µl of H_2O_2) was added to the wells and kept in dark room for 5-10 min. The reaction was stopped by adding 100 µl of 3 N H_2SO_4 to each well. The absorbance values were read at 492 nm in a microtitre plate ELISA reader (Bio-Rad, USA). The results, expressed as the mean of the optical density (OD) were obtained from duplicate samples. Cut –off value was determined by mean absorbance values of control sera +3 SD. Sera from those animals from which flukes were recovered from liver serve as positive control whereas sera collected from those animals which were having apparently healthy liver serves as negative control.

Results

In the present study native cysteine protease of *F. gigantica* which was purified to almost complete homogeneity was used as the antigen to determine the seroprevalence of fasciolosis in mithun. SDS-PAGE analysis of the purified protein revealed 28kDa protein. Using this protein ELISA was standardized. Seroprevalence of fasciolosis in mithun was recorded in free range condition as well as in semi intensive condition in Nagaland as well as Mizoram. Out of 156 animals tested, sera of 30 animals are found to be reactive in ELISA which yielded a percentage of 19.23%. Out of this 25.84% was recorded in free range condition and 10.44% was recorded in semi intensive condition.

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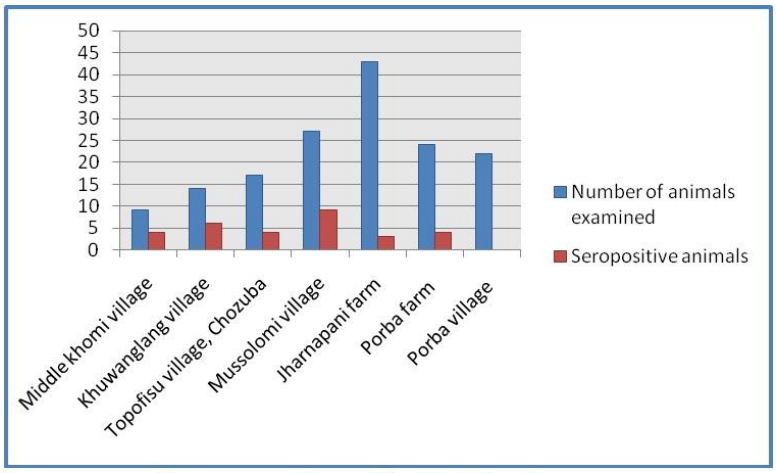

Fig. 1: Seroprevalence of fasciolosis in mithun

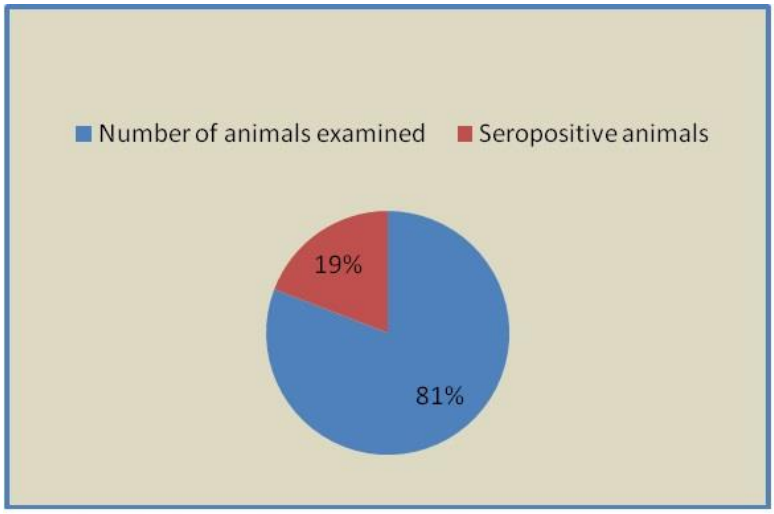

Fig. 2. Proportion of seropositive animals

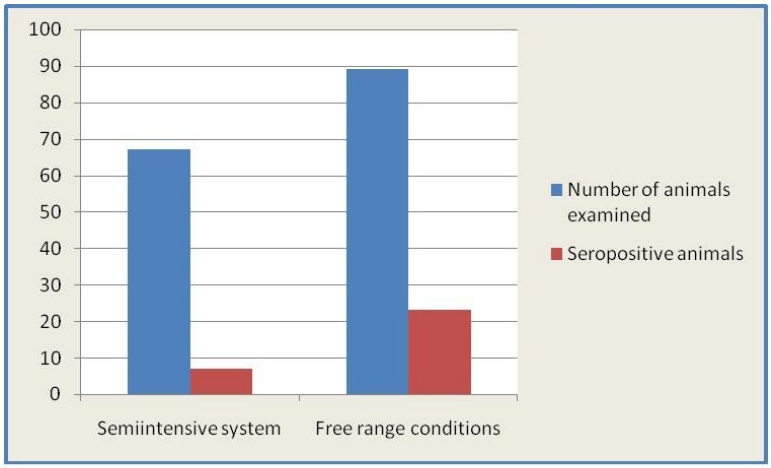

Fig. 3. *F.gigantica* infection in different rearing systems

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Discussion

The causative agent for fasciolosis in mithun has been identified as *Fasciola gigantica* (Chamuah, 2005). According to a study conducted in Nagaland by Rajkhowa *et al.*, 1999-2000, incidence of *F. gigantica* in mithun is 10%. The geographical distribution of *Fasciola* spp. depends not only on the presence of suitable aquatic *Lymnaid* snail but also on favourable climatic and ecological conditions (Tandon *et al*., 2005). According to a study conducted in cattle and buffaloes in India by Garg *et al.*(2009), prevalence rate of fasciolosis is highest in tarai region followed by hills and plains.

In most of the tropical developing countries, the temperature is generally favourable for the development of both the fluke and their intermediate host, but due to the variations in the precipitation and humidity there are fluctuations in the development of snail and free-living stages of parasites. There is a marked increase in the reproduction of snails in the rainy season that leads to a peak in snail population towards the end of the season. This trend slows down or completely ceases during the dry or cold periods resulting in less snail population in the dry season. This is accompanied by considerable fluctuation of herbage infestation and survivability of metacercariae. The infective stage may survive up to 10 months in the humid tropics and the longevity of metacercariae have been reported to vary from a few weeks to 3-4 months in relatively hot and dry climate . The ultimate determinant of epidemiology of parasitic disease is the rate of egg production by the adult flukes which subsequently influences the degree of pasture contamination. In addition, the grazing habits and management of the animals may significantly influence the epidemiology of liver fluke infection.

Conclusion

Present study revealed that incidence of fasciolosis is low in semi intensive system of rearing compared to free range system. This may be due to practice of regular deworming and better system of management in farms. In free range condition, there is easy access to metacercarial infestation in natural grazing area of forest. The seroprevalance of fasciolosis in mithun was found to be 19.23%. The low prevalence may be due to peculiar geography of north eastern hilly region and climatic condition prevailing in this area.

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References

1. Chakraborty A. 2001. Pathology of *Fasciola gigantica* infection in captive wild herbivores with special reference to SEM and EDAX study. Ind. Vet. J.78: 43- 47.
2. Chamuah JK. 2005. Studies on some aspects of parasites of mithun (*Bos frontalis*). M.V.Sc. Thesis submitted to Assam Agricultural University, Khanapara, and Guwahati-22.
3. Fagbemi BO and Obarisiagbon IO. 1990. Comparative evaluation of the enzyme linked immunosorbent assay (ELISA) in the diagnosis of natural *Fasciola gigantica* infection in cattle. Vet. Quart. 12: 35-38.
4. Farrell CJ, Shen DT, Wescott RB and Lang BZ. 1981. An enzyme-linked immunosorbent assay for diagnosis of *Fasciola hepatica* infection in cattle. Am. J. Vet. Res.42: 237-240.
5. Garg R, Yadav C, Kumar R, Banerjee P, Vatsya S and Godara R.2009. The epidemiology of fasciolosis in ruminants in different geo-climatic regions of north India. Trop. Anim. Health. Pro. 41: 1695-1700.
6. Hillyer GV, Sanchez Z and de Leon D. 1985. Immunodiagnosis of bovine fascioliasis by enzyme-linked immunosorbent assay ELISA and immunoprecipitation methods. J. Parasitol. 71: 449-454.
7. Rajkhowa C and Verma ND. 1993. Observation of parasitic infestation. In: Annual Report of ICAR, 1991-92 and 1992-93. National Research Centre on Mithun. (NRCM). Jharnapani, Medziphema, Nagaland-16.
8. Rajkhowa S and Bujarbaruah KM. 1999-2000. Incidence of intestinal parasitism in different strains of mithun. In: NRCM Annual Report of ICAR, 1999-2000. Jharnapani, Medziphema, Nagaland-16.
9. Rajkhowa S, Bujarbaruah KM, Rajkhowa C and Kapenlo T. 2005. Incidence of intestinal parasitism in mithun (*Bos frontalis*). J.Vet. Parasitol. 19: 39-41.
10. Raina OK, Yadav SC, Sriveny D and Gupta SC. 2006. Immuno-diagnosis of bubaline fasciolosis with *Fasciola gigantica* cathepsin-L and recombinant cathepsin L 1-D proteases. Vet. Parasitol. 98: 145-51.
11. Rajkhowa S, Rajkhowa C and Bujarbaruah KM. 2003. Diseases of mithun (*Bos frontalis*) -A Review. Veterinary Bulletin. 73: 1R-6R.
12. Santiago N and Hillyer GV. 1988. Antibody profiles by EITB and ELISA of cattle and sheep infected with *Fasciola hepatica*. J. Parasitol. 74: 810
13. Sriveny D, Raina OK, Yadav SC, Chandra D, Jayraw AK, Singh M, Velusamy R and Singh BP. 2005. Cathepsin L cysteine proteinase in the diagnosis of bovine *Fasciola gigantica* infection. Vet. Parasitol. 135: 25-31.
14. Tandon V, Kar PK, Das B, Sharma B and Dorjee J. 2005. Preliminary survey of gastrointestinal helminth infection in herbivores livestock of mountainous regions of Bhutan and Arunachal Pradesh. Zoos. Print. J. **20**: 1867-1868.
15. Varghese A, Raina OK, Nagar G, Garg R, Banerjee PS, Maharana BR and Kollannur JD. 2012. Development of cathepsin-L cysteine proteinase based Dot-enzyme-linked immunosorbent assay for the diagnosis of *Fasciola gigantica* infection in buffaloes. Vet. Parasitol. 183: 382-385.
16. Yadav SC, Saini M, Raina OK, Nambi PA, Jadav K and Sriveny D. 2005. *Fasciola gigantica* cathepsin-L cysteine proteinase in the detection of early experimental fasciolosis in ruminants. Parasitol. Res. 97: 527–534.

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