Sero-Diagnosis of *Fasciola gigantica* Infestations in Goats Using Elisa and its Comparison with Fecal Sample Analysis

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Abstract: Fascioliasis, one of the most economically important helminth infestations of goats worldwide can be diagnosed using fecal examination and ELISA of serum samples. The aims of the present study were to compare ELISA and fecal examination for diagnosis of fascioliasis and to estimate the point prevalence of *Fasciola gigantica* in goats from two selected farms. Both serum and fecal samples (n = 100) were collected randomly from two goat farms. Antibodies against the f2 antigen of Fasciola were detected using ELISA. Fecal samples were examined using the modified McMaster technique. Among 100 goats, 38 serum samples showed the presence of antibody against fasciola and 21 of these had fasciola eggs. Overall, 27% goats were infested with fascioliasis with varying degrees of severity. The 28 sero-positive goats were retested for the presence of eggs and antibody 30 days after treatment with broad-spectrum anthelmintics. No eggs were found in the fecal samples but 24 goats remained sero-positive at varying degrees according to ELISA. The anthelmintics presumably destroyed the existing eggs and larvae but the antibody was still present and only detectable using ELISA. ELISA therefore offers a suitable technique for the rapid, specific and accurate detection of fascioliasis in goats.

Key words: Antibody, egg, ELISA, fascioliasis, goat, Bangladesh

INTRODUCTION

Goats are the second most important livestock in Bangladesh after cattle but their production is hindered by parasitic infestation. The agro-ecological and geo-climatic conditions of Bangladesh favor the high prevalence of parasitic infestation (Howlader *et al.*, 1994). The most important parasitic disease of ruminants throughout Bangladesh, fascioliasis of goats (Islam, 2002), causes vast economic losses in the form of retarded growth and mortality due to liver damage as well as reduced milk and meat productions. This disease is considered to be a major constraint to the development of goat farms in Bangladesh (Nooruddin and Islam, 1996). *Fasciola gigantica* is the only species of liver fluke affecting ruminants of Bangladesh due to the local availability of its two intermediate hosts, namely *Lymnaea rufescens* and *L. acuminata.*

Detection of parasitic eggs in feces by fecal sample examination is routinely practiced as a tool for diagnosis of fascioliasis in Bangladesh. This method is ineffective, however, particularly for early diagnosis as the prepatent period of the fluke is approximately 8-12 weeks and the parasite takes at least 10-15 weeks to attain sexual maturity and to release eggs into the feces. Moreover, in many infections, the fluke eggs are often not found in the feces, even after multiple fecal examinations. If they are found, the number of Eggs Per Gram (EPG) of feces is almost never proportionate to the number of adult worms present and provides no estimate of infection severity (Anderson *et al.*, 1999). Furthermore, Fasciola and Paramphistomum eggs have very similar morphologies which make them difficult to differentiate. An alternative and effective method for detection of fascioliasis is greatly needed.

Immunodiagnosis is an important procedure for the confirmation of fascioliasis and involves the analysis of antibody responses to fluke antigens as well as the detection of circulating antigens using defined sera and monoclonal antibodies (Spithill *et al.*, 1999). Serological

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diagnosis using indirect Enzyme-Linked Immunosorbent Assay (ELISA) can determine the presence of antibodies to the parasite from the 3rd week after infection and this technique has been used for epidemiological studies of *F. hepatica* (Ibarra *et al.*, 1998). In contrast to numerous studies on *F. hepatica*, little research has been reported on *F. gigantica*, particularly for diagnosis. Early *F. gigantica* infestation may be detected easily and accurately through the detection of specific antibodies in the sera of infected goats. The present study was undertaken to investigate the possibility of using ELISA as a tool for fast, specific and accurate diagnosis of fascioliasis in goats infested with *F. gigantica* in Bangladesh.

MATERIALS AND METHODS

Experimental design: The research work was conducted in the Animal Health Research Division of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh. Researchers examined 50 goats each from the BLRI Goat Farm (BLRIGF) and the KOICA-BLRI (Korea International Cooperation Agency-BLRI) Co-operative Goat Farm (KBCGF) with the intention of identifying eggs, especially of *Fasciola* sp. and other helminthes, in the feces and antibody levels against *Fasciola* sp. in the serum. Fecal samples were collected from all goats and were examined using the modified McMaster technique. Serum levels of antibody specific for *Fasciola* sp. were measured using ELISA. After the primary investigation, KBCGF goats received oral administration of broad spectrum anthelmintics, Levex® containing Livamizole HCl 600 mg and Triclabendazole 900 mg and the feces and sera from 28 sero-positive goats were retested using the modified McMaster technique and ELISA, respectively.

Fecal sample examination: Several methods are available for preparing feces for microscopic examination aimed at detecting the presence of eggs or larvae (Troncy, 1989). Individual egg counts for parasites were carried out using a modified McMaster technique (Thienpont *et al.*, 1986). Fresh feces were preferably collected directly from the rectum so that the identity of the animal could be established. Duplicate slides were prepared for each sample. In the examination, freshly prepared slides were observed under 10x magnifications for best identification. The severity of parasitic infestation was categorized into four classes: severe (>600 EPG), moderate (300-600 EPG), mild (<300 EPG) and no infestation (0 EPG). Similarly, a scoring system was applied to classify the severity of fasciola infestation.

Fasciola antibody detection by ELISA: Serum antibody specific to the fasciola f2 antigen was detected using an ELISA kit according to the manufacturer's instructions (Fasciola ELISA kit, Institute Pourquier, France). Briefly, undiluted duplicate serum samples were added to the pre-coated ELISA plates and incubated for 1 h at 37°C. After washing, antibody against fasciola was detected with peroxidase-labeled anti-bovine IgG conjugate (1:100 dilution). Finally, TMB substrate was used for color development and the reaction was stopped with 0.5M H_2SO_4. Optical densities were read at 450 nm using an ELISA reader. The validity of the test results was assessed based on criteria described in the kit instructions.

Following the validation values, the status of the tested serum was classified as: strong infestation, S/P600>100% (>50% infestation, +++); medium infestation, S/P600≤100% and S/P150≥100% (between 20 and 50% infestation, ++); low infestation, S/P between 50 and 100% (<20% infestation, +) and no infestation, S/P150≤50% (-).

Statistical analysis: Data obtained from the ELISA and fecal egg count was analyzed with Microsoft Excel program.

RESULTS AND DISCUSSION

Fecal and serum samples were collected randomly from 50 goats each from the BLRIGF and the KBCGF. Fecal samples were examined to detect fasciola eggs using modified McMaster technique and levels of antibody against fasciola f2 antigen were measured using ELISA in corresponding serum samples.

From fecal sample examinations, eggs of fasciola and some other gastro-intestinal parasites of goats like paramphistomum, stomach worm, hook worm, trichuris and haemonchus were detected. The point prevalences of paramphistomum, stomach worm, fasciola and mixed infection were found to be 20, 28, 16 and 18% in the BLRIGF and 14, 22, 38 and 12% in the KBCGF, respectively (Fig. 1). About 18 and 14% of goats were free from any infestation in the BLRIGF and KBCGF, respectively. Overall, 27% goats were infested with fasciola. The stomach worm infestation rate (28%) was higher than that of fascioliasis (16%) at the BLRIGF but fascioliasis (38%) was the most common infestation at the KBCGF.

At the BLRIGF, 16, 38 and 12% of goats were mildly, moderately and severely infested with parasites and 10, 4 and 2% of goats had mild, moderate and severe fasciola infestation, respectively based on fecal egg counts
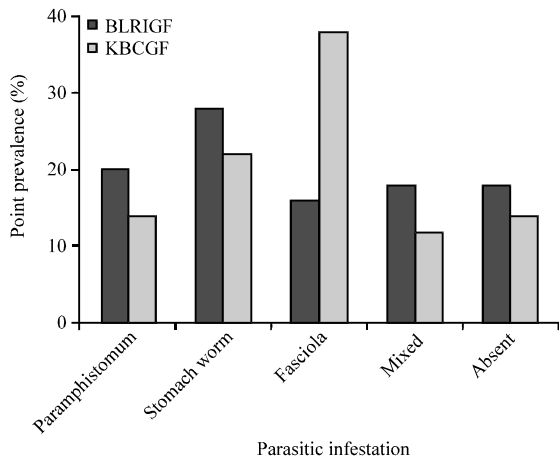
Fig. 1: Point prevalence of parasitic infestations in goats. Parasitic infestations were determined in 100 goats by direct smear egg count Method at the BLRI and KBC goat farms
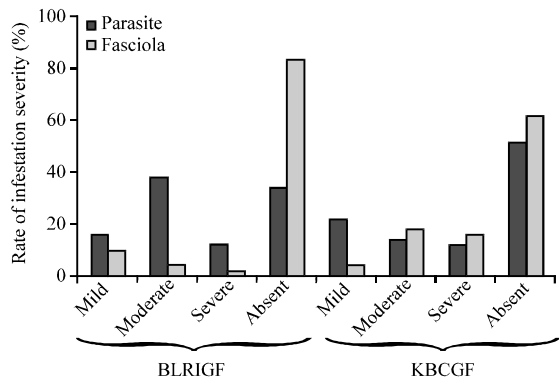

Fig. 2: Rates of parasitic infestation severity in goats. Parasitic infestation severity was designated as mild, moderate, severe or absent according to fecal egg count in goats at the BLRI and KBC goat farms

(Fig. 2). At the KBCGF, 22, 14 and 12% of goats were mildly, moderately and severely infested with parasites and 4, 18 and 16% of goats had mild, moderate and severe fasciola infestation, respectively based on fecal egg counts.

In contrast to fecal egg counts, ELISA detected mild infection at BLRIGF in 12% and moderate infection in 2% of goats. No severely infected sero-positive goats were detected at BLRIGF (Fig. 3). At KBCGF, 8, 18 and 36% of goats had mild, moderate and severe levels of antibody as detected by ELISA (Fig. 4). Between both farms, 38% of goats were sero-positive for fasciola.

Feces and serum samples from 28 sero-positive goats from KBCGF were retested 30 days after treatment with
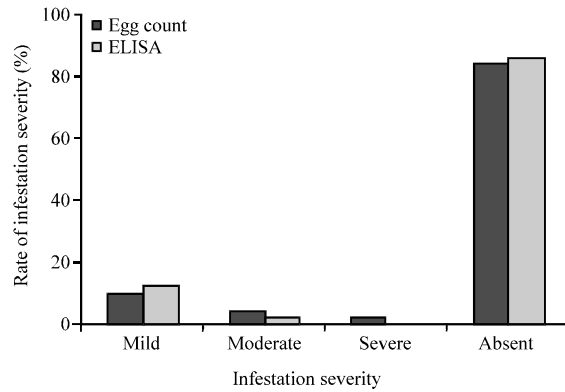

Fig. 3: Comparison on rates of fasciola infestation severity as measured by egg count and ELISA in goats at the BLRI goat farm. Fifty goats were examined for fascioliasis by fecal egg count and anti-fasciola antibody in sera by ELISA. Fasciola infestation severity was designated as mild, moderate, severe or absent according to fecal egg count and level of anti-fasciola antibody in goats
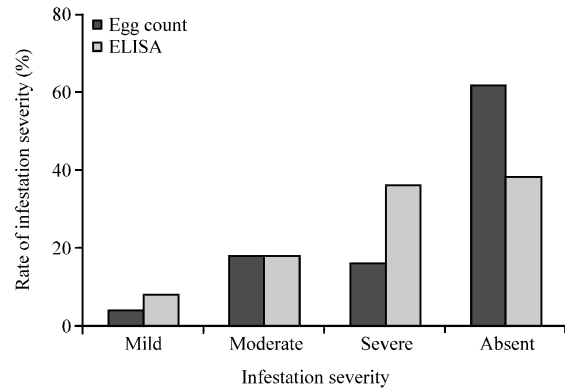

Fig. 4: Comparison on rates of fasciola infestation severity as measured by egg count and ELISA in goats at the KBC goat farm. Fifty goats were examined for fascioliasis by fecal egg count and anti-fasciola antibody in sera by ELISA. Fasciola infestation severity was designated as mild, moderate, severe or absent according to fecal egg count and level of anti-fasciola antibody in goats

broad spectrum anthelmintics. No parasitic eggs were found on fecal examination but ELISA revealed mild, moderate and severe infestation in 21.42, 10.71 and 53.57% of the goats after anthelmintics compared to 10.71, 28.57 and 60.71% of the goats before anthelmintics, respectively (Fig. 5). Among the 28 sero-positive goats 30 days after treatment, antibody levels decreased in 13, remained the same in 11 and increased in four goats.
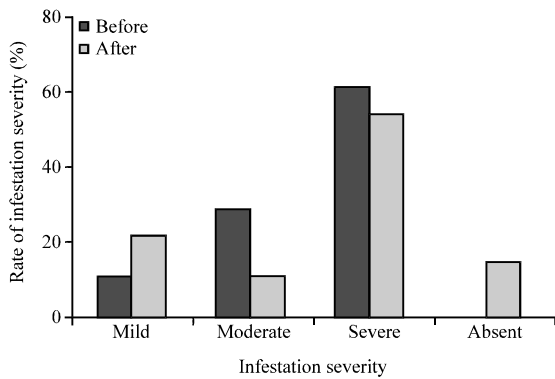
Fig. 5: Comparison on rates of fasciola infestation severity as detected by ELISA before and after giving anthelmintic in goats at the KBC goat farm. Twenty eight sero-positive goats were retested by ELISA 30 days after treatment with broad spectrum anthelmintics. Fasciola infestation severity was designated as mild, moderate, severe or absent according to the level of anti-fasciola antibody in goats

Researchers compared ELISA and fecal examination for the diagnosis of fascioliasis and estimated the point prevalences of *F. gigantica* in 100 goats from two selected farms.

Fecal sample examination revealed fasciola and some other gastro-intestinal parasites including paramphistomum, stomach worm, hook worm, trichuris and haemonchus. Out of 100 goats, 16 were free from any infestation observed by fecal examination. Overall, 27% of goats were infested with fasciola. This prevalence rate was much higher than that described by Munguia-Xochihua *et al.* (2007) who found a prevalence of 10.2% for the Guaymas and 12.8% for the Cajeme municipality in Mexico using a sedimentation test. The increased prevalence rate could be associated with the available environmental conditions for the fluke life cycle (Hurtrez-Bousses *et al.*, 2001). The stomach worm infestation rate (28%) was higher than that of fascioliasis (16%) at BLRIGF but fascioliasis (38%) was more prevalent than other infestations at the KBCGF.

The severity of infestation was probably higher at the KBC goat farm because the farm was newly established with recently purchased goats. The BLRI goat farm was well established and routinely treated their goats with anthelmintics.

The rates of fasciola infestation severity at both farms were measured both by egg count in feces and antibody level in serum using ELISA. At the BLRI goat farm, both egg count and ELISA yielded a similar infestation rate but some variations were noted at the

KBC goat farm. Taking both farms together, 38% of goats were found to be sero-positive against fasciola, higher than the levels of 17.8 and 13.16% reported in the Guaymas and Cajeme municipalities in Mexico, respectively. Based on egg count, 27% of goats were infested with fasciola while ELISA detected fasciola antibody in 38% of the animals. The detection of a higher proportion of positive animals using serological analysis than using the coprological test has been previously reported (Munguia-Xochihua *et al.*, 2007). Only mild infestation was detected both using egg count and ELISA in the BLRIGF but moderate to severe infestation was detected in the KBCGF using both egg count and ELISA. Wang *et al.* (1987) described a moderate prevalence according to the sedimentation test compared to a high proportion of antibody-positive goats using indirect ELISA.

Between the BLRI and KBC goat farms, 12 out of 100 goats were free from both parasitic and fasciola infestations based on negative fecal sample examination and the absence of an antibody response against fasciola by ELISA. Both tests provided similar results. Fecal sample examination identified 16 goats infested with only paramphistomum and one case with hemonchus. Among the 17 paramphistomum-infected goats, three showed antibody responses against fasciola. Twenty five goats were infested only with stomach worm, 15 had a mixed infestation (paramphistomum, stomach worm, trichuris and hookworm) and antibody response against fasciola was also detected in ten of these goats using ELISA. On the other hand, 27 goats were infested with fasciola either alone or in combination with other parasites and 21 of these had an antibody response. Based on these findings, researchers conclude that the antibody responses were against fasciola only, not against paramphistomum or stomach worm. Antibody responses against fasciola were detected using ELISA in four goats which were free from any infestation by fecal examination. Since, the eggs of paramphistomes are similar to that of fasciola in appearance, there might have been some error in differentiating the eggs in the feces but the antibody detected in ELISA was specific for fasciola. Eggs of Fasciola may also be missed in fecal examination if an animal is in the early stages of infestation.

Overall, the prevalence of positive serum antibodies and the infestation rate of Fasciola obtained from fecal examination were similar at the BLRIGF and more variable at the KBCGF (Fig. 3 and 4). These findings indicate that detection of antibody against Fasciola antigen using ELISA is effective and reliable and can be used to accurately identify fascioliasis infection. Because ELISA detects anti-fasciola antibody from the 2nd week

post-infection (Sinclair and Wassall, 1988) it can also provide an earlier diagnosis than the coprological test which is only effective when the parasite is 8-10 weeks old. Ibarra *et al.* (1998) have also described the suitability of serological techniques for the diagnosis of fascioliasis in large populations.

After goats were treated with broad spectrum anthelmintics, no parasitic eggs were found in fecal examination while the ELISA test still detected antibody against fasciola antigen. Out of 28 goats, antibody level decreased in 13, remained the same in 11 and increased in four after treatment. Researchers expected anthelmintics to lower the infestation rate, explaining the absence of eggs in the feces. As the antibody level in serum declines steadily after it reaches to its peak, yet still positive, decrease in antibody level in these 13 goats might be due to this fact. Thus, it can be assumed that the anthelmintics treatment destroyed the existing eggs and larvae in intestine but the antibody was still existed which can only be detected by ELISA. This observation indicates that detection of specific antibody using ELISA was more accurate for diagnosing fasciola compared to the fecal smear method. At the same time, the presence of antibodies does not always indicate active fasciola infection inside the host (Munguia-Xochihua *et al.*, 2007) suggesting that supplementary tests are needed to distinguish active infection after treatment.

Though the BLRIGF is established and goats are regularly treated with anthelmintics, there are some wetland areas adjacent to the farm that ensure the availability of an intermediate host (snail) and favor fasciola infestation at moderate to low levels during grazing. In contrast, the KBCGF is newly established with goats recently purchased from the market. Most farmers rear their goats in free ranging conditions and do not practice routine deworming prior to sale. Accordingly, the goats might become infested with parasites when they graze in the low lying areas that may contain snails. These differences might explain the higher level of fasciola infestation observed at the KBCGF compared to that of the BLRIGF. Low lying or irrigated areas support the presence of fascioliasis; infection prevalence is known to be higher in irrigated areas compared to that of non-irrigated lands associated with the dry season and increased altitude (Michael, 2004; Yilma and Malone, 1998).

CONCLUSION

In this study, ELISA and fecal examination were compared for the diagnosis of fascioliasis and estimated the point prevalence of *F. gigantica* in 100 goats from two selected farms. The prevalence of positive serum antibodies and the infestation rate of fasciola obtained from fecal examination were similar at the BLRIGF. This finding indicates that detection of antibody against Fasciola antigen using ELISA is effective and reliable and can be used to accurately identify fascioliasis infection. Researchers observed moderate prevalence of *F. gigantica* infestation in the goats of two selected farms in Bangladesh. ELISA was more suitable than fecal examination for the rapid, specific, accurate and early detection of fascioliasis in goats.

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