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Research Article

APPLICATION OF DIFFERENT COPROLOGICAL METHODS IN DIAGNOSING OVINE SCHISTOSOMOSIS

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ABSTRACT

Three laboratory methods viz. formol-ether, alkaline digestion and hatching methods were applied on faecal samples from fifty sheep for determining their sensitivity in diagnosing intestinal schistosomosis. Alkaline digestion method proved more sensitive than formol-ether for diagnosing eggs of schistosomosis in sheep. Eight (16%) sheep were positive by alkaline digestion whereas formol-ether method could detect only five (10%) cases. The hatching test, specific for schistosomosis, proved most sensitive by diagnosing 14 (28%) sheep being positive. The statistical analysis revealed a highly significant difference between hatching test and formol-ether ($p < 0.01$) and a highly significant difference between hatching test and alkaline digestion ($p < 0.05$) for detecting schistosomes infection. The present work advocated use of hatching test over egg detection coprological methods for diagnosing intestinal schistosomosis in domestic animals.

Keywords: Sheep, schistosomes, coprological diagnosis.

Along with grazing, sheep is also dependent on water sources like ponds, ditches and *nullahs* for drinking, thereby getting exposed to trematode infections. Among various diseases caused by flukes, schistosomosis is an important fluke infection transmitted by snails albeit with poor diagnosis by routine diagnostic methods, due to lower egg output (Agrawal, 2003). Efforts have been made to diagnose ovine schistosomosis by faecal examination (Chaudhri *et al.*1994; Bedarkar *et al.* 2000; Chaudhri *et al.* 2000; Tamloorkar *et al.* 2002) but scanty work of comparing different coprological diagnostic methods is available in literature. Therefore, in the present study, formol-ether, alkaline digestion and hatching methods were employed on faecal samples of sheep for detection of schistosomosis in and around Jabalpur.

MATERIALS AND METHODS

About 30 gram faeces each from 50 sheep were collected from small animal slaughterhouse of Jabalpur after taking into consideration of their sex and age i.e. young < 1 yr, adult = 1-3 yr and old > 3 yr. The faecal samples were examined for helminthic infection using hatching techniques, formol-ether technique and alkaline digestion method.

a. **Hatching method**: Twenty gram faecal material was diluted in a 500 ml conical flask as per method of Banerjee and Agrawal (1992) and exposed to light for 3 hr with further exposure for half an hour after covering the flask with a black cloth except brim. Ten ml of water from upper surface was poured in a Petri dish and examined under a stereoscopic microscope for detection of miracidia. In case of negative, additional 10 ml of water was examined.

b. **Formol-Ether method**: The formol-ether method was followed as per Vohra and Agawal (2006) using 1 gram of faeces dissolved in 5 ml of 10% formalin. This faecal suspension was filtered through brass mesh sieve (mesh size 50 pores per sq. cm) into 15 ml centrifuge tube. More 10% formalin was added making the volume of suspension up to 10 ml. To this, 2 ml petroleum ether was added and shaken vigorously for 1 minute and left for 15 minutes and later floating debris was removed. A part of the sediment was examined under a compound microscope (40X) but without putting a coverslip. In case of negative, whole sediment was examined.

c. Alkaline digestion: This was done as per method described by Soulsby (1982) with minor modifications. Ten gram of faeces was dissolved uniformly in 100 ml of 0.4 N sodium hydroxide (prepared in 1.7 % sodium chloride) with the help of glass beads. After keeping overnight, it was shaken vigorously and 10 ml solution, representing 1 gm of faeces, was filtered through 3 brass mesh sieves (30, 50 and 80 holes/sq cm). The filtrate was transferred in a glass centrifuge to stand for 10-15 minutes for settling of the eggs. After removing the supernatant, the sediment was diluted to 2 ml with 10% formalin from where 0.5 ml was examined (without putting coverslip) under a microscope (40X). If the first aliquot was negative, remaining sediment was examined. The number of schistosome eggs present in 0.5 ml was multiplied by 4 for calculating epg.

Statistical analysis: The three methods were compared among each other for schistosome diagnosis using McNemar's Chi-square (Binomial Test) test.

RESULTS AND DISCUSSION

Faecal samples of 50 sheep (3 young, 18 adult and 29 old including 9 male and 41 female) were examined during the present study. The result detected schistosome (14, 28%), amphistome (30, 60%), *Fasciola* (6, 12%), nematodes (47, 94%), cestode (3, 6%) and coccidian (12, 24%) eggs/ oocysts. All the faecal samples examined were positive for single or multiple infections of parasitic eggs/ oocysts.

The formol-ether technique detected schistosome eggs in 5 sheep (10%) and all eggs were of *Schistosoma indicum.* Cherian and D'Souza (2009) detected only 4 (0.615%) cases of ovine schistosomosis out of 650 faecal samples examined from 15 districts of Karnataka using formol-ether technique. A higher number of old animals

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(n=4, 13.8%) were positive than adult animals (n=1, 5.5%) while no young animal was found positive for schistosomosis. Our findings are in compliance with those of Agrawal (2012). All positive cases were of female animals.

The alkaline digestion method diagnosed eight animals (16%) positive for schistosome eggs. Out of these, seven were of *S. indicum* and one of *S. spindale*. A higher number of *S. indicum* positive cases than *S. spindale* were also observed in cattle and goats in different districts of Bangladesh by Islam *et al*. (2011). Cherian and D'Souza (2009) also reported majority positive cases of *S. indicum* (98.18%) over *S. spindale* in ovine faecal samples in Karnataka. Vohra and Agrawal (2007) during post mortem studies in sheep reported that *S. indicum* flukes occupy more area of intestinal mesentery than *S. spindale*. They also reported a higher number of eggs of *S. indicum* than *S. spindale* in the intestinal scrapping during post mortem. The egg per gram (EPG) of *Schistosoma* revealed 1.71±0.28 in females with range of 1-3. De Bont *et al*. (1991) also recorded very low faecal egg count in cattle in Sri Lanka, making egg detection method unreliable. It may be due to less egg produced by all fluke in comparison to other helminthes. Also some eggs of schistosomes are not able to cross the mucosa and are encapsulated by host immune system. Out of the total sheep examined, only one male animal (*S. indicum*) and seven female animals (six having *S. indicum* eggs and one with *S. spindale* egg) were found positive. No young animal was positive, however, two adult and six old sheep harboured the infection. These results clearly indicate that infection rate of blood flukes increases with increase in age. De Bont *et al*. (1991) also observed that animals less than two years were less infected (21.3%) than animals older than five years (47.97%). Similar were the findings of Cherian and D'Souza (2009) in cattle and goats at Karnataka. A higher number of alkaline digestion positive cases in comparison to formol-ether method may be attributed to higher amount of faeces taken for examination. However, the difference was statistically non-significant. It is pertinent to mention here that during faecal examination by formol-ether method or alkaline digestion method, no faecal sample was having schistosome with *Fasciola* or faeces positive for all the three fluke eggs i.e. schistosome, *Fasciola* and paramphistomes. The reason may be either different intermediate host or heterologous immunity among them (Agrawal and Southgate, 2000).

Hatching test especially designed for schistosomosis proved superior over the other two diagnositic methods by detecting 14 (28%) cases. The test detects moving miracidium of *Schistosoma* in water but has a limitation that it does not identify schistosome species. A higher efficacy of hatching test was also reported by Bhatia and Rai (1975), Agrawal and Panesar (1987), Banerjee and Agrawal (1992), Gupta (2002) and Cherian and D'Souza (2009) in various animal species. A higher number of females (13) in comparison to the males (1) are found positive by this method. As per the age, infection was absent in young animals, while four adult and ten old sheep were positive for schistosomosis. Higher infection in older animals was also observed by Agrawal (2012). As per the number of miracidia in water, higher number was observed in adult animals in comparison to old animals *i.e*. 6 animals contained one miracidium, 7, 2-3 and only one adult female contained 5 miracidia. The statistical analysis showed a highly significant difference ($p < 0.01$) between hatching test and formol–ether technique and significant different ($p < 0.05$) between hatching test and alkaline digestion method. From the present study, it is concluded that hatching method is the most sensitive parasitological method during ante-mortem for diagnosing schistosomosis.

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