Coprological Diganosis of Ovine Schistosomosis by different laboratory techniques

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Abstract

Schistosomosis, a chronic and wasting illness of livestock has been underestimated and undiagnosed due to various intrinsic factors. The diagnosis of the disease in small ruminants, especially in sheep, has not received due attention, inspite of the striking reports of mortality from many parts of the country. In the present investigation, the efficacy of four different methods *viz.,* Direct Examination, Formal Ether Technique, Alkaline Digestion and Miracidial Hatching Test were evaluated for the detection of ovine schistosomosis. Faecal samples from 650 sheep belonging to 15 districts of Karnataka were screened for schistosome egg or miracidia, out of which 44 (6.76%) samples were positive. Majority of the cases had *Schistosoma indicum* infection (93.1 %) and very few were positive for *S.spindale*. Miracidial Hatching Test was found to be superior in detecting natural ovine schistosomosis, followed by Alkaline Digestion and Formal Ether Technique. Direct examination detected none of the cases as positive.

Keywords: Livestock, Schistosomosis, Laboratory Technique, Coprological Diagnosis, Small Ruminant, Wasting illness.

Introduction

Schistosomosis or Bilharziosis is a disease of both man and animals and is mainly a problem of the tropical and subtropical zones of the world. In livestock it is a chronic and wasting illness, contributing to considerable economic losses. Schistosomosis is now recognized as the fifth major helminthosis of domestic animals in the Indian sub-continent. The species which commonly occur in India are *Schistosoma nasale* and *S.spindale* in cattle, *S.indicum* in equines and sheep and *S.incognitum* in pigs (D'Souza, 2006).

In India, there have been reports of *S.indicum* schistosomosis in sheep from some states including Rajasthan (Vashishta *et al.*, 1981), Haryana (Singh *et al.*, 1984), Karnataka (Chandra *et al.*, 2003) and Madhya Pradesh (Agrawal *et al.*, 2004). The disease seems to be highly prevalent, but is under diagnosed and has not received due attention. The routine methods such as direct smear test, floatation or the sedimentation method are ineffective in detecting schistosome eggs (Agrawal, 1999). In the routine post mortem examinations or in the slaughter houses no attempts are made to diagnose schistosomosis. Hepato-intestinal schistosomosis is diagnosed only in 30% positive cases, while the others go undetected (Agrawal, 1999).

Inspite of the striking reports of mortality due to the disease, little attention has been paid to diagnose schistosomosis in small ruminants, especially in sheep. There is a need for assessment of the coprological methods for their efficacy. Hence, the present study was undertaken to compare the efficacy of four different methods in detecting schistosomosis in sheep *viz.,* Direct examination, Formal Ether Technique, Alkaline Digestion and Miracidial Hatching Test.

Materials and Methods

The faecal samples were collected from sheep belonging to different parts of Karnataka. Faecal samples were collected in total from 650 sheep belonging to 15 districts.

Preliminary direct examination of the faecal samples was made for the detection of parasite ova according to the procedure described by Hendrix *et al.* (2006).

The faecal samples were subjected to Formal Ether Technique (Foreyt, 1997) for the detection of schistosome eggs. 1 g of faeces was mixed with 15 ml of Normal saline and the mixture was strained and centrifuged at 1000 rpm for 2 minutes in 15 ml centrifuge tubes. The washing was done at least twice. The supernatant was discarded and the tubes were allowed to stand for 10 minutes after adding 10ml of

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10% formalin to the sediment. Ether (3ml) was added to the tubes, and the mixture was centrifuged for 2 minutes at 1000 rpm after proper mixing. The debris on the top of the tubes was removed and the rest of the fluid was decanted. A part of the sediment was examined on a microscopic slide under a compound microscope, first under 10X and then under 40X for schistosome eggs. In case of negativity, the whole sediment was examined.

Alkaline Digestion Technique was done for the detection of schistosome eggs as per Vohra and Agrawal (2006). 10 g of faeces was dissolved uniformly in 100 ml of 0.4 N Sodium Hydroxide (prepared in 1.7% Sodium Chloride) and was allowed to settle for over night in a conical flask.10 ml solution (representing 1g of faeces) was filtered through three brass mesh sieves each of 30, 50 and 80 holes per square centimeter. The filtrate was allowed to stand for 10-15 minutes for setting of eggs, in a glass centrifuge tube. After removal of the supernatant, the sediment was diluted to 2 ml with 10 per cent formalin. 0.5 ml was examined (without putting a cover slip) under a microscope (40X). If the first aliquot was negative, then the remaining sediment was also examined (0.5 ml each). EPG (Eggs per Gram) was calculated as Number of fluke eggs in 0.5 ml x 4.

Miracidial Hatching Test (Vohra and Agrawal, 2006) was done to detect the presence of miracidia in the fresh samples. The faecal sample was suspended in an equal amount of physiological saline and was washed, sieved and concentrated atleast thrice. The sediment was diluted with distilled water or boiled and cooled tap water in a conical flask. The conical flask was exposed to a source of bright light for 4 hours. The flask was then covered, except at the brim with a black cloth and was further exposed to light for half an hour. Ten ml of water from the upper surface taken in a Petri dish was examined under stereoscopic microscope for miracidia. If the first sample was negative, an additional 10 ml of water was checked.

Results

The screening of 650 faecal samples of sheep from 15 districts of Karnataka revealed that 44 samples (6.76 %) were positive for schistosomosis. Two species of schistosomes were observed *viz., S.indicum* and *S.spindale*. Majority of the positive cases had *S.indicum* infection (93.18%). Only three cases were positive for *S.spindale* infection.

Miracidial Hatching Test alone detected the most number of positive cases (2.61%). Alkaline Digestion and Formal Ether Test alone could detect 7 (1.07%) and 4 (0.615%) cases respectively. Nine samples were positive by all the three tests. Direct examination could not detect any of the samples as positive. The details are given in Table 1.

The mean EPG as detected by the Alkaline Digestion Method was highest for the samples from Raichur, with a mean of 6.4 ± 3.577, and an EPG range of 4-12. The samples from Bellary had a similar EPG (6.28 ±3.147) with the same range. The EPGs of the samples from Bidar and Bagalkot were in the range of 4-8. The lowest EPG values were observed for the samples from Dhangur and Suthatti (4. 0).

Discussion

Agrawal and Panesar (1987) recommended the use of Hatching Test or Sieving Test in the field for the diagnosis of porcine schistosomosis. Agrawal (1999) opined that egg detection methods should be replaced by the Hatching Test for the diagnosis of schistosomosis. Vohra and Agrawal (2006) advocated the use of Hatching Test over egg detection methods for diagnosing natural hepatic schistosomosis in domestic animals, based on their studies on caprine schistosomosis.

In the present study, four different coprological methods were compared for the detection of ovine schistosomosis *viz.,* Direct examination, Formal Ether Test, Alkaline Digestion and Miracidial Hatching Test. The results were varied, *i.e.* a particular sample was detected positive by more than a single test. Miracidial Hatching Test was found to be superior as it detected a total of 32 (72.7%) out of the 44 positive samples. Alkaline Digestion could detect 19 positive cases (43.1%) and Formal Ether Test 18 cases (40.9%), in total. Direct examination failed to detect any of the samples as positive. The present study concluded that Miracidial Hatching Test was most suitable for the coprological diagnosis of natural ovine schistosomosis.

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Table-1. Detection of ovine Schistosomosis by four different methods.

Test	Number of samples screened	Number ofpositive samples	Percentage positive
MHT	650	17	2.61
ALK.DIG	650	7	1.07
FET	650	4	0.61
FET and MHT	650	4	0.61
ALK. DIG and MHT	650	2	0.30
FET and ALK.DIG	650	1	0.15
FET, ALK.DIG and MHT	650	9	1.38
Direct Microscopic Examination	650	0	0
Total	650	44	6.76

MHT: Miracidial Hatching Test , FET: Formal Ether Test, ALK.DIG: Alkaline Digestion

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