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IMMUNODIAGNOSIS OF PARAMPHISTOMOSIS IN SHEEP

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Paramphistomosis in sheep is a serious disease in the Kashmir Valley and the neighbouring countries due to the economic losses associated with retardation of growth and sometimes death. Paramphistomes of livestock are abundant in the tropical and subtropical countries. The disease causes high morbidity and mortality resulting in great economic losses through reduced productivity (Mukherjee and Chauhan, 1965). The adult worms inhabiting the rumen have low pathogenicity, while the migrating immature stages cause severe pathological disturbances (Horak, 1971). The eggs can’t be detected in faeces by routine parasitological investigation during the immature stages of the parasites. Therefore, immunological diagnosis would be of considerable significance during the non egg producing period.

Materials and Methods

Antigen: - Adult *P. cervi* ranging from 9.25 to 10.35 mm in length were collected from the rumen of infected sheep. Adult *Fasciola* sp. worms were also collected from the liver of naturally infected sheep at the abattoir. The worms were washed with physiological saline and stored at -20°C until use. Somatic antigen of adult flukes was prepared by the following technique of Yadav and Gupta (1966).

The paramphistome flukes were blotted over sterile filter paper and were freeze dried. Then 1gm of fluke was immersed in 5ml of PBS and then frozen and centrifuged at 5000 rpm for 30 min at 4°C. The supernatant of the flukes was used as antigen. Finally 0.1% of thiomersol was added to each antigen and stored at -20°C.

Hyperimmune sera: - Hyperimmune sera was raised in two rabbits. Three injections were given subcutaneously with equal quantity of antigen having protein concentration 2.5 mg/ml and Freund’s Complete Adjuvant at an interval of five days. Rabbits were bled after 7 days of last injection. The serum was separated and kept at -20°C for further use.

Agar gel diffusion test: - Immunodiffusion test was carried out as per method described by Ouchterlony (1958). 1% agar solution (Difco Agar) was prepared in 100ml barbitone buffer having pH
9.6. The agar gel solution obtained was poured onto slides and then the slides were kept at room temperature so that gel solidifies. With the help of blowpipe wells (holes) were made in the agar gel plate, with one central well and two peripheral wells at a distance of 3 mm between them. The central well was charged with antibody and the peripheral wells with antigens. The Petri-dish containing the gel slides were kept in a moist chamber at room temperature for four days and the reaction was observed daily. To rule out the possibility of cross reactions with other flukes, the gel diffusion test was also performed with the Fasciola antigen and the positive reference sera against paramphistome antigen.

Results and Discussion

In general, three precipitin lines were observed between the antigen and antiserum wells. No reaction was observed between Fasciola antigens. When the gel and antigen were stored in a refrigerator for 3 months and used for agar gel diffusion test (AGDT), clear reactions could still be observed and there was no fungal development. Serodiagnosis of the disease seems to be the only alternate to coproscopic detection of the fluke eggs. The double immunodiffusion (DID) test has been found suitable test of choice for serodiagnosis of flukes (Hillyer and Dewell, 1981; Gomez et al., 1984; Gorman et al., 1993 and Mousa, 1994) due to their specificity and satisfactory sensitivity.

However, suitability and reliability of this test in this host – parasite system, considerably depends upon the quality of the P. cervi antigen. The antigen used should be of high quality in respect of sensitivity and specificity. In DID test, after 22 days of antigen inoculation, three precipitin lines were detected after 24hrs of incubation using Paramphistomum cervi antigen. Bratanov et al. (1981) observed 6-precipitation lines by Paramphistomum sps.

References

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Mukherjee, R P., Chauhan, B S., (1965) J Zoo Soc Ind 17 150-225
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Sheep (*Ovis aries*) is infected with a variety of gastrointestinal helminths, of which notably *Fasciola hepatica* and *Paramphistomum cervi* are prominent and pathogenic causing a lot of morbidity and mortality. Immature stages of *P. cervi* are highly pathogenic but the routine parasitological diagnosis is difficult. Under the circumstances immunological test resorted to the present study, is aimed at investigating the relative immunodiagnostic reliability and sensitivity of the Ouchterlony gel diffusion test and ELISA in paramphistomiasis. Sheep blood was collected at the local abattoir naturally infected with *Paramphistomum cervi*. Using somatic antigen of whole worms derived from *P. cervi* was prepared by homogenisation, sonication and centrifugation at 10,000 rpm for 20 minutes at 4°C. Rabbits were immunized with the antigen mixed with Freund's complete adjuvant (1:1 ratio) for raising hyperimmune sera. Blood was collected at regular intervals by puncturing ear vein of the rabbit. The naturally infected sheep sera were also collected from the slaughter houses. The Ouchterlony test and ELISA were found to be positive as early as 2-4 weeks post-infection. By gel diffusion test, two precipitation bands were observed, and at 6th-8th weeks of post infection by ELISA. An indirect ELISA standardized for detection of anti-Paramphistomum antibodies using antigen concentration of 2μg ml. Indirect ELISA revealed antibody titre as high as 1: 12,800 in rabbit sera whereas in sheep sera 1: 6400.

**Key words:** *P. cervi*, Indirect ELISA, Freund's Complete Adjuvant (FCA), immunodiagnosis, sheep, rabbit.
Seasonal Incidence of *Paramphistomum cervi* in Sheep in Kashmir Valley

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A total of 512 sheep were examined during the period January to December 2004 to establish seasonal trends of infection and their relationship to the climatic factors caused by *P. cervi* in sheep. The sheep were chosen from different areas of valley, but the parasite *P. cervi* was found throughout the year with prevalence rate of 42%. Prevalence increased in the rainy and Post-monsoon seasons and decreased slightly in the winter and summer. Adult parasites were found predominantly in the oesophageal end, reticular end and papillers of the rumen. Immature parasites were predominant in dorsal, ventral saes of the rumen and duodenum.

**Key words:** *Paramphistomum cervi*, Prevalence, Rumen and Sheep