SERODIAGNOSIS OF HAEMONCHOSIS IN SMALL RUMINANTS

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ABSTRACT

In the present study an attempt was made to find out the incidence of haemonchosis in ovine and caprine through the DID and IHA tests. The results of the study showed higher incidence in sheep (72.5%) than goats (56%).The age wise incidence in sheep was recorded as 79.16% between 1 to 3 years of age whereas in case of goat it was 70.3% in the same age group. In sheep 16.7% animals suffered from mild form while for moderate and high forms the percentages recorded were 70.8 and 12.5. In goats only mild (33.3%) and moderate forms (66.7%) were recorded. Seasonal incidence showed that the parasitism was the highest in July/August (47.7%) while it was the least in September/October (3.1%). The sensitivity of faecal culture was recorded as 42 % while for DID and IHA it was 47 % and 56 %, respectively. The specifity of faecal culture was recorded as 89.5 % while for DID and IHA it was 94.7 % and 98.2 %.

Keywords: Haemonchosis, DID, IHA, Faecal culture.

INTRODUCTION

Livestock is an important and valuable asset of a country. The importance of small ruminants, which produce items of great demand, can never be ignored. The growth rate of sheep in Punjab is –0.84% while for goats it is +3.95%. Out of total mutton production the contribution of sheep is 53 thousand tons while goats contribute 178 thousand tons. In addition to mutton and wool, 65 thousand tons of edible offal, 1.196 million no's of skin and 5.55 thousand tons of horns/hoofs are being produced by the small ruminants. (MINFAL, 2006).

Almost 90% of total population of small ruminants is suffering from different parasitic infections (Mumtaz, 1998). The most common infection is parasitism by roundworms that causes great economic loss of meat, wool; skins and other consumable items (Hashmi, 1989).The blood sucking abomasal nematode Haemonchus are responsible for extensive losses in sheep and goats (Sharma *et al*., 1997). The history and clinical signs are sufficient for diagnosis especially if supported by faecal worm egg counts. Although in sheep which have undergone self cure or are in terminal stage of the disease, the bulk of worm burden may have been lost from the abomasums. Moreover, the routine coprological tests fail to detect patent low level infections which incur the risk of having a reservoir capable of perpetuating infections. (Urquhart *et al*., 1987). Since the response of gut to Haemonchosis is immunologically mediated (Parkhouse *et al*.1987), the present study was carried out to assess the immunodiagnostic efficacy of haemagglutination inhibition and double immunodiffusion tests along with routine faecal culture test for the diagnosis of Haemonchosis in order to record the incidence of Haemonchosis in different age groups of sheep and goat and during different seasons of the year.

It is anticipated that the results of this research will aid in detection of sub-clinical Haemonchosis that will in turn aid in timely treatment of this parasitism.

MATERIALS AND METHODS

Collection and categorization of samples: The reference population of sheep and goats was categorized into three age groups namely; 6months to 1 year, 1 to 3 years and above 3 years. Samples of faeces, venous blood and abomasae were collected from 100 diseased sheep and goats brought to Lahore, slaughter house on the basis of postmortem examination of abomasae. Fifty seven true negatives faeces, venous blood samples were collected from non diseased sheep and goats declared Haemonchosis free on necropsy examination.

Three hundred milliliter of blood sample was collected from worm free sheep in glass container having 30 mg of EDTA for preparation of tanned sensitized R.B.C s.(Hooda *et al*.,1999).

FAECAL EXAMINATION

Faecal Egg Counts: Faecal egg count was performed by McMaster technique (Gordon and Whitlock, 1939) and the species was later confirmed by identification of third stage larvae obtained through faecal culture.

Culture and Maintenance of Worms: The faecal culture was performed at 25^0C for 10 days (Litchenfels *et al*., 1986) and the larvae were stored at 4^0C in refrigerator. Pure culture of larvae was obtained by dissecting adult females. The adult females are cut into half on a sheet of glass and uteri extruded. The uteri were picked up with forceps and transferred to a mortar. The uteri were chopped, a little coarse silver sand was added and mixture was lightly ground. The mortar was filled

with water and well stirred. Water and sand collected in a separate receptacle. The suspension was poured through the mesh with aperture of 0.15mm and filtrate collected. The eggs were recovered after sedimentation and resuspended in 2% copper sulfate. After sedimentation the suspension is mixed with silver sand to a moist consistency. The material is placed in a Petri dish and incubated for 21 days at 27^0 C. After incubation the cultures were stored at 4^0C. (Gomez *et al*., 2000).

Examination and Identification: Permanent mounts were prepared and examined at 60X. The adults and larvae were identified according to keys and morphological characteristics. (Soulsby, 1988).

SEROLOGICAL TECHNIQUES

Preparation of crude antigen: Adult worms and larvae obtained after faecal culture were homogenized and lyophilized for the preparation of crude antigen according to the method described by Yoshihara *et al*., 1979. The antigen was stored at - 20^0C for use in serological tests.

Preparation of Hyper immune Serum: Experimental rabbits were injected with crude antigen. The rabbits were bled aseptically after 16 days for the collection of hyper immune serum (Kagan *et al*., 1958).

Preparation of sera from blood samples: The blood samples were centrifuged at 3000 rpm for 30 min for the collection of sera which were later stored at -20^0C for use in serological tests.

Double Immunodiffusion test: Agar gel plates were prepared and checked for sterility. Wells were made and filled with antigen, hyper immune serum and test sera according to the protocol. The charged plates were incubated for 48 hours at 37^0C as suggested by (Kagan *et al*., 1958, Yoshihara *et al*.,1979.).

Indirect haemagglutination test: Tannic acid sensitized R.B.C s of sheep were prepared from 300 ml healthy blood . Two fold dilution of sera were prepared and mixed with tanned sensitized R.B.C s of sheep in "V" plates. The plates were incubated at 37^0C for 16 hours. Negative and positive control wells were also made and results noted. (Tysker *et al*. 2000).

RESULTS AND DISCUSSION

Identification of parasites: The parasites were identified by knob like vulvular process and barber's pole appearance in female and wedge shaped spicules along with characteristic bursa in males.(Fig.1).

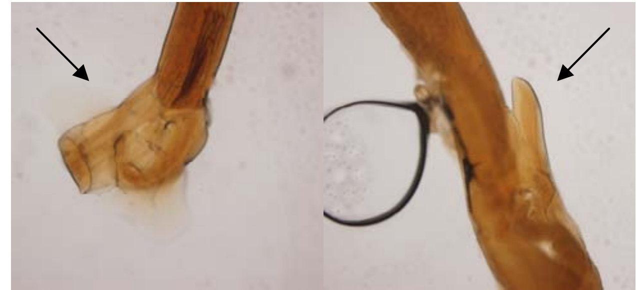
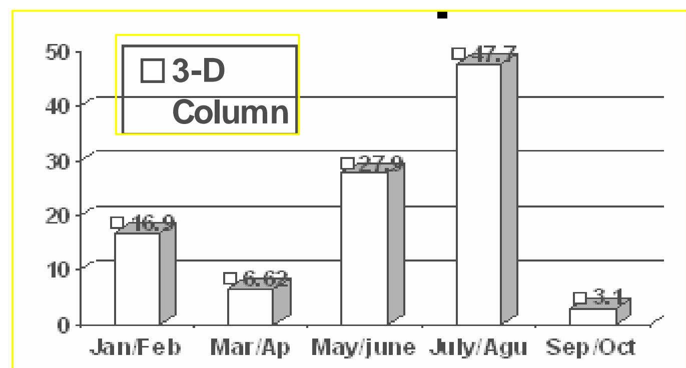


Fig. 1. Female (vulvular process) Male (Bursal lobes)

Incidence of haemonchosis: On the basis of postmortem examination 63.4% incidence was recorded. The results of the study showed higher incidence in sheep (72.5%) than goats (56%).The results also indicated incidence rate



of 64.7%, 79.16% and 71.42% in sheep in three age groups while in goats 51.5%, 70.3% and 50% incidence

Fig.2: Seasonal pattern of Haemonchosis in sheep and goats.

rate were recorded in age groups of 6 months to 1 year, 1 to 3 years and above three years, respectively. The levels of infections recorded were mild, moderate and high on the basis of egg count by Mc Master Method .In sheep 16.7% animals suffered from mild form while for moderate and high forms the percentages were 70.8 and 12.5. In goats only mild (33.3%) and moderate forms (66.7%) were recorded.(Table 1) Seasonal incidence showed that this parasitism was highest in July/August (47.7%) while it was least in September/October (3.1%).(Fig.2).

Efficacy of tests: The sensitivity of faecal culture was recorded as 42% while for DID and IHA it was 47% and 56%, respectively. The specifity of faecal culture was recorded as 89.5% while for DID and IHA it was 94.7% and 98.2% (Table 1) respectively The immunodiagnostic tests even detected those samples positive (16%) that were declared negative by faecal culture test. Statistical analysis showed a significant difference ($P<0.001$) amongst the diagnostic efficacy of the tests applied in the present study.

Table 1: Sensitivity & Specificity of Diagnostic tests.

Parameter	Sensitivity	Specificity	Predictive Value for +ve test result	Predictive Value for –ve test result.
Feacal Culture	42%	89.5%	87.5%	46.8%
DID	47%%	94.7%	94%	46.8%
IHA	56%	98.2%	98.2%	56%

In the present study, the sensitivity of IHA test was found to be 56% as compared to 42% in case of faecal culture test. This showed that IHA test detected immune responses even against immature stages of *H. contortus* and low antibody titers. These findings were in accordance with Bruce *et al*.;(1988).and Ian (1987) who reported that 0.05 ug /ml antibody level can be detected by IHA test. Raman *et al*., (1999) reported drawbacks in the conventional coprological tests.

In the accomplished study the higher incidence rate in sheep than in goats may be due to difference in feeding habits between two species. These observations were in accordance with Gibson *et al*., (1987). Similar observations were made by Sydney *et al*., (1983) who reported that difference in immune and non-immune factors exists between sheep and goats which may be responsible for variation in incidence of diseases. The positive percentage was higher in animals from 1 to 3 years of age i.e 79.16% in sheep and 70.3% in goats .Similar findings were made by Parkhouse *et al*. (1987) who explained the phenomenon of age resistance in Haemonchosis. Roth *et al*.,(1985) also reported the higher incidence of Haemonchosis in lambs and kids. The seasonal pattern indicated that mostly the infection occurred during the months of July/August (47.7%) which might be due to the presence of moisture that favored the development of larvae. Similar observations were made by Hashmi (1989) and Soulsby (1988) and Rizvi *et al*., (1999) who reported that the development of larvae was favored in the presence of moisture.

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