World Journal of Agricultural Sciences 3 (5): 562-566, 2007
ISSN 1817-3047

Clinicopathological Changes in Sheep Experimentally Infected with *Haemonchus contortus*

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Abstract: This study was designed to evaluate the clinicopathological response of native sheep of Kashmir to blood feeding nematode, *Haemonchus contortus*. Gross and histopathological changes noted in the present study were severe and parasitic nodules were concentrated towards the fundic region. The infected abomasa showed thickened walls characterized by minimal glandular dilation, mucosal hyperplasia, marked cell accumulation and hemorrhages on the mucosal surface. The mean abomasal pH (5.2±0.6) was significantly higher ($p<0.05$) in infected animals corresponding to control animals (3.23±0.4). Plasma pepsinogen and ESR were Significantly ($p<o.o5$) elevated wheras decreased values of Hb, RBC, PCV, total serum proteins were observed in infected animals compared to control.

Key words: Abomasum · *Haemonchus contortus* · hyperplasia · sheep

INTRODUCTION

Small ruminants play an important socio-economic role within traditional farming system in many developed and developing countries including India. Gastrointestinal nematodes impose severe economic constraints on the sheep and goat production worldwide. Losses occur due to subclinical parasitism through mortalities, reduced production, decreased weight [1, 2]; decreased fertility [3] and milk yield [4]. Haemonchosis is ranked among the most important diseases of small ruminants in Kashmir valley. *Haemonchus contortus* is a major pathogen primarily affecting the abomasa of sheep and other small ruminants. Adult worms feed on blood and can cause severe anemia, weight loss and even death. The purpose of the present study was to examine clinicopathological changes associated with single experimental infection of *Haemonchus contortus* in Native breed of Kashmir.

MATERIALS AND METHODS

Experimental design: Twelve lambs aged between 5-6 months were acquired from local breeders, reared and housed under controlled conditions so as to avoid the accidental infection. All the animals were treated with broad spectrum anthelmintic, Albendazole (Valbazene, 10 mg kg^{-1}) and Acaricide (Malathion 0.6%) to rule out any parasitic infection. Six lambs were allocated to infected group remaining six served as uninfected control.

Infection: Each animal of the infected group was inoculated with L_3 larvae of *Haemonchus contortus* collected from donor animal faeces, cultured in an incubator at 27°C for 7 days according to the method of Roberts and Oscillivan [5]. From 14th day of infection the faecal samples were collected for confirmation of *Haemonchus contortus* infection.

Measurement of pH: All the experimental animals were killed at the end of experiment. Abomasum of each animal was opened and pH was recorded. The pH meter was not used for measuring the pH, because it was not suitable for use on abomasal surface. Thus less accurate method of pH sticks was used. The pH sticks were placed at different sites of each abomasum for 10 seconds and mean pH was taken.

Histopathology: During the course of study the sheep abomasa were subjected to detailed histopathological examination. Abomasal tissues were fixed in Bouin's fixative for 24 hours. After fixation three washes in 70% alcohol were given to fixed tissues followed by dehydradration and dealcholization. Sections were cut on microtome, which were processed and stained with Haematoxylin and Eosin.

Estimation of plasma pepsinogen: Modified colorimetric method of Scott *et al.* [6] was followed for the estimation of plasma pepsinogen concentration. This method is

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based on the conversion of ovine serum albumin into small peptide fragments at pH<2 by active pepsin. The small peptide fragments are measured colometrically using copper complex

Haematology: Blood samples were collected from the animals for haematological investigation. For determination of haematocrit, Hb levels, RBC counts, techniques as recommended by Schalm *et al.* [7]; were employed

RESULTS

During the course of study macroscopic lessons were first seen in abomasa. The cardiac regions of infected abomasa had thickened walls and edematous folds. Infected gastric mucosa of cardiac region showed white spots. Marked congestion of hemorrhages were seen in the entire abomasa of infected sheep. No pathological changes were detected in abomasa of uninfected control animals. Mucosal hyperplasia and abundant mucus secreting gastric cells were noted in infected animals. The cellular inflammatory reactions were more marked because of infiltrated eosiniophils, mononuclear cells and plasma cells in the mucosa of infected animals. Mean pH measured during the study period was 5.2±0.6 in infected animals compared to 3.23±0.4 of control animals. Changes in pH, plasma pepsinogen, total serum proteins, ESR, Hb, PCV and TRBC count observed during the course of experimentation are presented in table. There was a gradual fall ($P<0.05$) in Hb, PCV and TRBC from infection mean values till 35th day of infection. After words these values again started rising slowly. The initial PCV values observed were within the range of 30-40% and observed differences were not significant. These values gradually decreased in the infected lambs reaching minimum of 27% during the forth week. Raised ESR was found which attained the peak in the fifth week of infection. Total serum protein levels observed in infected and uninfected groups were 5.6±0.9 and 6.8±1.2 respectively (Table 1). Though the lowest weekly mean of TSP (5.0±0.7) in infected group was observed in 4th week.

DISCUSSION

Macroscopic observations revealed greater involvement of abomasa of sheep due to *Haemonchus contortus* infection, though the extent of damage depended upon the intensity of infection and formation of nodules in the infected organ. Significantly heavier mucousa observed in infected animals corresponding to control was primarily due to mucous cell hyperplasia. Hyperplasia of cells is attributed to their over activity due to raised plasma gastrin levels similar to that described by Titchen [8]; Mapes and Coop [9]. It has been suggested that increased plasma gastrin level is responsible for thickness of abomasal mucosa [10]. Hemorrhages on the mucosal surface of the cardiac and occasionally pyloric regions is attributed to active feeding habit of parasites due to their well equipped mouthparts causing traumatic damage. Raised pH of infected animals observed in the present study is similar with the findings of Simpson *et al.* [11]; who reported that emergence of L_4 larvae from the mucosa of sheep abomasa is responsible for increased pH. The histopathological lesions were seen in fundic part of stomach but the pH was same in all parts suggesting that the uniform pH is maintained via circulation. Significant increase in plasma pepsinogen level noted in the present investigation is attributed to the damage of HCl producing cells caused by the emergence of L4 larvae, thus altering the integrity of the epithelium and allowing for the leakage of macromolecules. Damage to these HCl producing cells must have resulted in the increased plasma pepsinogen concentrations that were observed infected animals, because pepsinogen was not converted into active pepsin. Our results are in accordance with the findings of Maitu *et al.* [12] and Malan *et al.* [13]; who observed increased concentrations of plasma pepsinogen elevated abomasal pH. Decline in total serum proteins in infected animals opposed to control animals was similar to Kuttler and Marble [14]; Knox *et al.* [15] and Raisinghani *et al.* [16] who described decreased concentrations of total protein in sheep haemonchosis. Decrease in total serum proteins observed in the present study may be attributed to haemodilution, a compensatory mechanism for the

Table 1: Showing mean and standard deviation of various clinical parameters recorded during the present study

Animal group	pH	Plasma pepsinogen i,u l^{-1}	Total serum protein (g l^{-1})	PCV (%)	Hb (mg/100ml)	RBC count (10^6/mm^3)	ESR/hr
Uninfected	3.23±0.4	1.74±o.2	6.8±1.2	35.2±5.0	14.6±2.8	7.6±2.2	36±5.4
Infected	5.20±0.6	4.02±0.3	5.6±0.9	29.9±2.2	11.6±1.6	7.0±2.1	42±3.3

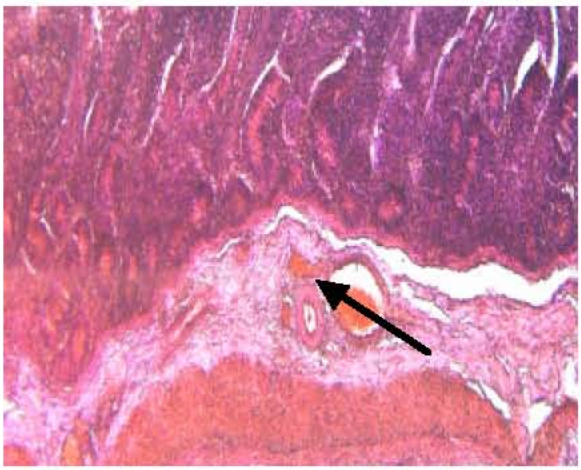

Fig. 1: Showing the infiltration oeosinophil cells

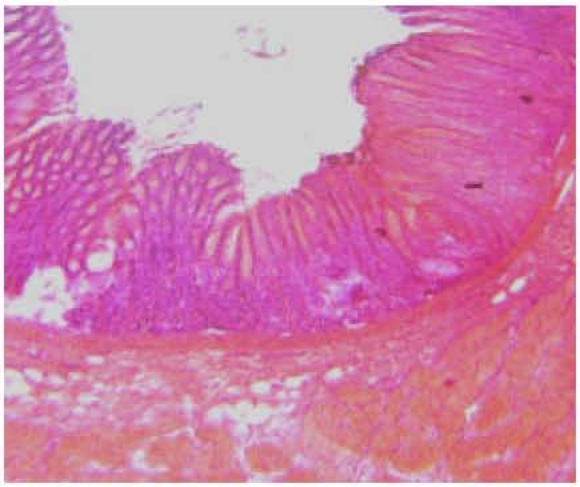

Fig. 2: Showing the white spots in Infected abomasums

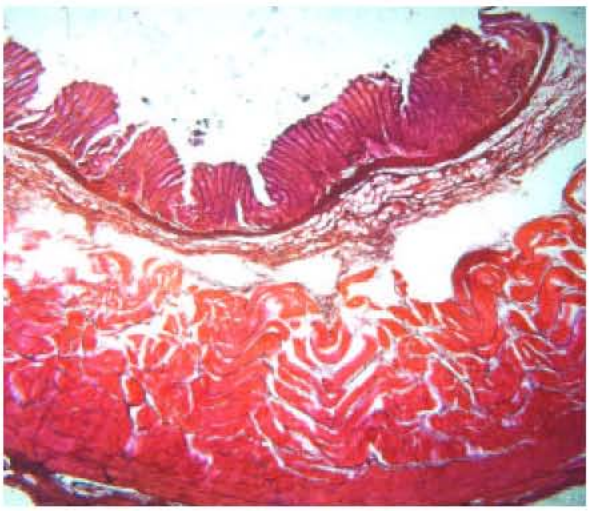

Fig. 3: Abomasum of uninfected control sheep

abomasal haemorrhages caused by the invading larvae and later on due to loss of large quantities of serum proteins into gut and consequent increased fractional catabolic rate of albumin. The present study revealed a marked reduction in haematocrit, haemoglobin and RBC count which confirmed the observations of early workers [17] who observed decreased values of haematocrit, haemoglobin and RBC counts in lambs in relation to nematode and Amphistome infection. The reduced RBC counts, Hb and PCV values in infected group may be attributed to the bleeding of abomasa due to injuries caused by the parasites similar to that described by Abdel [18]; Ahmed and Ansari [19]. However, marked blood loss in the infected animals is to be attributed to the blood sucking activities of *Haemonchus contortus*. Eosinophillia and increased lymphocyte count observed in present investigation is in agreement with the findings of Ackerman *et al.* [20], Baker [21], Bhat and Sharma [22] who concluded that eosinophillia is associated with antigenic stimulation or parasitic burden. Increased lymphocyte count might be due to proliferation of lymphocytes due to excretory secretory product of *Haemonchus contortus*.

CONCLUSION

Alteration in haematobiochemical parameters observed in the present study carry importance as they may indicate the extent of abomasal damage and help in better understanding of the pathogenesis of aneamia.

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