Environment & Ecology 29 (3) : 1134—1137, 2011
 ISSN 0970-0420

Experimentally Induced Haemonchosis in Garole Sheep : Changes in Serum Mineral Concentration

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Abstract

Haemonchus contortus is one of the widely prevalent gastrointestinal nematode of sheep and it causes severe pathogenic effects in infected animals. The effects of experimentally induced *H. contortus* infection on serum mineral concentration were studied in Garole sheep. Based on phenotypic characteristics and persistent low fecal egg count 10 Garole sheep were selected and divided into two equal groups. One group of sheep was orally infected with 700 third stage larvae of *H. contortus* per kg body weight and the other group was maintained as uninfected control. Blood samples were collected from all the experimental sheep on seven occasions at 7 day interval from 0 day-post-infection (DPI) onwards and serum was separated for estimation of serum calcium (Ca), inorganic phosphorus (Pi), iron (Fe) and zinc (Zn) concentration. Haemonchosis caused significant decrease of serum Ca and Fe from 21 to 35 DPI in the infected sheep compared to the control group. Serum concentration of inorganic P did not differ significantly due to haemonchosis. Serum Zn concentration declined significantly on 28 DPI in infected sheep compared to control group. Haemonchosis hampered the absorption of dietary Ca and produced negative impact on serum iron concentration due to anaemia.

Key words : *Haemonchus contortus*, Mineral concentration, Serum, Sheep.

The Sunderban delta of South 24 Parganas district in West Bengal is the home tract of a popular sheep breed, the Garole sheep (1) and it plays an essential role in the economy of rural India constituting small and marginal farmers and landless laborers. Garole sheep are highly prone to gastrointestinal (GI) parasitic infection particularly GI nematodosis which causes severe economic losses due to reduced weight gain (2). *Haemonchus contortus* is the predominant helminth species of sheep and it is responsible for severe pathogenic effects in terms of blood loss resulting in decreased erythrocyte and lymphocyte counts, hemoglobin concentration, packed cell volume, body weight and wool growth (3). The principal feature of *H. contortus* infection is anemia. The average blood loss due to *H. contortus* infection is 0.03 ml/parasite/day (4). Both adult and fourth stage larvae suck blood and in addition, move and leave wounds which hemorrhages into the abomasum (5). Haemonchosis causes severe damage to the abomasal mucosa in infected animals (5) and results in alteration of local environment of GI tract and thereby hampering the utilization and absorption of mineral through GI tract. Decreased level of serum mineral concentration has been recorded in natural by occurring GI nematodosis in Garole sheep and goat (6, 7). The present study was therefore carried out to estimate some mineral concentration in serum of Garole sheep experimentally infected with *Haemonchus contortus*.

(The authors thankfully acknowledge the financial assistance of the Indian Council of Agricultural Research, New Delhi in conducting this study).

Methods

Experimental Animals

Based on phenotypic characteristics and persistent low fecal egg count for one year 10 Garole sheep in the age group of 18 to 24 months were selected in Dosra Bhagabanpur, a village in the South 24 Parganas district (West Bengal), the native tract of this sheep and were purchased from the respective owners. These animals were then maintained under intensive

Table 1. Changes in serum calcium and phosphorus level due to *Haemonchus contortus* infection in Garole sheep. Values bearing superscripts x, y in a row and a, b, c, ...in a column differs significantly ($P<0.05$).

Day post infection	Mean (±SE) of serum Ca concentration			Mean (±SE) of serum inorganic P concentration		
	Infected	Control	*P* value	Infected	Control	*P* value
0	7.73 ± 0.26	7.79 ± 0.09	0.798	3.89 ± 0.49	4.23 ± 0.39	0.615
7	7.73 ± 0.19	7.91 ± 0.09	0.405	3.99 ± 0.44	4.22 ± 0.38	0.698
14	7.72 ± 0.11	7.93 ± 0.09	0.19	3.90 ± 0.33	4.04 ± 0.39	0.795
21	7.58^{y} ± 0.06	7.91^{x} ± 0.08	0.01	4.00 ± 0.38	4.08 ± 0.38	0.888
28	7.37^{y} ± 0.17	7.91^{x} ± 0.06	0.018	4.28 ± 0.37	4.61 ± 0.41	0.567
35	7.53^{y} ± 0.06	7.84^{x} ± 0.07	0.013	4.26 ± 0.38	4.15 ± 0.37	0.831
42	7.92 ± 0.05	7.81 ± 0.04	0.146	4.27 ± 0.37	4.32 ± 0.29	0.917
P value	0.242	0.79	N=5	0.973	0.950	N=5

system of management with adequate green fodder and concentrate feed and *ad libitum* clean drinking water. Pre-existing gastrointestinal parasites, if any, after coprological screening were eliminated by treatment with Fenbendazole (Panacur, Intervet) at the dose rate of 5 mg/kg body weight. Thereafter, all possible precautions were observed to preclude extraneous parasitic infections during the course of the experiment.

Experimental Infection

The infective third stage larvae (L_3) were obtained by culturing (5) the eggs separated from the adult female worms recovered from the abomasums of slaughtered sheep (8). The infective L_3 at the dose rate of 700 per kg body weight were orally administered in one donor sheep, after overnight withdrawal of feed, to get sufficient stock of infective L_3 for artificial infection. After the patency of the infection the faeces of the donor sheep was cultured and the L_3 were harvested (9). These L_3 were used for artificial infection of five Garole sheep as stated earlier keeping the remaining five sheep as uninfected controls.

Fecal samples of all the infected sheep were qualitatively examined daily by salt floatation technique (5) from second week post- infection onwards to determine the prepatent period of the infection.

Collection of Blood and Estimation of Serum Mineral Concentration

Blood samples were collected from all the experimental sheep at weekly interval from 0 DPI onwards till 42DPI. Serum samples were separated following the standard procedure. Serum calcium (Ca) and inorganic phosphorus (P) were estimated by UV-vis-spectrophotometer (ELICO, India) by O-cresolphthalin complex and UV- molybdate methods, respectively (10). The concentration of serum iron (11) and zinc (12) was estimated by UV-vis-spectrophotometer (ELICO, India).

Statistical Analysis

All the parameters for each group on different post-infection days were compared (analyzed compared means) for obtaining the mean value along with standard error (SE). Then they were analyzed separately i.e. between groups and between post-infection days by Duncan method (One-way-ANOVA) and the significance (*P*-value) was recorded at 5% ($P<0.05$) level and 1% ($P<0.01$) level. The complete statistical analyses were done with the help of Statistical Package for Social Scientist (SPSS), Windows Version 10.0.

Results and Discussion

The prepatent period of *H. contortus* infection in Garole sheep in the present study was 17 days. Prepatent period of the infection in sheep is 2—3 weeks (4, 5). In the present study, the prepatent period of the infection in Garole sheep was well within the previously established range.

Serum Ca values gradually decreased following the *H. contortus* infection and this decrease was significant ($P<0.01$) from 21 to 35 DPI in infected Garole

Table 2. Changes in serum iron and zinc level due to *Haemonchus contortus* infection in Garole sheep. Values bearing superscripts x, y in a row and a, b, c,...in a column differs significantly ($P<0.05$).

Day post infection	Mean (±SE) of serum Fe concentration			Mean (±SE) of serum Zn concentration		
	Infected	Control	*P* value	Infected	Control	*P* value
0	$178.94^{a} \pm 6.29$	180.23 ± 5.91	0.885	$47.54^{a} \pm 4.49$	45.58 ± 4.60	0.760
7	$176.69^{a} \pm 3.83$	176.11 ± 4.96	0.928	$43.67^{ab} \pm 2.32$	45.67 ± 1.61	0.499
14	$175.22^{a} \pm 4.05$	179.31 ± 4.02	0.494	$42.18^{ab} \pm 3.85$	43.42 ± 3.64	0.820
21	$160.34^{bcy} \pm 4.66$	$178.48^{x} \pm 3.44$	0.014	$41.19^{ab} \pm 1.48$	44.20 ± 1.69	0.218
28	$149.06^{cdy} \pm 4.27$	$173.37^{x} \pm 3.14$	0.001	$37.54^{by} \pm 1.68$	$43.66^{x} \pm 1.22$	0.018
35	$139.82^{dy} \pm 5.25$	$174.12^{x} \pm 5.21$	0.002	$37.77^{b} \pm 1.79$	42.99 ± 1.54	0.373
42	$169.95^{ab} \pm 1.43$	171.44 ± 4.06	0.738	$38.91^{ab} \pm 1.50$	43.89 ± 1.21	0.624
P value	0.000	0.780	N=5	0.140	0.152	N=5

sheep compared to the respective control values. Serum inorganic P concentration did not differ significantly ($P>0.05$) due to *H. contortus* infection in Garole sheep (Table 1).

Dietary Ca and inorganic P absorbed chiefly in the upper small intestine, particularly the duodenum. Absorption of Ca and inorganic P is facilitated by a low intestinal pH and thus normal gastric secretion of HCl is necessary for absorption (13). There is a significant rise of abomasal pH soon after *H. contortus* infection accompanied by increased plasma pepsinogen and gastrin concentration (14). Higher abomasal pH causes higher duodenal pH, which might have hampered the binding of Ca and protein (albumin) which is essential for absorption of Ca and thereby reducing serum Ca concentration in the present study (15). Hypocalcaemia results from a decreased concentration of negatively charged proteins and there of protein bound Ca^{++} (15). Hypoproteinaemia (hypoalbuminaemia) and protein loosing enteropathy is a common feature in haemonchosis (16—18), which also contributed to the reduced serum Ca concentration.

Due to *H. contortus* infection, serum Fe concentration in Garole sheep significantly ($P<0.05$) declined from 21 to 35 DPI. Compared to the respective control groups, the serum Fe level was significantly lower from 21 to 35 DPI in infected Garole sheep (Table 2).

Decreased level of serum iron is associated with blood sucking activities of *H. contortus* (19). Anaemia is a constant feature in haemonchosis (4, 20, 21). Haemonchosis in the present study might be responsible for anaemia. This has resulted in stimulation of erythropoietic activity with increased demand of iron, which might be ascribed to the fall in serum iron level (7).

Haemonchosis resulted in significant decrease ($P<0.05$) in serum Zn level from 28 to 35 DPI in infected Garole sheep compared to the preinfection value. Although serum Zn level was consistently lower in the infected sheep, it was significant ($P<0.05$) only on 28 DPI compared to the control group (Table 2).

Serum zinc concentration in infected sheep decreased significantly on 28 DPI compared to control sheep. It is known that the immune system has specific requirement for certain micronutrients such as zinc (22, 23). Zinc is an essential mineral for the development of T-cell and helps in cell mediated immune (CMI) response (24). The CMI response in the infected Garole sheep is activated due to *H. contortus* infection resulting in greater involvement of zinc (7). This might have also contributed to the lowered serum zinc level in the infected sheep.

The present study indicated that *H. contortus* infection hampered the absorption of dietary Ca and caused severe negative impact on serum iron concentration due to anaemia and thereby aggravating the anaemic condition of the host. *Haemonchus contortus* is the predominant parasite of small ruminants and hence the reduced concentration of serum mineral may be a common feature in the infected animals. Therefore mineral mixture should be provided along with feed of small ruminants to combat the negative impact of *H. contortus* infection.

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