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Parasitological and haemato-biochemical response to *Haemonchus contortus* infection in Garole sheep

GAUTAM BORDOLOI[1], RUMA JAS[2] and J D GHOSH[3]

West Bengal University of Animal and Fishery Sciences Kolkata, West Bengal 700 037 India

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ABSTRACT

Garole sheep is commonly known for its lower susceptibility to parasitic diseases. Possible existence of resistance and/or resilience to *Haemonchus contortus* infection was explored based on the parasitological and haemato-biochemical responses in experimentally induced infection. Female Garole sheep (15) with persistently low faecal egg count (FEC) since last 1 year were selected for the experiment. Sheep were orally fed with 700 infective larvae of *H. contortus* (L_3) per kg body weight. Prepatent periods (PP) of the infection, faecal egg count (FEC), total worm count (TWC) and selected haemato-biochemical parameters were studied. PP was found to be16 - 17 days and FEC on 21th days post-infection (DPI) was 90±18.71 but after 24 DPI onwards, no egg was detected both on qualitative as well as quantitative faecal examination. Infected animals were sacrificed on 49 DPI and no worm in the abomasum and upper small intestine was detected. Haemoglobin value significantly declined from 21 DPI but PCV (packed cell volume) remained constant between infected and control groups. Total erythrocytic count (TEC) was reduced significantly in infected group between 21 to 28 DPI. Serum protein concentration including serum albumin and globulin did not change significantly due to *H. contortus* infection. Parasitological and haematological findings indicated that although *H. contortus* infection was established in Garole sheep, the parasites were eliminated at the early patent period of the infection with gradual restoration of the TEC and PCV and serum proteins concentration.

Key words: Garole sheep, Haemato-biochemical parameters, *Haemonchus contortus*, Resilience, Resistance

The Sunderban delta in West Bengal is the home tract of the Garole sheep –a popular sheep breed (Sharma *et al*. 1999). Husbandry practices adopted by the sheep rearers make the breed highly susceptible to gastrointestinal (GI) parasitic infection particularly GI nematode (Pandit *et al*. 2009). *Haemonchus contortus* is the predominant species of the GI nematodes of sheep and is responsible for severe pathological impact in terms of blood loss leading to erythrocytopenia, lymphocytopenia, anaemia, decreased packed cell volume, body weight and wool growth (Hayat *et al*. 1996). As resistance to *H. contortus* which is measured by faecal egg count (FEC) test, is a moderately heritable trait, selective breeding of sheep having phenotypically expressed this trait could possibly increase the resistance ability against *H. contortus* (Bishop *et al*. 1996, Woolaston and Piper 1996, Morris *et al*. 1997). Studies on the genetics of resistance to internal parasite in sheep have been conducted in Australia and New Zealand and United Kingdom (Bishop *et al*. 1996, Bishop and Stear 2001). It has been found that Garole sheep of West Bengal exhibited some degree of resistance/resilience against *H. contortus* infection (Annual Report 2008-09, Nimbkar *et al*. 2000, Bose 1995, Ghalsasi *et al*. 1994) but no systematic study was conducted regarding this aspect (Hooda *et al*. 1999). In view of the above, the present study was undertaken to elucidate the possible existence of resistance and/or resilience against *Haemonchus contortus* by studying the parasitological and haemato-biochemical response of Garole sheep to experimentally induced haemonchosis.

MATERIALS AND METHODS

Experimental animals: Based on phenotypic characters (i.e. small size, rough wool, ear length and tail length) and persistently low monthly faecal egg count (mean epg 128) as compared to the unselected group (mean epg 704.73) for 1 year, 15 female Garole sheep of 18–24 months-old were selected in a village of South 24 Parganas district (West Bengal). No anthelmintic treatment was given to the sheep during pre-selection period. Selected sheep were transported and maintained under intensive system of management with adequate green fodder, concentrate feed and *ad lib.* clean drinking water. Pre-existing gastrointestinal parasites, if any, were eliminated by treatment with fenbendazole @ 5 mg/kg

Present address: [1]Ph. D. student (gauti_022@rediffmail.com), [2]Research Associate (ruma_k007@yahoo.co.in), [3]Professor and Head (jdg_wbuafs@yahoo.co.in), Department of Veterinary Parasitology.

body weight with subsequent precautions to preclude extraneous parasitic infections during the course of the experiment. The animals were then randomly divided into group 1 comprising 10 sheep and group 2 comprising 5 sheep.

Culture of infective larvae (L_3) of H. contortus*:* Infective third stage larvae (L_3) were obtained by culture of the eggs obtained from adult female worms recovered from the abomasums of slaughtered sheep (Soulsby 1982). Cultured larvae (L_3) were administered orally @ 700/kg body weight in a helminth free indigenous sheep, after overnight withdrawal of feed, to serve as donor of eggs/L_3 for artificial infection.

After patency of the infection L_3 were harvested by coproculture of donor sheep (Soulsby 1982). Sheep of group 1 were artificially infected with the L_3 while group 2 served as uninfected control.

Examination of faecal samples and estimation of worm burden: Faecal sample of the infected sheep was qualitatively examined by standard salt floatation technique (Soulsby 1982) from second week onwards to determine the prepatent period (PP) of the infection. Quantitative faecal examination of all the infected sheep was done by Modified McMaster's technique (Soulsby 1982) from 18– to 30 day-post infection (DPI) at 3-day-interval and thereafter at 6-day-interval till the end of the experiment. Five sheep from group 1 were sacrificed for recovering worms from the abomasum and the upper small intestine.

Collection of blood samples for haemato-biochemical studies: Blood samples of all the experimental sheep were collected at weekly interval from day–0 onwards till 42 DPI. Haemoglobin (Hb) value, packed cell volume (PCV) and total erythrocyte count (TEC) were estimated by cyanomethaemoglobin, Wintrobe's haematocrit and haemocytometer methods, respectively (Jain 1993). Total serum protein (TSP) and serum albumin (SA) were estimated spectrophotometrically by the method of Reinhold (1953) and Dumas *et al*. (1971), respectively. Serum globulin (SG) was estimated by subtracting the value of SA from that of TSP.

Statistical analyses: Statistical analysis was done using one way ANOVA test (Duncan method) between 2 groups and post-infection days.

RESULTS AND DISCUSSION

Prepatent period (PP), faecal egg count (FEC) and worm burden of the infection: The prepatent period (PP) of *H. contortus* infection in Garole sheep was 16-17 days. After patency of infection, mean epg on 18, 21 and 24 DPI was 70±10.20, 90±18.71 and 60±10.0, respectively, and thereafter no egg was detected in both qualitative and quantitative faecal examinations. Interestingly, at necropsy on 49 DPI no worm was recovered from abomasum and upper small intestine of slaughtered sheep. Although, prepatent period of *H. contortus* in Garole sheep was 2–3 weeks which was similar to other sheep breed (Urquhart *et. al.* 1996, Soulsby 1982), but fecundity of parasites was significantly lower as compared to other breeds of sheep. Fecundity of the parasite, in terms of epg, is generally considered as a useful indicator of resistance/immune response to nematode infections (Mugambi *et al*. 2005). In this regard, Garole sheep in the present study had the highest epg of 90±18.71, which was even lower than th epg (128) that constituted the major criterion for their selection. This was indicative for greater degree of resistance or stronger immune response of Garole sheep to *H. contortus* infection (Urquhart *et al*. 1996).

Reduced worm count at necropsy is an important indicator of resistance to *H. contortus* in sheep (Woolaston and Baker 1996, Mugambi *et al*. 2005). In this study, no parasite was recovered on 49 DPI in the sheep, which had patent infection till 24 DPI. Absence of worm burden in Garole sheep corresponded with the result of faecal egg count (FEC), which became negative after 24 DPI. Expulsion of the worms is a clear indication of rejection of the adult worms along with the immature ones, if any, after 24 DPI in Garole sheep. Association of the mast cells and globule leucocytes with rejection of challenge larvae of *H. contortus* is a well-recognized phenomenon in sheep (Amarante *et al*. 2005, Kemp *et al*. 2009). Parasite specific IgA, IgG1 and IgE in the abomasal mucosa are also involved in the response against gastrointestinal nematodes including *H. contortus* (Harrison *et al*. 2003, Amarante *et al*. 2005). However, involvement of such mechanisms in expulsion of existing adult worms need to be explored in a systematic manner.

Haemato-biochemical profiles: Infected sheep revealed a significant ($P < 0.01$) decline in haemoglobin level from 21 DPI onwards compared to pre-infection value (Table 1). Infected sheep showed significantly ($P < 0.01$) lower PCV level between 21 and 28 DPI and thereafter PCV level restored to normal level on 42 DPI. Total erythrocyte count significantly decreased ($P < 0.01$) between 21 and 28 DPI and thereafter it gradually increased and reached to normal (Table 1). Concentration of TSP, SA and SG did not alter significantly ($P > 0.01$) due to haemonchosis in Garole sheep compared to pre-infection value as well as control group (Table 2).

Resistance to helminth was the characteristic of having fewer parasite numbers which was measured indirectly by FEC, whereas resilience is the ability of the host to completely or partially combat the effects of the infection on productivity (Woolaston and Baker 1996). Anaemia was the major effect of *H. contortus* infection and it was measured in terms of Hb concentration, PCV and TEC. Haemonchosis produced negative impact on Hb concentration with reduced value in infected sheep as compared to control group. PCV value did not vary due to haemonchosis in Garole sheep. Although the TEC value declined at the early stage of the infection, it was restored near to the normal level at the later stage.

Table 1. Changes in haematological parameters due to *Haemonchus contortus* infection in Garole sheep

Day post-infection	Hb concentration (g/dl)		PCV (%)		TEC value (× 10^6/cmm)	
	Infected	Control	Infected	Control	Infected	Control
0	$9.80^{a} \pm 0.26$	10.00 ± 0.35	$38.6^{ab} \pm 2.54$	37.8 ± 1.88	$20.86^{a} \pm 0.78$	20.74 ± 0.55
7	$9.60^{a} \pm 0.37$	10.20 ± 0.46	$39.0^{a} \pm 2.66$	37.4 ± 2.60	$19.91^{a} \pm 0.90$	20.35 ± 0.62
14	$9.20^{ab} \pm 0.26$	9.40 ± 0.24	$33.0^{bc} \pm 1.64$	36.4 ± 2.44	$18.69^{a} \pm 0.69$	19.39 ± 0.77
21	$8.10^{by} \pm 0.33$	$9.20^{x} \pm 0.34$	$31.3^{c} \pm 0.86$	35.9 ± 0.87	$12.41^{cy} \pm 1.02$	$19.52^{x} \pm 0.76$
28	$8.30^{b} \pm 0.54$	9.30 ± 0.37	$31.0^{c} \pm 2.07$	33.1 ± 0.87	$14.92^{by} \pm 0.47$	$19.36^{x} \pm 0.81$
35	$8.20^{by} \pm 0.37$	$9.7^{x} \pm 0.20$	$34.4^{bc} \pm 1.69$	35.8 ± 1.16	$17.69^{a} \pm 0.61$	19.21 ± 1.22
42	$8.10^{by} \pm 0.19$	$9.3^{x} \pm 0.12$	$35.6^{b} \pm 1.54$	35.4 ± 0.87	$18.69^{a} \pm 0.57$	19.33 ± 0.95

Values bearing superscripts x, y in a row and a, b, c, in a column differs significantly (P<0.05).

Table 2. Changes in serum protein concentration due to *Haemonchus contortus* infection in Garole sheep

Day post infection	TSP concentration (g/dl)			Serum albumin concentration (g/dl)			Serum globulin concentration (g/dl)		
	Infected	Control	P value	Infected	Control	P value	Infected	Control	P value
0	7.31 ± 0.15	7.49 ± 0.17	0.351	3.76 ± 0.06	3.93 ± 0.05	0.377	3.55 ± 0.14	3.56 ± 0.16	0.696
7	7.44 ± 0.38	7.48 ± 0.30	0.512	3.75 ± 0.11	3.92 ± 0.12	0.328	3.69 ± 0.15	3.56 ± 0.18	0.719
14	7.03 ± 0.25	7.29 ± 0.27	0.933	3.46 ± 0.14	3.85 ± 0.05	0.126	3.57 ± 0.31	3.44 ± 0.24	0.550
21	6.56 ± 0.31	7.29 ± 0.32	0.8	3.27 ± 0.24	3.67 ± 0.17	0.101	3.29 ± 0.17	3.62 ± 0.13	0.387
28	6.85 ± 0.37	7.29 ± 0.25	0.44	3.36 ± 0.31	3.81 ± 0.14	0.063	3.49 ± 0.21	3.48 ± 0.22	0.948
35	7.18 ± 0.21	7.37 ± 0.14	0.428	3.47 ± 0.15	3.75 ± 0.06	0.138	3.71 ± 0.14	3.62 ± 0.13	0.751
42	7.43 ± 0.30	7.52 ± 0.16	0.477	3.61 ± 0.08	3.9 0± 0.15	0.210	3.82 ± 0.10	3.62 ± 0.25	0.804
P value	0.316	0.565	N=5	0.484	0.613	N=5	0.133	0.649	N=5

Generally, PCV is considered as a haematological marker of resistance to nematodes, particularly the blood sucking nematodes like *H. contortus* and PCV in *H. contortus* resistant breed of sheep remained stable (Miller *et al*. 1998, Bricarello *et al*. 2002). Hence the response of *Haemonchus* infected Garole sheep in respect of PCV as observed was a good expression of its resistance to the infection. Besides, in this study the existence of resistance in Garole sheep was also substantiated by the results of Hb and TEC values, the two other important haematological indicators of anaemia. Haemonchosis causes significant negative impact on serum protein concentrations, macro- and micro-mineral levels and the enzyme activities (Bricarello *et al*. 2002). Results of the present study clearly indicated that Garole sheep has greater ability to combat the negative impact of the infection i.e. higher degree of resilience in respect of serum protein concentrations. The resilience of Garole sheep in terms of the serum proteins (TSP, SA and SG) was significantly higher, particularly during the later stage of the infection and it corroborated with the findings of Saddiqi *et al*. (2009).

This study indicated that Garole sheep although initially failed to completely resist the establishment of *H. contortus*, but it exhibited marked resilience to the infection by successfully combating the pathogenic effect of the infection in terms of the major haematological parameters. As PCV was considered as haematological marker for resistance to *H. contortus* infection and it has been found to be low to moderately heritable/repeatable traits (Morris e*t al.* 1997, Charon *et al.* 2000, Vanimisetti *et al.* 2004), selective breeding involving the sheep with fair degree of resilience as measured by the FEC and the indicators of anaemia, especially PCV could therefore be a promising option for ensuring sustainable improvement in productivity of Garole sheep.

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