

Clinical Response to Experimental *Haemonchus contortus* Infection in Desert Lambs

Abakar, A.D.¹; El Amin, E.A.² and Osman, A.Y.³

(1) Faculty of Veterinary Science, University of Nyala, Sudan (2) Faculty of Veterinary Science, Khartoum North, P.O.Box 32, University of Khartoum, Sudan (3) Central Veterinary Research Laboratories, Soba, P.O.Box 8067, Animal Resources Research Corporation, Sudan.

ملخص البحث

توضح هذه الدراسة بعض التغيرات المرضية التي تنجم عن إصابة الحملان الصحراوية بالطور الثالث المعدي لدودة الهيمونكس كونتورتس. استغرقت الدراسة ٤٨ يوما وتمثلت النتائج في فقدان وزن الحيوان، ظهور الأعراض المرضية المرتبطة بالانيميا، نقص البروتين بالإضافة إلى نقص في معدلات الحديد والكالسيوم والصوديوم.

Summary

Changes in live body weight, faecal egg count, haematological and biochemical values were studied in lambs experimentally infected with 3500 *Haemonchus contortus* third stage larvae over a period of 48 days. Infected animals showed loss of body weight and development of clinical haemonchosis associated with anaemia, hypoproteinaemia and reduced serum iron, calcium and sodium concentrations.

Introduction

Haemonchus contortus is the most pathogenic helminth parasite of sheep and goats which causes great losses in production. The disease caused by this parasite is prevalent wherever small ruminants are raised but causes particularly great losses in tropical regions (Blood and Radostitis, 1989; Rahman and Collins, 1990; Rahman, 1994). Studies of growth rates in sheep infected with *H. contortus* were reported by Abbott *et al.* (1985) and Ahmed and Ansari (1989). Their results indicated that haemonchosis causes varying degrees of body weight loss. Changes in certain blood constituents of sheep infected with *H. contortus* have been reported by Mottelb *et al.* (1992). The objectives of the current study were to record the live weight, haematological values, serological values and faecal egg counts of lambs experimentally infected with this parasite.

Materials and Methods

Animals:

Ten lambs of 3-4 months of age were purchased from a local market and were kept at premises of the Central Veterinary Research

laboratories at Soba in Khartoum. Upon their arrival, they were ear-tagged housed in cleaned disinfected pens and subjected to thorough clinical examination and subsequently ascertained their freedom from internal parasites by faecal examination for three consecutive weeks using the flotation technique before the start of the experiment. The animals had free access to feed and water. Lambs were divided into two equal groups on body weight basis. In the first group each lamb was infected with 3500 L3 *H. contortus* *per os*; lambs of the second group served as un-infected control.

Parasitological Techniques:

Infective *H. contortus* larvae were cultured from adult worm collected from naturally infected sheep using standard the Baermann technique. The culture and harvesting of larvae were performed according to Anon(1977). The dose of infective larvae was administered *per os*. Faecal samples were collected directly from the rectum of each lamb and the faecal nematode egg count performed by a modified McMaster technique (Anon, 1977).

Blood Analysis:

Blood for haematological analysis was collected by jugular veinpuncture into heparinized vacutainers(Becton Dickinson, England). Red blood cell(RBC)count, haemoglobin (Hb) concentration, PCV and white blood cell(WBC)counts were determined according to the procedure of Jain (1986).

Blood for biochemical analysis was collected in plain vacutainer tubes containing no anticoagulant. The serum was harvested and stored at -20°C awaiting later analysis. Total serum protein was analyzed by Biuret method (King and Wooton, 1956) while serum albumin concentration was determined after Bartholomew and Delnay (1966). Serum globulin was calculated as the difference between total serum protein and albumin concentration.

Serum iron was measured by a commercial kit(iron-B-Bathophenanthroline, England). Serum phosphorus concentration was determined in accordance with the technique described by Varley (1967). Serum calcium level was measured using cresophthalein method kits. Analysis of electrolyte concentrations was determined after Wotton (1974). The analysis of variance was used to analyze the results. P values less than 0.05 were considered significant.

Results

Clinical observations:

Infected lambs showed signs of depression, inappetance pale mucous membrane and loss of body condition. Their live body weight (Table 1) was significantly decreased ($P < 0.05$) compared to the un-infected controls. Lamb No. 2455 died on day 30 post infection had 554 worms.

Faecal egg output:

Eggs of *H. contortus* were recorded for the first time during the 4th week after larval administration (Table 1). Maximum egg count, in terms of eggs per gram (EPG) was recorded on day 32 post infection ($95.6 \times 10^3 \pm 12 \times 10^3$). From day 32 post infection onwards, the mean EPG value fell gradually to its lowest value on day 48 post infection ($10^3 \pm 0.2 \times 10^3$). The patent period was 30 days.

Table 1: Mean body weight (Kg \pm S.E.M.) and faecal egg count in desert sheep (EPG \pm S.E.M.) experimentally infected with 3500 *H. contortus* larvae

Days post infection	Body weight (kg)		Egg count	
	Infected	Controls	Infected	Controls
0	13.20 \pm 1.30	13.00 \pm 1.55	0	0
4	12.95 \pm 1.86	13.50 \pm 1.46	0	0
9	12.70 \pm 1.92	13.50 \pm 1.23	0	0
13	12.60 \pm 1.87	14.00 \pm 1.44	0	0
18	12.50 \pm 1.87	14.00 \pm 1.15	1325 \pm 307	0
22	12.30 \pm 2.11	14.50 \pm 1.53	26600 \pm 1969	0
25	12.10 \pm 2.56	14.68 \pm 1.89	28860 \pm 4153	0
28	12.10 \pm 2.92	15.00 \pm 2.18	72100 \pm 6299	0
32	12.50 \pm 2.47	15.80 \pm 2.18	95600 \pm 1238	0
36	12.12 \pm 2.47	16.00 \pm 2.208	3500 \pm 4107	0
40	12.00 \pm 2.50	16.20 \pm 2.223	21600 \pm 3927	0
44	12.00 \pm 2.55	16.50 \pm 2.66	6000 \pm 326	0
48	12.00 \pm 3.07	16.60 \pm 2.35	1000 \pm 233	0

Haematological finding :

Haematological findings of infected and non-infected lambs are shown in table 2. Haematological values recorded for the controls

were found to fluctuate within the normal range for sheep throughout the observation period. *H. contortus* infection caused significant ($P<0.05$) reduction in means Hb, PCV and total RBCs. Moreover, the inoculation of lambs with the parasites resulted in significant ($P<0.56$) increase in total WBCs and eosinophils.

Changes in Serum Constituents:

Biochemical changes in the serum of infected and control lambs illustrated in tables 3 and 4. The mean total serum protein and serum albumin of infected lambs were significantly ($P<0.05$) decreased when compared with non-infected controls.

The mean serum phosphorus level of infected lambs failed to show any consistent pattern of changes throughout the observation period. On the other hand, serum calcium was significantly decreased when compared to that of control animals. Mean serum iron of infected lambs was significantly ($P<0.05$) reduced when compared with the non-infected controls. Analysis of serum electrolytes indicated significant ($P<0.05$) decline of serum sodium but serum potassium levels failed to show any significant changes

Discussion

The experiment was designed to investigate the clinical response of sheep to primary infection with *H. contortus*. The establishment of this parasite, as judged by faecal egg counts, followed similar patterns to those described by previous studies (A/Latif *et al.*, 1980; Abbott *et al.*, 1986; Ahmed and Ansari, 1989). The clinical signs demonstrated by the infected lambs in this trial were typical to those reported by Dargie and Allonby (1975); Hunter and Mackenzie (1982); Al-Quaisy *et al.* (1987) and Mottelib *et al.* (1992).

Mean live weight of the infected lambs showed that haemonchosis had a significant effect. Bezubik *et al.* (1978) and Barger and Cox (1984) also reported a loss of 1.5 and 2 kg in the weight of sheep infected with 3000 and 20000 L_3 of *H. contortus* respectively. A/Rahman and Collins (1990) reported loss of body weight in goats infected with 20000 larvae of sheep derived *H. contortus* strain. The current work documented that infected lambs

Table 2: Mean (S.E.M.) Haematological values of non-infected(N) and infected (INF) lambs receiving single doses of 3500 *Haemonchus contortus* larvae

Days*	Hb (g/dl)		PCV(%)		MCV(fl)		MCH(g/dl)	
	N	INF	N	INF	N	INF	N	INF
0	9.12±0.17	8.87±0.67	8.98±0.98	8.86±0.82	25.5±4.79	24.80±0.03	27.98±4.43	26.07±3.45
4	8.93±0.48	8.78±0.42	9.46±0.68	8.97±1.45	24.8±0.04	24.60±0.04	27.55±4.19	32.27±7.15
9	8.82±0.17	8.27±0.73	9.13±1.08	8.90±1.35	25.00±0.04	24.60±0.03	28.29±5.22	29.04±6.95
13	9.46±1.66	8.11±0.79	8.49±0.82	7.28±0.78	26.80±0.04	23.20±0.03	28.63±6.16	29.42±3.93
18	8.46±2.04	7.86±0.56	8.29±0.41	6.80±0.54	25.50±0.04	20.40±0.04	29.85±7.88	29.55±4.77
22	8.68±2.27	7.61±0.74	8.65±0.47	6.55±0.51	24.50±0.03	19.80±0.04	27.04±5.04	26.23±4.92
25	9.31±1.24	7.11±0.83	8.95±0.29	5.89±0.39	24.88±0.03	18.20±0.05	27.30±3.52	26.86±5.86
28	9.18±0.92	6.57±0.88	8.78±0.49	5.14±1.32	23.75±0.04	17.40±0.04	27.90±3.67	28.04±1.44
32	9.84±1.06	5.99±1.23	8.43±0.50	5.13±1.49	27.00±0.03	17.40±0.04	29.00±2.27	27.75±5.73
36	9.82±0.85	5.95±0.96	9.07±0.46	5.91±0.35	26.00±0.03	19.50±0.05	28.53±3.79	26.03±1.57
40	9.67±1.08	5.73±1.04	8.90±0.79	6.05±0.97	26.00±0.03	18.50±0.05	28.75±1.07	27.17±3.26
44	9.20±1.11	5.73±1.43	8.82±0.62	6.57±0.09	26.30±0.03	20.00±0.04	29±3.38	29.68±5.44
48	9.43±1.33	5.19±1.30	8.57±0.57	6.03±0.09	27.33±0.03	19.25±0.05	28.60±2.05	29.55±0.49

Table 3: Mean (\pm S.E.M.) protein values of non-infected and infected lambs receiving single doses of 3500 *Haemonchus contortus* larvae

Days post infection	Total protein		Albumin		Globulins	
	Control	Infected	Control	Infected	Controls	Infected
0	6.40 \pm 0.03	7.10 \pm 0.16	3.60 \pm 0.01	3.82 \pm 0.30	2.80 \pm 0.30	3.27 \pm 0.23
4	6.90 \pm 0.03	7.14 \pm 0.13	3.50 \pm 0.02	3.58 \pm 0.25	3.40 \pm 0.45	3.50 \pm 0.42
9	6.90 \pm 0.04	6.43 \pm 0.07	3.04 \pm 0.12	3.47 \pm 0.01	3.80 \pm 0.32	2.50 \pm 0.01
13	6.80 \pm 0.01	6.10 \pm 0.06	3.55 \pm 0.02	3.43 \pm 0.12	3.30 \pm 0.25	2.67 \pm 0.22
18	6.63 \pm 0.04	5.65 \pm 0.12	3.33 \pm 0.15	3.22 \pm 0.34	3.30 \pm 0.01	2.43 \pm .48
22	7.00 \pm 0.34	5.70 \pm 0.04	3.43 \pm 0.16	2.93 \pm 0.06	3.57 \pm 0.35	2.77 \pm 0.38
25	6.93 \pm 0.02	5.20 \pm 0.04	3.25 \pm 0.10	2.85 \pm 0.15	3.68 \pm 0.36	2.35 \pm 0.13
28	6.66 \pm 0.13	5.33 \pm 0.13	3.38 \pm 0.05	2.64 \pm 0.13	3.28 \pm 0.06	2.69 \pm 0.40
32	7.33 \pm 0.20	5.29 \pm 0.01	4.27 \pm 0.43	2.75 \pm 0.35	3.10 \pm 0.05	2.54 \pm 0.01
36	7.00 \pm 0.10	5.05 \pm 0.08	3.27 \pm 0.12	2.60 \pm 0.32	3.73 \pm 0.74	2.25 \pm 0.43
40	6.50 \pm 0.03	5.41 \pm 0.03	3.33 \pm 0.01	2.87 \pm 0.07	3.17 \pm 0.63	2.45 \pm 0.01
44	6.65 \pm 0.19	5.22 \pm 0.01	3.31 \pm 0.30	2.42 \pm 0.16	3.34 \pm 0.53	2.80 \pm 0.12
48	7.60 \pm 0.12	5.00 \pm 0.01	3.43 \pm 0.20	3.09 \pm 0.01	3.60 \pm 0.07	2.00 \pm 0.38

Table 4: Minerals values of non-infected (N) and infected (INF) lambs receiving single dose of 3500 *Haemonchus contortus* larvae

Days*	Calcium (mg/dl)		Phosphorus (mg/dl)		Iron (µg/dl)		Sodium (mEq/l)		Potassium (mEq/l)	
	N	INF	N	INF	N	INF	N	INF	N	INF
0	9.06 ± 0.98	9.20 ± 0.28	4.02 ± 0.58	4.23 ± 0.40	18.00 ± 1.03	19.76 ± 1.05	143.5 ± 2.20	143.00 ± 1.14	5.00 ± 0.06	5.00 ± 0.05
4	9.46 ± 0.35	10.13 ± 0.57	4.91 ± 0.13	4.01 ± 0.13	18.00 ± 0.93	18.75 ± 1.00	144.5 ± 2.90	143.40 ± 1.14	5.20 ± 0.20	5.10 ± 0.07
9	8.66 ± 0.12	7.52 ± 0.47	4.17 ± 0.13	4.01 ± 0.43	20.90 ± 0.99	19.22 ± 1.08	143.0 ± 2.20	143.50 ± 2.38	5.00 ± 0.17	5.10 ± 0.08
13	8.80 ± 0.23	7.14 ± 0.48	5.12 ± 0.26	5.30 ± 0.44	19.22 ± 1.12	19.66 ± 1.02	143.0 ± 3.50	142.50 ± 1.41	5.10 ± 0.10	5.08 ± 0.06
18	8.88 ± 0.32	8.06 ± 0.22	4.55 ± 0.19	5.26 ± 0.24	19.22 ± 1.00	18.35 ± 0.90	141.6 ± 3.80	140.70 ± 0.44	5.00 ± 0.10	5.00 ± 0.05
22	7.50 ± 0.42	6.89 ± 0.23	4.80 ± 0.15	4.10 ± 0.26	19.19 ± 0.95	15.73 ± 1.02	140.00 ± 1.50	139.5 ± 1.50	4.95 ± 0.10	4.90 ± 0.09
25	7.73 ± 0.37	6.91 ± 0.33	5.61 ± 0.37	5.28 ± 0.30	22.72 ± 1.26	15.14 ± 1.06	140.00 ± 1.41	138.00 ± 1.63	4.95 ± 0.10	4.90 ± 0.08
28	8.00 ± 0.36	6.66 ± 0.13	4.99 ± 0.22	5.98 ± 0.50	20.97 ± 1.08	17.12 ± 1.06	139.9 ± 1.91	138.00 ± 1.41	5.00 ± 0.05	4.92 ± 0.05
32	7.75 ± 0.10	6.92 ± 0.29	4.42 ± 0.36	4.29 ± 0.23	22.72 ± 1.06	16.16 ± 1.01	142.00 ± 0.95	137.50 ± 1.00	4.90 ± 0.09	4.88 ± 0.06
36	7.75 ± 0.24	5.92 ± 0.32	4.94 ± 0.15	5.13 ± 0.75	142.00 ± 0.95	136.00 ± 1.52	141.60 ± 1.90	136.50 ± 1.53	4.95 ± 0.05	4.82 ± 0.07
40	8.85 ± 0.42	5.66 ± 0.17	5.28 ± 0.05	5.71 ± 0.33	141.60 ± 1.90	136.00 ± 1.52	139.00 ± 0.95	136.00 ± 1.52	4.90 ± 0.10	4.90 ± 0.07
44	8.00 ± 0.42	6.66 ± 0.37	5.16 ± 0.05	3.81 ± 0.15	139.00 ± 0.98	137.00 ± 0.50	140.00 ± 2.00	137.00 ± 0.50	5.00 ± 0.10	4.90 ± 0.07
48	8.20 ± 0.23	6.46 ± 0.11	5.81 ± 0.23	5.20 ± 0.27	21.48 ± 0.98	17.00 ± 0.96	140.00 ± 2.00	138.00 ± 1.00	4.90 ± 0.05	4.83 ± 0.02

*Mean ± S.E.M

developed macrocytic hypochromic anaemia as indicated by a decrease in haemoglobin concentration, erythrocytic counts and haematocrit values. This might be related to the blood loss resulting from invasion of the abomasal mucosa by fourth stage larvae and adult worms. Our results conform to those of Abbott *et al.* (1986) and Rahman and Collin (1990).

In this study, lymphocytosis and eosinophilia were evident in blood of infected lambs. Rahman and Collins (1990) stated that infection with *H. contortus* did not lead to significant changes in total white cell counts. However, a decrease in lymphocyte and increase in neutrophils numbers were reported. The increase in the number of eosinophils is a common feature observed during infection with *Haemonchus* (Butterworth, 1980; Salaman and Duncan, 1985).

Total serum protein and albumin levels were reduced by infection that may simply be related to anorexia and blood loss. This phenomenon of hypoproteinaemia and hypoalbuminaemia was documented by Abbott *et al.* (1986); Dukkak (1988) and Rahman and Collins (1990). Ahmed *et al.* (1990) reported a marked reduction in serum globulins particularly alpha and beta globulins while gamma globulins fraction showed a significant increase.

Analysis of the mineral contents of the serum revealed reduction of iron, calcium and sodium concentrations. Serum phosphorus and potassium failed to show a fixed pattern of changes. The reduction of serum iron level in infected lambs could be attributed to the expanded erythropoiesis to compensate for blood loss leading to depression of iron stores. Albers and others (1990) noted a decrease in the serum iron levels of lambs receiving a single dose of 10000 L3 *H. contortus*. Moreover, Rahman and Collins (1990) reported reduction in calcium levels in goats infected with sheep-derived strain of *H. contortus*. The hyponatremia reported here may also be attributed to increase permeability of abomasal mucosa that led to leakage of plasma. Pradhan and Johnstone (1972) stated that lambs infected with *H. contortus* not only showed a reduction in serum sodium but also a rise in potassium concentration.

In conclusion, the present experiment provides evidence that infection with *H. contortus* in lambs results in the development of macrocytic hypochromic anaemia, hypoproteinaemia, and reduced levels of iron

and calcium together with marked disturbances in the serum electrolytes.

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