The Sudan J. Vet. Res. (2001) **17** *E-mail:cvrlcenterl@hotmail.com*
With 1 table and 3 figures in the text.

The Interaction of Coccidian and *Haemonchus contortus* Infections in Desert lambs

Abakar[1], A.D; El Amin[2], E.A., and Osman[3], A.Y.
(1) Faculty of Veterinary Science, University of Nyala P.O. Box 155 Nyala, Sudan. E-mail adamfaf@hotmail.com (2) Faculty of Veterinary Science, P.O.Box 32 Khartoum North University of Khartoum, Sudan. (3)Central Veterinary Research Laboratories,P. O. Box 8067 Al Amarat, Animal Resources Research Corporation Sudan..

[illegible]

(*Haemonchus contortus*) [illegible] (*Coccidia*) [illegible] (*Sporocysts*) [illegible]

Summary

The effect of experimental interaction between a mixture of *Eimeria spp*. and *Haemonchus contortus* caused reduced feed intake, diarrhoea and some deaths but had no significant effect (P>0.05) on the live bodyweight. Combined infections with the parasites caused sharp inappetence, severe scouring, and reduction in live body weight and death. The output of oocysts was significantly (P<0.05) increased by the presence of *H. contortus* in simultaneously infected lambs but it was not affected when *Haemonchus* infection was separated from Eimeria infection by 15 days interval. Egg output was vastly increased when the worm was administered together with *Eimeria spp.* compared with coccidian infection administered two weeks apart from *Haemonchus* infection or with single infection.

Introduction

Sudan is a vast country and has a livestock population of 23.042 million sheep (Arab Organization for Agricultural Development, 1992). Desert sheep are frequently infected with coccidia in the Sudan (Abakar, 1996, Abakar *et al*., 1999-2000).

The stomach worm *H. contortus* was reported to affect both sheep and goats causing considerable economical losses (Abakar *et al*.,

1999-2000). There is a meager information on the effects of concurrent and sequential infections of desert sheep with coccidia and gastrointestinal helminth parasites. Catchpole and Harris (1989) working with *Eimeria* and *Nematodirus battus* in lambs showed that concurrent infections caused more severe disease than single infections. Bristol *et al*. (1983) have indicated that concurrent infections with different species of parasites not only affect each other but also may alter the host immune system. Rahman (1994) has proved that, in subclinical coccidiosis, super infection of goats with *H. contortus* increased the fecundity of the worms.

Mixed infections with coccidia and helminth are common in the tropical sheep (Vercruysse, 1988; Osman *et al.,* 1990; Kanryi, 1993). The interaction of enteric coccidia and *H. contortus* infections in Sudanese sheep has not been studied before. The present work was proposed to investigate the effect of experimental sequential and concurrent infections with ovine coccidia and the stomach worm *H. contortus* in desert lambs.

Materials and Methods

Experimental animals:

Twenty nine desert sheep lambs, 2-3 month-old with average bodyweight of 13 kg were used in this study; they were purchased from Kosti, Central Sudan, and transported to the premises of the Central Veterinary Laboratories at Soba, Khartoum. All lambs were ear-tagged and housed in clean pens. The pens were disinfected using quaternary ammonium before admission of animals. Each lamb was provided with food ration consisting of 0.5 kg dura and sorghum stalks *ad-libitum*. Lambs had free access to water. Upon their arrival, lambs were subjected to thorough clinical examination and were proven to be free of internal parasites by faecal floatation examination for three consecutive weeks before the beginning of the experiment.

The parasite:

Eimeria species:

Cultures of *Eimeria spp*. were made from coccidial oocysts voided by naturally infected sheep. The sporulated oocysts of each

Eimeria species were propagated in a coccidia-free lamb (Two-month old). Faecal samples were subsequently collected and the coccidial

oocysts were sporulated for seven days in 2.5% potassium dichromate solution at room temperature with constant aeration.

Haemonchus contortus:

Adult females of *H. contortus* were obtained from the abomasum of naturally infected sheep encountered during routine post-mortem examinations at Omdurman and Nyala slaughterhouses. The females were isolated from the abomasal contents and the eggs were freed by maceration and then cultured in sterile sheep faeces and incubated at 28 °C for 10-14 days.

The infective third stage larvae (L3) were harvested in a Baermann apparatus and stored at 4ºC until used. The preparation of the inocula and the infective doses were made according to Jqrgan and Perry (1994).

Experimental Design:

Unless otherwise indicated, each lamb was experimentally infected with a dose of 3000 L3 *H. contortus* and/or 1×10^6 sporulated oocyts of *Eimeria spp.* The coccidial inoculum contained a mixture of *E. ovina* (33%), *E. ovinoidalis* (40%), *E. ahsata* (12%), *E. crandallis* (7%), *Emiria faurei* (5%) and *E. parva* (3%).

The experimental lambs were divided into six groups according to their body weight by a complete randomized design. These groups of animals were as follows:

Group I:

Consisted of four animals served as uninfected controls.

Group II:

Consisted of five animals infected with *H. contortus,* viz *Haemonchus* infection.

Group III*:*

Consisted of five animals infected with *H. contortus* and 15 days later were dosed with the coccidial inoculum, viz. Sequential infection with *H. contortus* and coccidia.

Group IV*:*

Consisted of five animals inoculated with the coccidial inoculum, viz coccidial infection.

Group V:

Consisted of five animals inoculated with the coccidial inoculum and infected after 15 days with *H. contorus*, viz. sequential infection with coccidia and *H. contortus*.

Group VI:

Consisted of five animals simultaneously infected with sporulated oocysts and *H. contortus*.

The lambs were kept under clinical observation for 52 days. Bodyweight was taken twice a week. Daily faecal samples were collected from the rectum of each lamb from day 7-post infection (PI) onwards and the number of oocysts per gram faeces (OPG) and egg per gram faeces (EPG) were determined as described by Anon (1977).

Statistical analysis:

The statistical analysis of the collected data i.e. prepatent period, patency, bodyweight, OPG and EPG was done using the complete statistical system (CSS) with database management and graphics (Release 3.1, Copyright 1986-1990 stat soft, Inc. Japan) in A commodore personal computer. Analysis of variance (ANOVA) tests were used to compare body weight, EPG, OPG, prepatency and patent periods in the treatment groups and when the results were significant ($P<0.05$) the treatment effect between different groups was computed using least significant difference (LSD).

Results

Clinical observations:

Lambs of all infected groups showed signs of depression, in-appetence, and anaemic mucous membrane and loss of body condition. The live body weight was significantly ($P<0.05$) decreased in the concurrently infected lambs and was highly significant ($P<0.01$) in simultaneously infected lambs (Fig. 1). Diarrhoea developed in lambs of all groups that were infected with cocidia and it became more severe and accompanied with dehydration and soiling of hindquarters in the concurrently infected animals. Death occurred with varying proportions in infected lambs of all groups. The mortality rate was 25% in lambs of group II, 60% in lambs of group III and 40% in lambs

infected with coccidia only. The latter animals succumbed within 20-35 days PI while *H. contortus* infection killed the lambs within 30 days

PI. Lambs succumbed to concurrent infection with both parasites in 25-42 days PI and to simultaneous infection within 27-44 day PI. The worm burden of *H. contortus* infected lambs that died during the experimental period is shown in table 1.

Faecal Oocysts output:

The mean OPG of infected lambs is shown in Fig. 2. The faecal oocyst shedding started on day 11 PI for lambs that were infected with *Eimeriae* alone. The prepatent period was 11 days. Maximum mean OPG production was recorded between day 20 and day 31 PI and the highest mean OPG value was recorded on day 25 PI ($4.8x10^5 \pm 9.4x10^4$). Thereafter, oocysts shedding declined and reached its lowest value on day 41 PI ($5x10^2 \pm 1.410^2$). The patent period was 30 days. Lambs that were infected firstly with *Eimeria spp*. and superimposed by *H. contortus* infection began to shed oocysts in their faeces after a prepatency of 11 days PI that peaked 17-27 days PI. Maximum mean OPG occurred on day 21 PI ($1.4x10^7 \pm 1.7x\ 10^5$). Subsequently, the mean OPG fell gradually to reached its lowest mean value by day 49 PI (5×10^2). The prepatent period for this group was 38 days. The excretion of oocysts in faeces of lambs that were initially infected with *H. contortus* and 15 days later inoculated with coccidia had a prepatency of 9 days. The peak of oocysts shedding was recorded between days 21 and days 29 PI and the maximum mean OPG value occurred on day 25 PI ($8.4\pm2.9x10^4$). From day 29 onwards the faecal oocysts shedding declined gradually until it reached its lowest mean value level on day 53 PI ($1.5x10^3 \pm 0.5x10^2$). The prepatent period of coccidial infection in lambs of this group was 44 days. Lambs that were infected simultaneously with *Eimeria spp*. and *H. contortus* started to shed coccidial occysts in their faeces 11 days PI. The level of oocysts shed by lambs of this group was highly significant ($P<0.01$) in comparison with lambs with either single or sequential infections. Faecal oocysts shedding was observed between days 17 and 35 PI and the maximum mean OPG was recorded on day 25 PI ($3.0x10^7\pm 5.1x10^5$). From day 35 PI the mean OPG

value fell gradually and reached its lowest value on day 44 PI ($8.5x10^3$) when the last animal on this group died.

Faecal egg output:

Fig. 2 shows the mean EPG of faeces excreted by lambs infected with *H. contortus* in the different groups. Lambs infected with *H. contortus* only started to void eggs in their faeces on day 18 PI. The mean EPG production in lambs of this group increased gradually and reached its highest values between days 23 and 34 PI. The maximum EPG was recorded on day 28 PI ($2.7x10^3 \pm 1.3\ x10^3$). From day 34 PI onwards the mean EPG values fell gradually until its lowest value was recorded on day 44 ($10^3 \pm 0.8\ x\ 8^2$). The prepatent period was 26. The prepatency in lambs infected first with *H. contortus* and 15 days later with *Eimeria* spp was 18 days. The mean EPG increased significantly ($P<0.05$) to reach its highest values between days 24 and 42 PI. The maximum mean EPG value was recorded on day 28 PI ($6.8x10^4 x\ 8.4x10^3$). From day 44 PI egg shedding began to fall gradually and the lowest value was recorded on day 52 PI ($10^3 \pm 4.2\ x\ 10^2$). The patent period was 34 days. Superimposition of *H. contortus* infection in lambs infected with coccidia had a prepatency of 15 days.

Table 1: Worm burden of dead lambs following infection with 3000 L3 *H. contortus* in the different treatment groups.

Infective agents	**Lamb No**	**Death (D.P.I.)**	**No. worms recovered**	**Total**	**% establishment of infection**	
					Individual Recovered	**Group Average**
Haemonchus	2455	30	554	554	18.30	18.30
Haemonchus	2453	28	600		20.00	
followed by	2459	42	150	1387	05.00	15.00
coccidia	2461	29	637		21.23	
Coccidia	2457	35	714		23.80	
followed by	2469	20	936	2650	31.20	29.00
Haemonchus	2492	28	1000		33.00	
Simultaneous	2451	44	550		18.30	
infection	2456	32	1013		33.70	
	2463	27	610	2377	20,30	22.40
	2471	27	634		21.10	
	2495	35	570		19.00	

D.P.I. = Days post-infection.

The maximum mean values of EPG were recorded between days 20-42 PI and the highest value were recorded on day 24 PI ($6.9x10^4 \pm 2.6$ $x10^3$). Subsequently, the mean EPG value gradually fell and by day 52 PI, it reached the its lowest value ($10^3 \pm 0.5$ $x10^2$). Lambs simultaneously infected with both parasites produced significantly ($P<0.05$) higher EPG counts compared to lambs of all other group. Shedding of eggs began on day 16 PI. The mean EPG increased progressively and reached its maximum values between days 21 and 38 PI. The maximum EPG value was recorded on day 34 PI ($1.1x10^5 \pm$ $9.5x10^3$). The mean EPG fell gradually reaching its lowest value (2.2. x 10^4) on day 44 when the last lamb of this group died.

Fig. (1) Mean body weight of infected and non-infected lambs

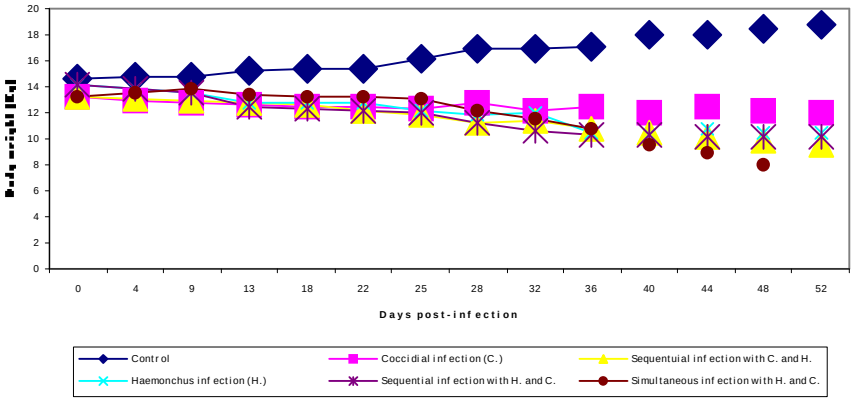


Fig. (2) Mean OPG values of lambs infected with Coccidia in ther various groups

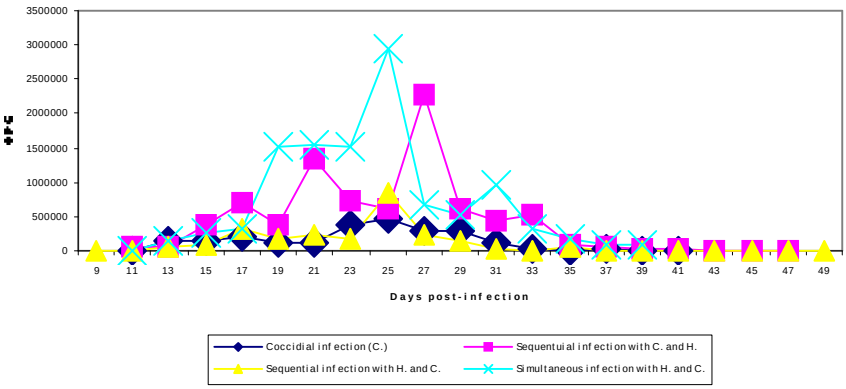
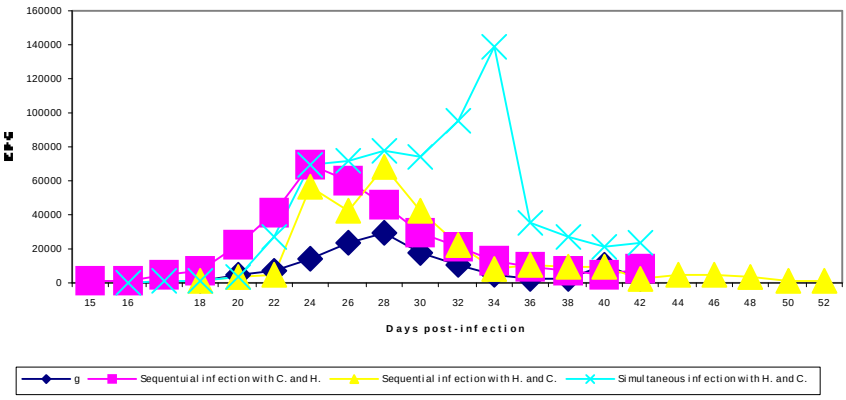


Fig. (3) Mean EPG of lambs infected with Haemonchus in the various groups



Discussion

The present work was undertaken to evaluate the effects of the dual infections of lambs with the stomach worm, *H. contortus,* and the enteric coccidia, *viz. E. ovina, E. ovinoidalis, E. crandallis, E. faurei, E. parva* and *E. ahsata. H. contortus* parasitizes the abomasum of sheep where its larvae burrow into the mucosal epithelium; they either become hypobiotic or emerge and develop to maturity in the lumen of the abomasum and start to lay eggs 16-18 days PI. *E. ovina, E. ahsata* and *E. faurei* develop in small intestine producing oocysts 13-17 day PI, while *E. crandallis* infects the lower small intestine and the large intestine producing oocysts 16-21 days PI (Pout *et al*., 1973, Gregory and Catchpole, 1987). Infection with *E. ovinoidalis* produced its first generation schizonts in the lower small intestine and completes its life cycle in the caecum and colon (Wacha *et al*., 1970). Meronts of *E. parva* were found throughout the small intestine while the sexual stages occur mostly in the caecum, colon and to lesser extent in the small intestine. Therefore, infection of lambs with stomach worm and coccidia may almost involve the entire gastrointestinal tract with potential disruption of its absorptive and digestive functions.

In this study, exclusive infection of lambs with coccidia caused diarrhoea, reduction in live body weight and death. Likewise, lambs infected with *H. contortus* alone expressed slight decrease in live body weight, anaemia and caused some deaths. When the two parasites were given together to the same animal severe scouring was noticed. Weight loss and high mortality rates presented additional manifestation. Catchpole and Harris (1989) reported similar results when lambs were concurrently infected with *Coccidia* and *Nematodirus battus*. The clinical signs manifested by the combined infections, therefore, could be attributed to the presence of the two type of parasites. In this study, high mortality rates, severe diarrhoea and the specific coccidial lesions were detected in both solely coccidia-infected lambs (group IV) and concurrently infected lambs (group III, V, VI) suggesting that coccidiosis was the possible cause of the rapid death of these lambs.

The prepatency of coccidial infection was shortened when the infection was proceeded by *H. contortus* establishment. This could be attributed to lowered immune status of lambs, which were sequentially

infected with coccidia and *H. contortus*. On the other hand, the prepatency or simultaneously occurring with coccidial infection. This might be related to the impaired immunity of the concurrently infected lambs. Similar observations on the effect of concurrent infection on the prepatency of nematodal infections mobilize adequate immune response elements to the multiplication of the coccidia because of probably too many antigens (*Coccidia* and *Haemonchus*) within defined period. Under normal conditions coccidial infection usually shows a high initial peak of oocysts production followed by rapid decline, which is due to the development of immunity (Pout *et al*., 1973). The patency of coccidial infection became rather prolonged in cases of concurrent infection with *Haemonchus* because of impairment of the immune function. Bristol *et al* (1983), De Fuencte *et al* .(1993) and Rahman (1994) have reported similar results. On the other hand, the occurrence of coccidial infection either sequentially or simultaneously had markedly affected the prepatency, patency and fecundity of *Haemonchus* infection in lambs. These findings are in a agreement with those reported by Davis *et al.* (1959;1960a and b), Bristol *et al.* (1983); Catchpole and Harris (1989), De Fuente *et al.*

(1993); Rahman (1994). This phenomenon could be linked with the subsequent spread of haemonchosis during concurrent coccidial infection. This study shows that the establishment of *H. contortus* in lambs was favoured by pre-existing coccidial infection.

Acknowledgements

This study was made possible by a research grant from the German Academic Exchange Services (DAAD). The authors wish to thank Dr. Abubaker M. Abbas for his help in the statistical analysis of data. The assistance of Dr. Hisham I. Seri in typing the manuscript is highly appreciated.

References

Abakar N. D. (1996). Ph. D Thesis. University of Khartoum 208. pp

Abakar, A. D, Alamin, E. A. and Osman, A.Y. (2000). *Sudan J.Vet.Res*.**16:** 1-10.

Abakar, A. D, Bashar, A. E and Ismail, A. A (2000). *Sudan J. Vet. Sci.Ainm.Husb.*

Anon (1977). Manual of Veterinary Parasitological Laboratory Techique, Ministry of Agriculture, Fisheries and Food.

Agricultural Development Advising Services, Technical Bulletin No. 18 2nd edition.

Bristol, J. R., Pion, A. L., Lillian, A. J. and Mayberry, L. F.(1983). *J.Parasitol*.**. 69:** 372 – 374.

Catchpole, J. and Harris, T. J**.** (1989) *Vet.Rec*. **10**: 603 – 605.

Davis, L. R., Herilich, H. and Bowman, G. W**.** (1959 b). *Am. J.Vet.Res*. **20:** 181 – 187.

Davis, L. R. Herilich, H. and Bowman, G. W. (1960 a*). Am. J. Vet.Res*. **21:** 181 – 187.

Davis L. R., Herilich, H. and Bowman, G. W. (1960 b). *Am. J.Vet.Res*. **21:** 188 – 194.

De Fuente, C., Cuquerella, M., Carrera, L. and Alunda, J. M. (1993). *Vet.Parasitol*.**45:** 177 – 183.

Gregory, M. W. and Catchpole, J. (1987). *Deutsch.Tieraez.Woch. Chrif*. **94:** 521 – 525.

Jqrgan, H. and B., Perry (1994). The Epidemiology, Diagnosis and Control of Helminth Parasities of Ruminants. Published by ILRAD Nairobi, Kenya.

Kanryi, N. W. P. (1993).*Vet.Parasitol*. **51:** 137 – 141.

Osman, H. M., Osman, A. Y., Mohammed, Z. A., Gasmir, G. S., Omer, F. A. and Shommein, A. M. (1990).*Sudan J.Vet. Sci.Anim.Husb*. **29** (1): 35 – 39.

Pout, D. D., Norton, C. C. and Catchpole, J. (1973).*Br.Vet.J*. **129:** 568 – 581.

Rahman, W. A. (1994).*Vet.Rec*.**134:** 235 – 237.

Vercruysse, J. (1988). *Bull. Anim. Hlth. Prod. Afri*. **36:** 186 – 187.

Wacha, R. S., Hammond, D. M. and Miner, M. L. (1971). The development of endogenous stages of Eimeria Ninakohl-yakimvae in domestic sheep. Proc. Helm. Soci. Washington. **38:** 167 – 180.