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EXPERIMENTAL INFECTION OF SHEEP WITH TRICHOSTRONGYLE NEMATODES OF THE CAMEL WITH SOME TRIALS ON THEIR CHEMOTHERAPEUTIC TREATMENT USING THE DRUG IVERMECTIN (With 6 Tables)

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عدوي الأغنام التجريبية بواصطة بيـــدان الجمال الترايكومترونجال الخيطية ومحاولة علاجها بواسطة عقــار ايغرمكتيــــن

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شملت الدرامة اجراء تجارب تم فيها نقل طغيل ديدان الجمال الاسطواني (
ترايكوسترونجلس بروبيولورس) الى الأغنام مع دراسة الآثار الاكلينيكية والفسيولوجي الناتجة من عدوي الطغيل للأغنام وبالاضافة الى ذلك تمت دراسة السفات المورفولوجي الماتهات المعنية والأطوار البالفة مدا وقد عولجت هذه الأغنام المعنية بواسطة عقي الإيفرمكتون بجرعة مقدارها ٢٠٠ ملليجرام عن كل كيلوجرام من وزن المعيوان وقد كانست نسبة نجاح العلاج مائة في المائة أي أن عقار إيفرمكتين قد قني على جميع طغيلي المدورة في أمعاء الأغنام .

SUMMARY

This study deals with transmission exsperiments of Trichostrongylus probolurus from camels to lambs. The effect of this worm on lambs was studied. No apparent clinical signs or post-mortem lesions were detected. Haematological parameters showed moderate eosinophilia, but no signs of anaemia were observed during the course of infection. The morphology of the adult worm and the infective larvae was also studied. The efficacy of Ivermectin (Ivomec-MSD), administered subcutaneously at a dose rate of 200 mg/kg body weight to these experimentally infected lambs against Trichostrongylus probolurus was investigated. The drug was found to be 100% effective against this worm.

INTRODUCTION

Data on cross transmission of trichostrongyle parasites between camels and sheep, to determine the role played by the latter host in the infection of camels by these parasites, was lacking; except for some trials made by BEVERIDGE, et al. (1974) in Australia where sheep were experimentally infected with Camelostronglylus mentulatus obtained from camels. The parasite was successfully transmitted to sheep and produced functional and morphological changes in the ovine abomasum.

In the present study some trials were made to determine the role of desert sheep in the transmission of <u>Trichostrongylus</u> sp. infections of camels.

The anthelmintic agent Ivermectin has recently been used to treat these trichost-rongyle infections. This drug is one of the members of the family Avermectins which are fermentation products produced by the actinomycete Streptomyces avermitilis. The drug acts by stimulating the release of inhibitory neurotransmitter gamma – amino-butyric acid (GABA) and enhancing its binding to the post synaptic receptors in the worm leading to paralysis and death (CAMPBELL and LEANING, 1983).

ARMOUR, at al. (1980) showed that, oral doses of 100 ug or subcutaneous injection of 100-200 ug/kg of Ivermectin killed all adults and inhibited larval stages of the common gastro-intestinal nematodes of cattle in the United Kingdom. LYONS, at al. (1981) stated that the percentage removal of Ivermectin (Ivomec-MSD), administered subcutaneously at the rate of 200 ug/kg body weight to calves infected with Ostertagia spp. and Trichostrongylus spp., was 100% effective against fourth-stage and 99% against mature Ostertagia pp. while on Trichostrongylus spp. it was 100% effective against both fourth-stages and mature worms. ARMOUR and BOGAN (1982) reported that, the efficacy of Ivermectin (Ivomec-MSD) against developing larvae, adult worms and arrested larvae of common gastrointestinal nematodes was 90%, and the drug has withdrawal time of 21 days.

Studies done in several countries showed that, the efficacy of Ivermectin at oral dose of 200 mcg/kg in sheep was independent of the strain of parasite, breed of sheep and husbandry conditions. HOTSON (1983) summarized the results obtained during these studies where Ivermectin was found to be 95% or more effective against both immature and adult stages of the following nematodes of sheep: Haemonchus contortus, H. placei, Trichostrongylus axei, T.colubriformis, Ostertagia circumcincta, Ccoperia curticel, C. onchophora, Nematodirus battus, N.spathiger, N.filicollis, Gaigeria pachyscelis, Strongyloides papillosus, Oesophagostomum columbianum, Oe.venulosum, Trichuris ovis, Chabertia ovine and Dictyocaulus filaria.

In the present work, the efficacy of subcutaneous injection of Ivermectin (Ivomec-MSD) on experimental trichostrongylosis in sheep was studied.

MATERIAL and METHODS

Six apparently healthy lambs were used in this experiment. These were dewormed using a single oral dose of Albendazol - tablets. The drug was administered in a dose rate of one tablet per 20-25 kg body weight. The animals were then ear-taged, and were devided into three equal groups. The animals were kept under worm-free conditions in sheded pens with cement floor. They were fed a daily ration of lucerne and a liberal supply of water. The animals were weighed each week.

Fresh faecal samples were collected directly from the rectum of naturally infected camels, and faecal cultures were made using the method of ROBERTS and O'SULLIVAN (1950). One hundred larvae were identified following the keys of LEVINE (1968) and ANON (1971), and the percentage of each genus was determined, which was 90% Trichostrongylus and 10% Haemonchus. The required number (1000-2000) of larvae was then taken from the thoroughly shaken suspension by the automatic pipette and administered to the experimental animals in a single oral dose using a stomach tube. Group I received 1000 larvae, group II, 1500 and group III received 2000 larvae.

Faecal samples were collected four times a week from the experimentally infected animals. A modified WILLIS technique (SOULSBY, 1982) was used to detect eggs in the faeces.

The modified McMASTER technique (SOULSBY, 1982) was used to determine the number of eggs per gram of faeces. Egg counting was performed four times a week, and the results obtained were expressed as weekly means.

Blood samples were collected weekly from the jugular veins of the experimental animals, into clean test tubes containing disodium salt of ethylenediamine-tetraacetate (EDTA) as an anticoagulant, for the determination of hematological indices. Haemoglobin content (Hb) total blood cells count per mm³ (RBCs and WBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and differential leucocytic count were carried out according to SCHALM, et al. (1975).

Four weeks following infection (i.e. 10 days after the prepatent period) one animal from each group was treated with a single dose of subcutaneous injection of Ivermectin (Ivomec-MSD)* in a dose rate of 200 ug/kg body weight. The remaining three lambs were kept to act as the untreated controls. These were kept in a separate pen.

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^{*} Chemotherapeutic drug manufactured by Merck Sharp and Dohme, Hoddesdon, England.

Five weeks following infection (i.e. one week after treatment) the animals were slaughtered. The gastro-intestinal organs (abomasum, small intestine, colon and caecum) were tied each at its anterior and posterior ends, and then removed from the animal. The organs were kept separately in white enamelled trays, and their contents removed. The mucosal surfaces were washed with tap water. The washings and the contents were then strained through a 100 um mesh sieve under running water until the drainage became clear. The debris on the sieve was then diluted with 2500 ml of tap water, thoroughly shaken and aliquots of one tenth of the total volume were removed into glass jars. Small amount of 4% formalin is added and the samples were preserved until examination. The samples were then searched in petri-dishes, using a long needle, under a dissecting microscope and the worms were removed, counted and preserved in 5% glycerine alcohol.

For morphological studies the worms were put in petri-dishes containing 5% glycer-ine alcohol for several days till the alcohol evaporated leaving the worms in the glycer-ine. Worms of each sex were randomly chosen for morphological studies. Septimens were mounted in glycerine jelly. The species were identified following the keys of YORKE and MAPLESTONE (1969). Measurements were done using an eye piece micro-meter.

RESULTS

All animals inoculated became infected. The pre-patent period ranged from 19-20 days. In animals receiving higher inocula, 1500-2000 larvae, the pre-patent period was 19 days and in those receiving 1000 larvae the pre-patent period was 20 days.

In all slaughtered animals only <u>Trichostrongylus probolurus</u> (RAILLIET, 1896) was detected mainly in the small intestine.

The number of adult worms of both sexes detected from each animal in relation to the number of inocula and egg counts during the last week of experiment are represented in Table (1), where animals receiving higher number of infective larvae harboured a high number of adult worms, and passed more eggs with their faeces.

Morphology of the parasite:

Trichostrongylus probolurus (RAILLIET, 1896) as obtained from the experimental lambs is a small worm, with no obvious cuticular structures at the anterior end. The excretory pore lies in a conspicious depression near the anterior extremity. The male spicules are stout, irregularly shaped and brown in colour. The female vulva has prominent lips and there are two ovijectors. The hind end of the female is pointed. The measurements of the worms are shown in Table 2.

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Criterion	Range		Avera	ige
	Male	Femals	Male	Female
Total length	4.4-5.9 mm	5.9-7.1 mm	5.4	6.5 mm
Length of oesophagus	736-800 u	800-896 u	764 u	853.3 u
Excretory pore from anterior end	144-160 u	144-160 u	154.6 u	152 u
Length of spicules	120.9-150.1 u		138.7 u	
Vulva from posterior end		1.45-1.49mm		1.46 mm

Infective third-stage larvae:

The infective larva of T.probolurus is of medium size, with pointed tail and a conical sheathed tail. Its measurments shown in Table (3).

	Crit	erion	R	ange		Avera	ge
Tot	al len	gth	643.	5-726	u	687.5	u
Lei	gth of	cesophagus	180	-184	u	182	u

Pathology of the disease:

Clinical signs and post-mortem lesions:

No obvious clinical signs or post-mortem lesions were detected.

Blood picture:

Tables 4 & 5 explain the haematological parameters encountered during the course of the disease. In the erythrocyte series (Table 2) there were no obvious changes in the total number of red blood cells per mm³ blood, in packed cell volume (PCV) and in haemoglobin concentration (Hb) except in animal 378 in which the PCV was slightly reduced. No considerable changes were detected in the values of mean corpuscular volume and mean corpuscular haemoglobin concentrations.

In the leukocyte series (Table 3) the total number of white blood cells per mm³ blood was increased except that of animal 375 where it decreased. The eosinophils percentage increased in all animals. However, the percentage varied in other types of cells, except that the monocytes percentage decreased as the condition became advanced.

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Chemotherapy:

Animals numbers 378, 372 and 373 were treated with Ivermectin (Ivomec-MSD) four weeks following infection (i.e. 10 days after the pre-patent period). The passage of eggs from all treated animals ceased on the third day dollowing treatment. Table 6 showed the effect of this drug on egg counts, where there was a considerable reduction in th counts as compared with that of the previous weeks and that of untreated controls. No worms were detected in the treated animals while considerable numbers of adult worms were obtained from the untreated controls.

Efficacy of Ivermectin (Ivomec-MSD):

The total number of parasites in the untreated control animals was 1461 worms, and that in the treated animals was zero. If we apply the control test of MOSKEY and HARWOOD (1941); where he efficacy of the drug was calculated according to the formula:

$$\frac{a-b}{a} \times 100 = \%$$
 efficacy

a = number of parasites in control animals

b = number of parasites in treated animals

The efficacy of Ivermectin on experimental trichostrongylosis in lambs will be:

$$\frac{1461 - 0}{1461} \times 100 = 100\%$$

So this drug is 100% effective against <u>Trichostronglus probolurus</u> in experimental lambs.

DISCUSSION

In the present study, <u>Trichostrongylus probolurus</u> obtained from naturally infected camels was susccessfully transmitted to lambs, where all the experimental lambs became infected indicating that sheep can act as a source of infection to camel, since natural infection with <u>T.probolurus</u> was reported in sheep (ANON, 1958).

Clinically, except for reduction in body weight which was accompanied by high egg counts in one lamb (No. 371), no apparent abnormal clinical signs were observed in the infected animals. The decrease in egg counts in animals No. 375 and 377 as compared with that in animal No. 371 was due to the presence of lesser number of female worms harboured by these animals. Correlations between egg counts and changes in body weights were observed in infections with other species e.g. in camel haemonchosis (ARZOUN, et al. 1984b) and in H.contortus infection of sheep (ROBERTS and SWAN,

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1982) and during H.longistipes infection of goats (ARZOUN, et al. 1983). The absence of clinical signs may be attributed to the lower worm burden harboured by the experimental animals. According to LELAND, et al. (1960) and KATES and TURNER (1960) the infective dose and the worm burden observed in this study reflected mild infection.

The pre-patent period ranged from 19 to 20 days. This range resembles that sted by KATES and TURNER (1960) in lambs experimentally infected with Trichostronglyus axel. Since only one day elapsed between the lower and higher limits of this range, the pre-patent period was not related to the number of larvae in the infective dose. Similarly LELAND, at al. (1960) did not observe significant variations in the pre-patent periods with variations in the number of larvae in the infective dose. KATES and TURNER (1960) observed no differences in the pre-patent period in 6 lambs experimentally infected with different doses of T.exei infective larvae except in lamb No. 6 which received the highest inoculum, where the pre-patent period was prolonged.

Haematologically no anaemia was observed in the infected lambs. This was in agreement with the findings of BAKER and DOUGLAS, 1966). Anaemia was generally considered to be absent in pure Trichostrongylus infections (BAKER and DOUGLAS, 1966) and there might even be an abnormally high erythrocyte count due to dehydration. KATES and TURNER (1960) also observed polycythemia and it was attributed to the effect of dehydration and reduced fluid intake; the authors also stated that, anaemia if present would be of nutritional type. Moderate eosinophilia was observed during the course of infection in the present study. Similarly LELAND and DRUDGE (1957); LELAND, et al. (1960) observed eosinophilia during experimental Taxei infections in rabbits and shoop respectively. however, LELAND, et al. (1959); KATES and TURNER (1960) did not observe such eosinophilia.

Morphologically the measurements of the males and females of T.probolurus recovered from experimentally infected lambs were comparable to those reported by LEVINE (1968). However, the length of the spicules differed from that stated by SOULSBY (1982). This difference could be attributed to geographical variations, phenomena that was detected in other species. ARZOUN (1981) attributed the increment in the length of spicules of Haemonchus longistipes, obtained from Sudanese camels, to geographical variations. The total lengths of T.probolurus infective larvae obtained from faecal cultures from the infected lambs were also comparable to those of T.axei infective larvae stated by LEVINE (1968) and OKPALA, et al. (1978), and also to those of T. vitrinus reported by LEVINE (1968) and ANON (1971).

Haemonchus longistipes infective larvae obtained from naturally infected camels failed to infect lambs when administered in a dose less than 200 larvae/animal in a mixture of 90% Trichostronglylus and 10% Haemonchus larvae in the present study. Similarly 300 larvae failed to infect sheep (EL BIHARI, et al. 1984).

Chemoherapeutic trails, in this study, using the drug Ivermectin (Ivomec-MSD) were carried out for the treatment of experimental Trichostrongylus infection in lambs. It was found that subcutaneous injection of Ivermectin at a dose rate of 200 ug/kg body weight given to lambs experimentally infected with T-probolurus was 100% effective against this worm in as far as the removal of the mature stages from the gut is concerned. This result confirmed that of LYONS, et al. (1981) in dealing with calves naturally infected with Trichostrongylus and Ostertagia spp. Ivermectin administered orally to sheep was found to have 95% or more efficent against the common sheep nematodes (HOTSON, 1983). Therefore, Ivermectin as has been pointed out previously, was shown to have the same effect on killing of adult worms whether injected subcutaneously or administered orally to sheep.

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Table 1: Number of adult worms in relation to number of inocula and egg counts in lambs experimentally infected with Trichostrongylus probolurus

Animal	Number of	Number	of adult	worms	
Number.	larvae	Total	Female	Male	a.p.g
377	1000	304	188	116	475 ± 1.32
371	1500	537	476	61	1250 ± 1.35
375	2000	620	357	263	563 ± 2.14

^{* =} untreated controls.

e.p.g. = eggs per gram of faeces as a mean count during the last week of experiment

Table 2: Haematological parameters in lambs experimentally infected with Trihostrongylus Probolurus (Erythrocytes series)

Veeks	e		MCHC (g/d1)	9/41				1		HCV (MCV (f1)	MCV (f1)
following	Group I		Grou	11 d	Grou	111 d	Group	- 1	;	Group	Group II	Group II Group III Group I Group III
	377 3	78	378 371 372 373	372	373	375	377	378		371	371 372	375 377 378 371 372 373 375
1	34.1 5	1.3	35	37.9	36.1	40.6	41.4	28	5	.5 44.9	.5 44.9 45.9	34.1 51.3 35 37.9 36.1 40.6 41.4 28.5 44.9 45.9 44.9 34
2	35.9 4	8.3	1.8	44.9	40.6	49.4	39.2	27	. 7	.7 38.1	.7 38.1 39.4	35.9 48.3 41.8 44.9 40.6 49.4 39.2 27.7 38.1 39.4 42.4 34
· (3	36.7 37.2	7.2	38	37.2	40.3	46.7	37.0	2.8	.9	35.0	35.0 38.3	37.2 40.3 46.7 37.0 28.9 35.0 38.3 41.9 33.1
_	34.5	5	0	27 1		,						

4	3	N B	1 8	1	90	Vooka
.0	8.1	.17	. 21	377	Group	
8.78	8.66	8.67	10.52	378		RBC
8.69	8.56	8.92	7.13	371	Grou	×
8.5	8.35	8.88	7.19	372	P 11	10 %
8.0 8.78 8.69 8.5 7.92 6.12 29 24 30 31	8.66 8.56 8.35 7.39 5.43	8.17 8.67 8.92 8.88 7.55 5.0	8.21 10.52 7.13 7.19 6.91 5.0	378 371 372 373 375 377 378 371 372	Group	RBC (X 10 %/C mm)
6.12	5.43	5.0	5.0	375	Ξ	
29	30	32 24 34 35	34 30 32 33	377	Grou	
24	25 30 32	24	30	378	p 1	P
30	30	34	32	371	Gro	PCV (%)
31	32	35	33	372	11 dn	*
31	31	32	31	373	Group II Group III Group I Group III Group I Group II Group III	
18	18	17	17	375	111	
31 18 10.0 8.4 11.4 11.5 13.0 8.7	18 11.0 9.3 11.4 11.9 12.5 8.4	17 11.5 11.6 14.2 15.7 13.0 8.4	31 17 11.6 15.4 11.2 12.5 11.2 6.9	373 375 377 378 371 372 373 375	Grou	НЪ
° 30	9.3	11.6	15.4	378	D I	con
11.4	11.4	14.2	11.2	371	Group	centra
11.5	11.9	15.7	12.5	372	11 6	tion
13.0	12.5	13.0	11.2	373	Group	Hb concentration (g/dl)
8		8.	6.	375	=	-

following infection Table 3 (Contd) Group I Group III Group III 377 378 Monocytes (%) 372 373 £ : Group 1 378 371 Group II Fosinophils (%) Group III Group I Basophils (%) Group II Group III

Ueeks	Tota	- E	Total WBC (X 103/C mm)	103/	(C mm)				 L.ymphc	Lymphocytes	Lymphocytes (%)	Lymphocytes (%)	Lymphocytes (%)	Lymphocytes (%)	Lymphocytes (%)	Lymphocytes (%) Neutrophils	Lymphocytes (%) Neutrophils
following	Group	1	Grou	p 11	Grou	111 d	Gro	1 du	Grou	Group II	Group II Group	Group II Group III	Group II Group III Grou	Group II Group III Group I	Group II Group III Group I Grou	Group II Group III Group I Group II	following Group I Group III Group I Group II Group II Group I Group II Group II Group III
	377 378 371	78	371	372	373	375	377	378	371	371 372	372 373 375 377 378 371 372 373	375	375	375	375	375	
	6.55 3.75 4.3 6.3 6.65 7.75 55	.75	4.3	6.3	6.65	7.75	55	52			26 15 25	26 15 25	26 15 25	26 15 25		26 15 25	26 15 25 31 21 25 51
	6.85 6.25 8.9	.25	8.9	7.7	7.7 8.0 6.3	6.3	5	50	 21		21 25 44	2.5	25 44	25 44 43 34	25 44 43	25 44 43 34	25 44 43 34 40 62
w	9.1 8.8 9.35 10.9 9.25 5.8		9.35	10.9	9.25	5.8	20	24	28	28 25	28 25 22	25	25 22	25 22 43 55	25 22 43	25 22 43 55	25 22 43 55 65 51
	10.2 7.1 8.03 7.8 8.6 5.6	-	8.03	7.8	8.6		47	46	5	15 41	15 41 32	15 41 32 45	15 41 32 45 31	15 41 32 45 31	15 41 32 45 31	15 41 32 45	33 46 31

Table 3:

lus probolrus (Leckoytes series)

Haematological parameters in lambs experimentally infected with Trichostrongy-

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Table 4: The effect of ivermectin (Ivomec MSD) on egg counts in lambs experimentally infected with Trichostrongylus probolurus.

Veeks			Eggs/gram	faeces		
following	Group	I	Group	II	Group	111
Intection	377	378	371*	372	375*	373
1	0	0	0	0	0	0
2	0	0	o	0	o	0
3	188±1.31	38+2.5	225±1.55	450±3.34	238+1.80	475±3.77
4	288±1.80	225+9.6	525+2.18	1025+1.19	475± .87	1000±4.14
5	475+1.32	13+2.5	1250+1.35	213+4.25	563+4.25	63+1.25

^{* =} untreated controls

^{** =} weeks following treatment