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THE PATHOLOGICAL PHYSIOLOGY OF *GAIGERIA PACHYSCELIS* INFESTATION

R. J. HART* and ADRIANA M. WAGNER, Veterinary Research Institute, Onderstepoort

ABSTRACT

HART, R. J. & WAGNER, ADRIANA, M. The pathological physiology of *Gaigeria pachyscelis* infestation. *Onderstepoort J. vet Res.*, 38 (2), 111-116 (1971).

In two experiments, sheep under controlled conditions were artificially infested with the hookworm *Gaigeria pachyscelis* Railliet & Henry, 1910.

The effects of the helminth infestation on the host's metabolism were monitored by a series of blood, chemical and enzyme analyses.

Significant changes recorded were the development of a macrocytic normochromic anaemia, hypoproteinaemia, hypocalcaemia, hyperglycaemia and eosinophilia.

The disease and death of some of the sheep were due primarily to loss of blood.

INTRODUCTION

The hookworm *Gaigeria pachyscelis* Railliet & Henry, 1910 was considered by Ortlepp (1937) to be one of the most serious parasites of sheep in South Africa. Since that time no information has appeared on the pathological changes in *Gaigeria* infested sheep to explain why this worm has such a deleterious effect upon its host.

The experiments described were undertaken in an attempt to characterize the effects of the worm burden on the host and to offer an explanation for fatalities which occur in cases of hookworm infestation.

In this study of sheep artificially infested with *G. pachyscelis,* two experiments were carried out. Firstly a small number of animals, kept under rigidly controlled conditions, was used as a basis for haematological measurements and blood chemical studies. The regular monitoring of changes in blood constituents, it was hoped, would give some indication of the altered metabolism of the host. A larger number of sheep was used in the second experiment to confirm the significance of effects observed in the first test.

MATERIALS AND METHODS

Experiment 1

Six worm-free Merino lambs were housed individually and paired according to body weight. One lamb of each pair was infested and the other remained worm-free.

While all sheep had free access to water, the uninfested control sheep were given the amount of food that their respective infested partners had consumed from an *ad libitum* supply on the previous day. Sheep 2, 4 and 6 were infested with 450, 300 and 150 infective larvae of *G. pachyscelis* respectively. The larvae, suspended in a few drops of water, were applied to a small area of shaven skin at the base of the neck, and the sheep were immobilized until the skin was no longer wet.

Water and feed (chopped lucerne hay) consumption and rectal temperatures were recorded daily. Blood analyses and body weight checks were carried out weekly. Respiratory rate measurements, faecal egg counts and tests for the presence of faecal blood were also carried out periodically.

At death or on slaughter, worms were recovered by washing out the whole of the gut contents with water and examining these, after suitable dilution, in a black tray. Infestation rates and worm burdens are given in Table 1.

Experiment 2

On completion of the initial study, 10 worm-free Merino lambs and 10 worm-free Dorper lambs were divided into groups balanced as far as possible by weight. Six infested groups (3 Merino and 3 Dorper) containing two lambs each and two control groups (1 Merino and 1 Dorper) containing four lambs each were formed. Lambs in the infested groups were dosed percutaneously with 900, 600 or 300 *G. pachyscelis* larvae according to the plan in Table 1. These sheep were housed together on concrete with free access to food and water. The purpose of these subsequent infestations was to confirm some of the results obtained from the individually housed sheep.

Blood samples were taken weekly for analysis. Faecal egg counts and body weights were also recorded. At death or slaughter of the surviving animals (in the 18th week of the infestation) worms were recovered and counted as in Experiment 1.

TABLE 1 *Infestation rates and worm burdens*

	Experiment 1						Experiment 2									
Merino No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Larvae given	0	300	0	450	0	150	300	300	600	600	900	900		Controls		
Adult worms recovered .	—	115+	—	283+	—	75	89	66	20	216	63	290	—	—	—	—
Dorper no.							17	18	19	20	21	22	23	24	25	26
Larvae given							300	300	600	600	900	900		Controls		
Adult worms recovered .							44	63	179	103*	290	191	—	—	—	—

+Died eleven weeks after infestation
*Died six weeks after infestation

*Present address: Cooper Technical Bureau,
Berkhamsted, Hertfordshire,
England.

Investigations carried out while a guest worker at Onderstepoort.

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Erythrocyte and leucocyte counts were done according to standard methods.

The following blood analyses were done by the methods indicated:

Blood sugar:	Hawk, Oser & Summerson (1954)
Blood urea nitrogen:	Hench & Aldrich (1926) modified by Bild (1955)
Plasma bicarbonate:	Van Slyke, Stillman & Cullen (1919)
Plasma phosphate:	Wootton (1964)
Plasma calcium:	Ferro & Ham (1957)
Glutamic oxalate transaminase and glutamic pyruvate transaminase:	King (1958)
Lactate dehydrogenase:	Wroblewski & La Due (1955)
Total plasma proteins:	Weischelbaum (1946)
Serum iron:	King & Wootton (1956)
Haemoglobin:	King & Wootton (1956)
Packed cell volume:	Standard Wintrobe tube method
Cortisol	S. J. van Rensburg (In Press)

Electrophoresis of plasma proteins was carried out using a Beckman Microzone apparatus on a cellulose acetate membrane and subsequent scanning of the stained membrane was done on the Beckman model RB Analytrol Densitometer, according to the manufacturer's procedures.

Examination for the presence of blood in the faeces was performed according to the method of Wootton (1964).

Results

Food and water intake and body weight (Table 2)

In Experiment 1 a very slight decline in food intake was seen in Sheep 4 and 6 from the sixth week after infestation. There was a corresponding decrease in the body weight of these two sheep and their controls (Sheep 3 and 5). The body weight of Sheep 1 (control) fell slightly over the whole experimental period. Sheep 2 and 4 ate very little food in the last 3 days before they died in the eleventh week of the infestation.

There was a slight general increase in the water consumption of all six sheep from time to time; this was probably due to increasing ambient temperatures from September to January.

Table 2 *Food and water intake, Body weights, Temperature and Respiration Rate*

Experiment 1:

	Sheep No.	Weeks after infestation										
		1	2	3	4	5	6	7	8	9	10	11
Average daily food intake g.	2	546	503	78	679	779	725	583	569	701	701	476
	4	631	662	703	653	676	623	447	469	389	391	320
	6	710	547	624	750	717	653	485	545	553	542	588
Average daily water intake l.	1	1,6	1,9	1,9	2,4	1,8	2,3	2,5	1,7	2,0	2,1	1,7
	2	1,3	2,2	2,1	2,0	2,2	2,2	1,8	1,8	2,3	2,8	2,4
	3	1,7	2,1	2,0	2,4	2,1	2,3	2,2	1,6	2,0	2,2	1,6
	4	1,7	1,9	2,3	2,1	2,3	1,8	1,6	1,3	1,7	1,9	1,6
	5	2,0	2,0	2,3	2,4	2,7	2,7	2,2	2,4	2,4	3,0	3,0
	6	2,0	2,3	2,3	2,5	3,3	2,3	2,0	2,3	2,3	2,5	2,1
Body weight kg.	1	21	20	19,5	20	19,5	19,5	19	19	18,5	19	19
	2	20,5	20	21	22	20	20	20	20	20,5	20	20
	3	17,5	17	17,5	19,5	17,5	17	15	15,5	15	15	15
	4	16	16	17	19	17,5	16	15,5	15,5	15	15	14
	5	24,5	22	22,5	25,5	24	23	22	22	21,5	22	22,5
	6	23	22	22,5	25	23	22	22	21,5	22	22	22
Rectal temperature °F	1	102,3	100,5	102,5	102,5	101,7	101,9	102,3	102,2	102,4	102,3	102,6
	2	101,0	99,9	101,2	101,3	101,4	102,7	101,4	102,5	102,5	102,4	100,7
	3	100,1	101,5	102,3	102,0	101,5	102,0	101,6	101,7	101,8	101,9	102,7
	4	100,6	100,3	102,1	102,2	101,8	102,4	102,7	102,4	103,1	103,1	103,1
	5	101,7	102,0	101,7	101,8	101,6	102,4	101,8	101,4	101,9	101,9	101,9
	6	101,7	101,5	102,6	102,3	102,2	102,6	101,6	102,7	102,5	101,9	102,6
Respiration per min	1	—	—	—	—	—	20	—	26	—	35	—
	2	—	—	—	—	—	166	—	144	—	130	—
	3	—	—	—	—	—	44	—	36	—	26	—
	4	—	—	—	—	—	73	—	76	—	50	—
	5	—	—	—	—	—	38	—	34	—	34	—
	6	—	—	—	—	—	60	—	54	—	50	—

Table 3 *Body weight*

Experiment 2:

	Weeks after infestation						
Mean group weights - kg.	0	3	6	9	10	11	15
Merino test:	18,5	20	22	23,5	22,5	23,5	24
Control:	21	22	23	27	26	28,5	31,5
Dorper test:	19	20	21,5	22	20	21,5	21,5
Control:	19	18	21,5	21,5	20	21,5	23,5

With free access to food in Experiment 2, the sheep showed no decrease in body weight although most of the controls, particularly the Merinos, gained weight more rapidly than the infested animals (see Table 3).

Temperature and respiratory rate (Table 2)

No detectable effect on rectal temperature was observed with the exception of a slight rise in Sheep 4 a week before death.

There was a marked increase in the respiratory rates of all infested animals as compared with the controls.

Blood chemistry

There were marked changes in packed cell volume, haemoglobin concentrations, plasma proteins, blood sugar levels and plasma calcium levels among the infested sheep recorded in the weekly tests during Experiment 1. Random tests in Experiment 2 confirmed these results.

No changes outside the limits set by the controls were noted in the plasma inorganic phosphate, plasma bicarbonate, plasma transaminases and plasma lactate dehydrogenase.

Blood analyses of the heavily infested Sheep 2 and 4 revealed erratic elevations of blood urea nitrogen but similar high values occasionally occurred in the controls, particularly Sheep 3, and in a check among the sheep used in Experiment 2 no discrepancy was noted between control and infested animals.

Packed cell volume and haemoglobin concentrations (Fig. 1, 2 and 3, Table 4)

The packed cell volume fell markedly after the fifth week of the infestation in Sheep 4, after Week 6 in Sheep 2, and only slightly after Week 7 in the most lightly infested animal (Sheep 6). The lowest level recorded in Experiment 1 was 12 per cent in Sheep 4 a week prior to death.

Packed cell volumes of individually infested animals started to fall by the fourth week of Experiment 2, but a decline in the Dorper group mean was not evident until the sixth week and in the Merino group mean until the ninth week.

Haemoglobin levels of the infested sheep in both experiments followed the decline in packed cell volumes; the lowest recorded level was 2,6 g/100 ml in Sheep 19, Week 16.

Total Plasma Proteins (Fig. 4, Table 4)

A fall in plasma protein concentration was evident from the fourth week in Sheep 2 and 4 in the initial trial and although a similar trend was recorded for Sheep 6 from the sixth week onwards it was less marked.

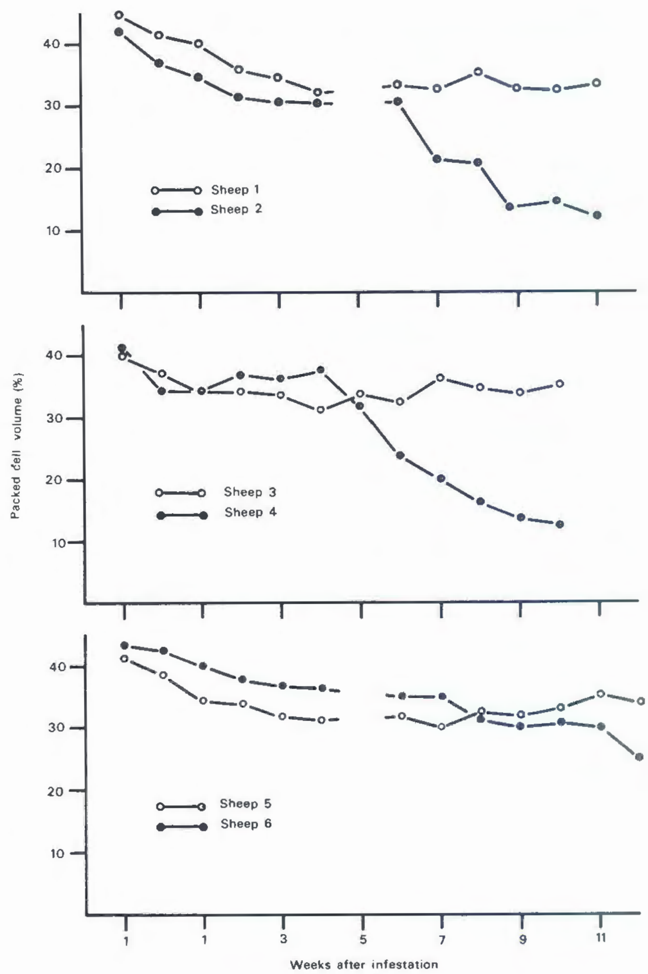

FIG. 1 Experiment 1: Packed cell volume per cent

In Experiment 2 it was confirmed that infested animals had lower plasma protein levels than the controls, the Dorpers being slightly more affected that the Merinos.

Electrophoretic analysis of the plasma revealed, in both experiments, that the lower protein levels were due to a drop in the plasma albumin concentration.

There was no increase in the globulin fractions.

TABLE 4 *Confirmatory test results*

Experiment 2:

Test	Blood sugar mg/100 ml	Total plasma protein g/100 ml	Calcium mg/100 ml	Haemoglobin g/100 ml	Eosinophils %
	week 10	week 10	week 13	week 16	week 12
Merino					
mean	52,6	5,3	8,8	8,0	10,2
Standard error	3,26	0,17	1,24	1,20	3,4
Control					
mean	48,9	6,8	11,1	12,2	7,5
Standard error	1,41	0,22	0,4	0,49	1,8
Dorper					
mean	47,8	4,7	9,2	6,3	24,6
standard error	3,34	0,24	0,59	1,49	6,8
Control					
mean	39,8	6,7	10,8	13,2	8,78
standard error	2,14	0,18	0,24	0,49	2,9

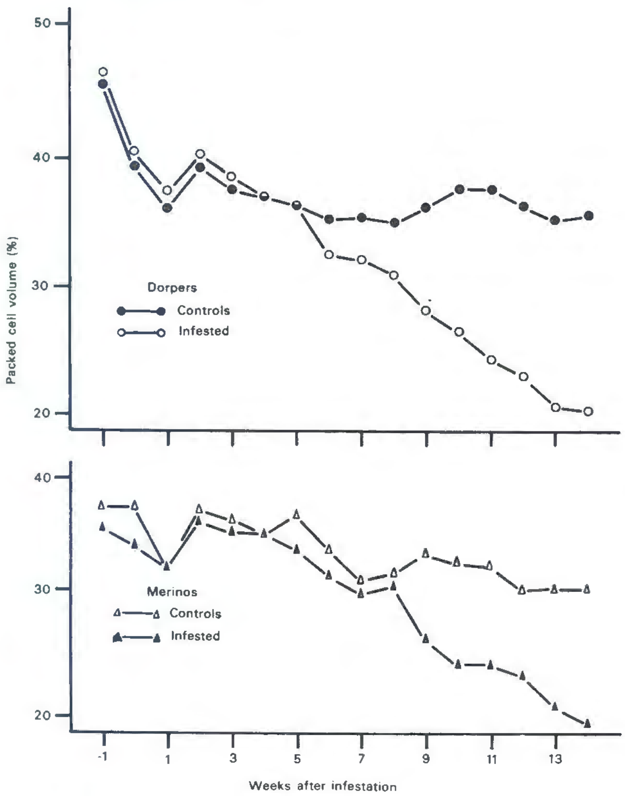

FIG. 2 Experiment 2: Packed cell volume per cent

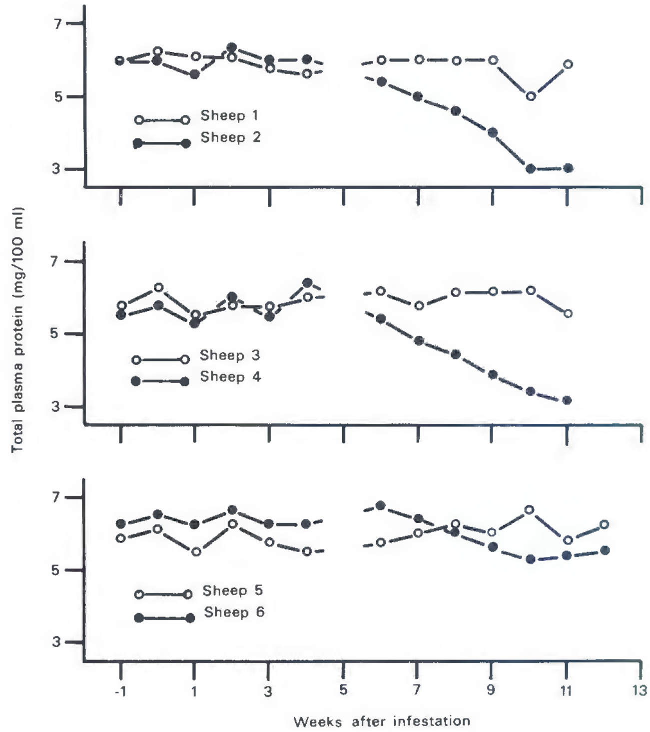

FIG. 4 Experiment 1: Total plasma protein mg/100 ml

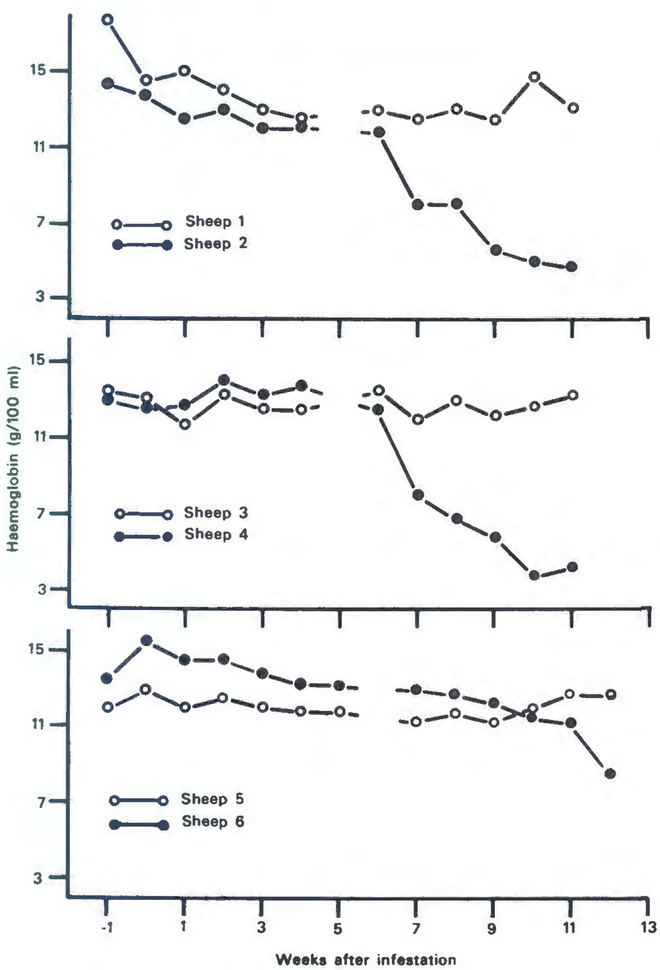

FIG. 3 Experiment 1: Haemoglobin g/100 ml

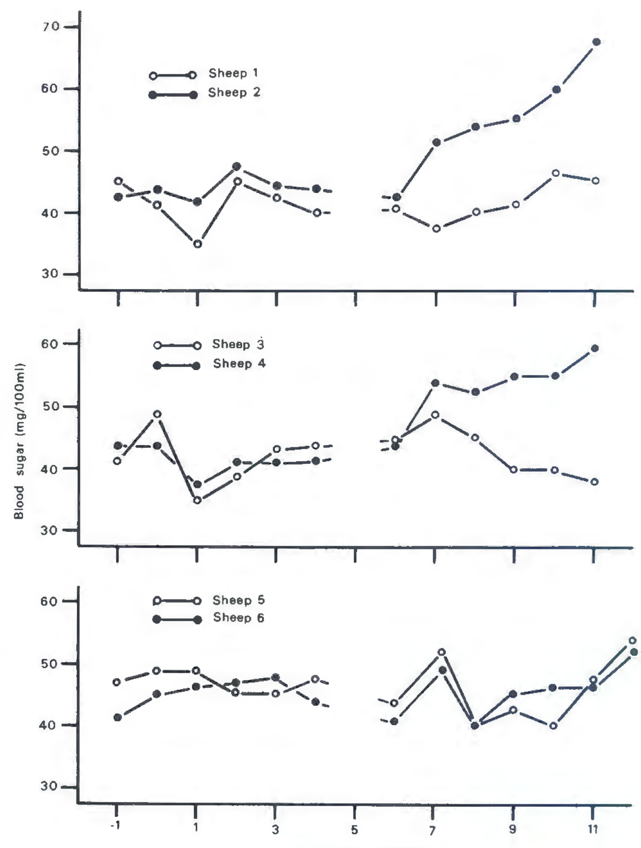

FIG. 5 Experiment 1: Blood sugar mg/100 ml

Blood sugar (Fig. 5 Table 4)

The blood sugar concentration of the two heavily infested Merino lambs in the first experiment began to rise during the sixth week after infestation. These levels continued to rise reaching a maximum of 68 mg/100 ml in Sheep 2 at the last analysis before death (see Fig. 5). That of Sheep 6 showed no clear difference from its control.

Blood sugar levels in the second experiment were estimated in the tenth week of the infestation. Six of the infested groups, two Merinos and four Dorpers had levels above the highest level of their respective controls.

Plasma calcium (Fig. 6 Table 4)

A decrease in calcium concentration was observed in plasma from Sheep 2 and 4 in the first experiment. The downward trend appeared to start after the fourth week of infestation. Reduced levels of plasma calcium were confirmed in the larger groups of sheep infested subsequently in Experiment 2.

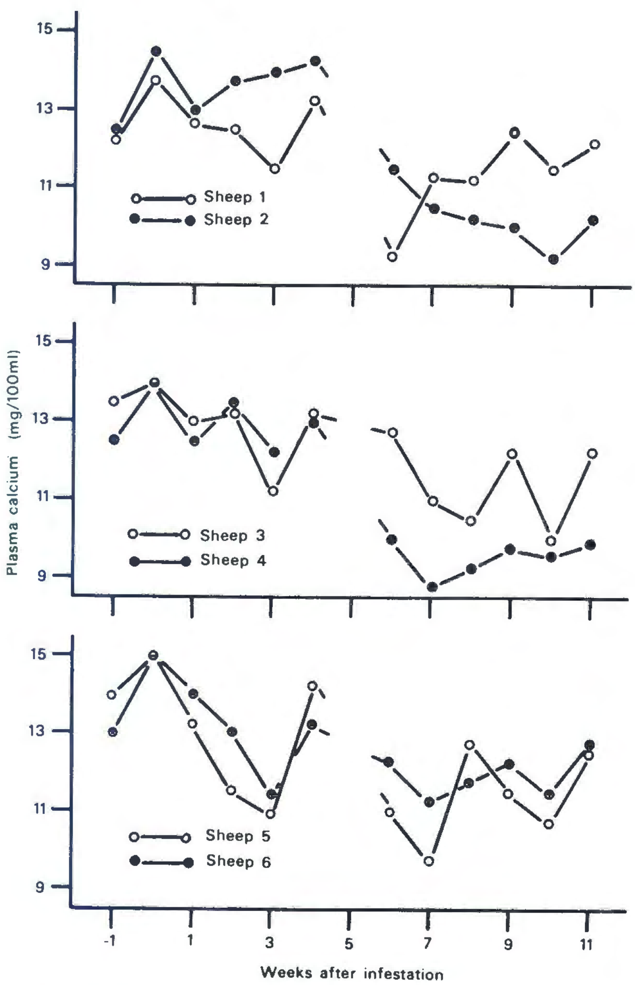

FIG. 6 Experiment 1: Plasma calcium mg/100 ml

Faecal egg counts and a test for blood in the faeces

Worm eggs were recovered in the faeces from Sheep 2 for the first time 66 days after infestation. The other infested sheep (No. 4 and 6) were positive by Day 73.

In the second part of the experiment, eggs first appeared in the faeces of some of the infested sheep on the 64th day. The faeces of all sheep were positive 79 days after infestation.

These observations confirm that the usual pre-patent period is about 10 weeks.

Blood was first detected in the faeces on Day 43 when the worms would still have been immature. All infested sheep were passing occult blood by the 64th day after infestation.

Haematology

As might be expected from the haematocrit values already mentioned, the erythrocyte counts were lower in the infested animals than in the controls. The erythrocyte counts were, in fact, lower than expected and measurements showed that the cells had increased in size. In the first experiment, from an average diameter of 3,8 μ for all sheep at the start of the trials, infested Sheep 2 and 4 had cells gradually increasing in diameter after the sixth week, to reach a maximum just before death (Week 11) when many cells were over 5 μ in diameter.

Mean corpuscular volumes were calculated in the second experiment and a maximum value of 87 μ^3 (Week 13) was found compared with the control mean of 26 μ^3. Mean corpuscular haemoglobin values were normal when compared with the control values.

No general trend could be seen in the fluctuating total leucocyte counts. There was, however, a rise in eosinophil counts, particularly marked among the Dorpers of Experiment 2. The establishment of eosinophilia appeared to take place in the seventh week of infestation. The maximum rise was to 43 per cent for Sheep 18, a Dorper, in Week 13. Values at the general peak of the eosinophilia (Week 12) are shown in Table 4.

Discussion

Helminth infestations in domestic animals frequently cause a marked loss of appetite. This has been reported in fascioliasis (Sinclair, 1962), paramphistomiasis (Horak & Clark, 1963), ostertagiasis (Horak & Clark, 1964), trichostrongylosis (Gordon, 1950) and oesophagostomiasis (Horak & Clark, 1966). In comparison the anorexia noticed in Experiment 1 after 6 weeks was mild, but must be presumed to be an effect of the helminth burden.

No change in water consumption during the experiment could be attributed to the infestation.

At no time were abnormal rectal temperatures recorded so it may be assumed that the observations here are not complicated by secondary bacterial infections as sometimes occurs in helminth disease.

The increase in respiratory rates was probably due to the anaemia and concurrently reduced haemoglobin concentration.

The only other clinical sign noted was the development of a submandibular oedema in several of the Dorpers, the first two cases occurring in the tenth week of infestation; this could have been a secondary effect resulting from the severe anaemia and the hypo-albuminaemia. Although not obvious in the infested Merinos during the experimental period, oedematous tissue was seen in the post mortem examinations.

The infestations caused fewer deaths than expected although other fatalities might have ensured had the experimental period been longer. The three sheep which did die harboured 103, 118 and 283 worms respectively, yet five other sheep with between 179 and 290 worms were still in fair condition up to the time of slaughter. A burden of 290 worms is therefore not invariably lethal though it can be postulated that death can be caused by a much smaller number in sheep grazing and living under field conditions.

The physiological findings that were attributed to the infestation were elevated blood sugar, lowered plasma calcium and plasma protein concentrations and high eosinophil counts together with a macrocytic normochromic anaemia. Statistical analysis showed that the lowered plasma protein and haemoglobin were the most significant of these observations.

It is particularly interesting to note that the onset of most of the symptoms occurred six weeks after infestation which is some three to five weeks before the worms are fully mature.

A combination of high sugar levels, low calcium levels and high eosinophil counts is indicative of stress-induced adrenal cortical hyperfunction. However, the tests for high cortisol levels did not confirm this, some infested sheep having levels below normal limits.

Eosinophilia is a symptom commonly associated with helminth infestations (Britton, 1963). As a rise in eosinophil counts is known to occur on the injection of any foreign protein into humans (Britton, 1963) it seems possible that a similar reaction would occur in sheep. It may be postulated that the worms by their intimate contact with the vascular tissues of the gut and probably by secreting a proteinaceous anticoagulant might well be the source of foreign proteins circulating in the bloodstream of the host and thus causing the observed eosinophilia.

The lowered plasma protein levels in the infected sheep may be produced by three factors. First, by a compensatory replacement of haemoglobin at the expense of circulating plasma proteins (Britton, 1963), second, by leakage of plasma proteins into the gut [Bremner (1969) has shown that cattle suffering from oesophagostomiasis lost an average of 40 g of protein a day in this way] and, third, by the loss of the blood taken by the parasites. The non-specific loss of body fluid by leakage would also account for the lowered plasma calcium values, the calcium being associated with plasma albumen would be lost with the protein.

The anaemia which developed was macrocytic and normochromic (consistent with a lack of protein - Britton, 1963) and is the same as was recorded by Sprent (1946) in cattle infested with *Bunostomum phlebotomum* (Railliet, 1900). This contrasts with the hypochromic, microcytic form usually found, at least initially, in other hookworm anaemias (Watson, 1960).

It seems likely that death in fatal cases of *Gaigeria* infestation can be explained by a completely debilitating blood loss.

Standard works of parasitology (e.g. Soulsby, 1968) suggest that Merino sheep are more susceptible than Persian sheep to the effect of *Gaigeria* infestation. No clear differences in the susceptibility of the Dorpers and Merinos were demonstrated in this experiment. If anything a very slightly greater tolerance was shown by the Merinos which might be accounted for by their slightly greater weight. In the field, the foraging ability of a particular breed could conceivably play a larger part in any observed differences than would susceptibility to or tolerance of the disease.

Summary

Six Dorpers and nine Merinos which were infested with *G. pachyscelis,* developed a macrocytic normochromic anaemia and a fall in plasma protein due to a drop in plasma albumin concentration. These changes were evident in all sheep 6 weeks after infestation. Plasma calcium had started to fall by the fourth week while the blood sugar rose from the sixth week onwards, followed by eosinophilia from the seventh week. Although five sheep survived burdens ranging from 179 to 290 worms, three sheep died with 103, 118 and 283 worms respectively, 6 to 11 weeks after infestation.

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