Experimental studies with *Strongyloides papillosus* in goats

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ABSTRACT

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Unusual clinical and pathological observations in the field in goats and sheep suffering from *Strongyloides papillosus* infection prompted experimental work on this parasite. Goats were infected percutaneously with either single or multiple, low or high levels of *S. papillosus*. Young goats up to 12 months of age were found to be the most susceptible. Some animals, however, showed substantial resistance to infective doses. Clinical signs included transient diarrhoea, misshapen, elongated faecal pellets terminally, dehydration, anorexia, cachexia, gnashing of teeth, foaming at the mouth, anaemia and nervous signs such as ataxia, a wide-based stance, stupor and nystagmus. A 'pushing syndrome' was seen in 22 % of the animals. The pathological changes are described and included enteritis, status spongiosus in the brain, hepatosis leading to rupture of the liver, nephrosis, pulmonary oedema, interstitial pneumonia and pneumonia. About 6 % of the goats died acutely from fatal hepatic rupture.

The development of an acquired immunity was determined. The immunity elicited an allergic skin reaction at the application site of larvae or injection sites of larval metabolites. This immunity, however, could be breached by large doses of larvae.

The most profound clinicopathological changes induced by the parasites were an anaemia (most pronounced in the young goats) and hypophosphataemia.

Trace element analyses provided evidence of Cu, Mn and possibly Se deficiencies in some goats.

Keywords: Goats, pathology, *Strongyloides papillosus*

INTRODUCTION

It is generally believed that severe and fatal strongyloidosis is likely to occur only in man or animals debilitated through faulty nutrition or other factors (Jones & Hunt 1983; Jubb, Kennedy & Palmer 1993).

For many years *Strongyloides papillosus* in particular has been considered to be of doubtful pathogenicity in ruminants (Beveridge 1934; Wood 1935; Neveu-Lemaire 1936; Marek 1941; Vaidyanathan 1942—all quoted by Enigk 1952a) and Hutyra, Marek & Manninger (1938). Clunies-Ross & Gordon (1936) stated that the parasitic females have not been credited with any definite pathogenic effects in sheep, which often suffer massive infections without evidence of ill effects. Mönnig (1938) regarded the genus *Strongyloides* as not very pathogenic, especially *S. papillosus* in sheep. Orlov (1938, quoted by Turner 1959a) maintained that this parasite is not a pathogen of sheep, while Round (1963) declared that infections of ruminants with this worm are usually mild and overshadowed by infection with other gastrointestinal nematodes. According to him it is probably

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for this reason that *S. papillosus* was formerly considered to be harmless (Round 1963).

Turner (1959a) pointed out that reported deaths of ruminants ascribed to a natural infection of this parasite are extremely rare. He stated that it was not regarded as a dangerous pathogen because few experimental studies had been made of its effects on the natural host. Woodhouse (1948), Messerli (1950), Vegors & Porter (1950), Enigk (1952a), Vegors (1954) and Supperer & Pfeiffer (1960) presented evidence that *S. papillosus* should indeed be regarded as a pathogen for ruminants. Turner & Wilson (1958) described the death of three lambs. These workers claimed that they were unable to find any record of naturally acquired fatal strongyloidosis in sheep, while according to Turner (1959a) deaths of goats attributable to strongyloidosis have not been reported in the literature.

More recently there has been an increasing awareness of the pathogenic potential of this parasite in ruminants. Round (1963) in Kenya recorded mortalities in sheep under natural conditions, which he ascribed to *S. papillosus* infection. Radke, Gräfner & Neetzow (1967) in West Germany found 81 % of sheep flocks examined infected while lambs in 9,9 % of these flocks showed clinical signs of strongyloidosis. In the Plateau area of Northern Nigeria, Ikeme (1970) described deaths in natural mixed infections of calves with *S. papillosus* and *Toxocara vitulorum*.

Turner (1959a) could find no reports in the literature on experimental studies in goats with this parasite apart from the cross-transmission experiment of Vegors & Porter (1953). These authors observed no pathogenic effects in any of their experimental animals. Experimentally, Turner (1959a, b) studied the pathogenicity of this helminth in sheep and goats, and Supperer & Pfeiffer (1960) made limited observations in cattle. Turner, Shalkop & Wilson (1960) reported on the migration and pathology in lambs. Astfalk & Buchwalder (1971) studied the effect on wool quality. Buchwalder (1971a) examined the influence of artificial infection with this worm on the performance of sheep of different age groups, while Moczon (1972) made detailed observations on the histopathology produced experimentally by *S. papillosus* in rabbits. Immunological studies have been reported by Turner (1959b), Stankiewicz (1969a, b) and Bezubik (1972). Haematological changes were studied by Stankiewicz (1969a, b; 1970) in sheep, Bezubik & Turner (1964) in sheep and goats, Buchwalder (1971b) in sheep, Stankiewicz & Brzozowska (1972) in sheep and Ratynska-Prill (1975) in goats. Chomicz (1967) and Lewandowska (1967) studied the effects of *S. papillosus* on the haematology of the rabbit.

This paper reports the results of a study conducted during 1966–1972 on the pathogenicity and pathology of *S. papillosus* in domestic goats.

BACKGROUND

During the period 1955–1960 a number of outbreaks of mortality in young lambs and kids during April and May were investigated in the Windhoek, Rehoboth and Mariental districts of Namibia (P.A. Basson, unpublished observations). The clinical signs seen were loss of condition, sunken eyes, chewing movements with foaming at the mouth, evidence of abdominal pain, dehydration, and occasional diarrhoea but more often dried-out faeces terminally. On one farm, kids had nervous signs, which included gnashing of teeth, aimless wandering and pushing with the head against solid objects.

Common gross pathological findings were cachexia, anaemia, nephrosis, pneumonia, enlarged lymph nodes, mild enteritis with occasional hydrothorax, hydropericardium and ascites. On the particular farm where nervous signs were observed, odd animals at necropsy exhibited hepatomegaly with haemorrhages in the liver.

High egg counts of *S. papillosus* occurred in affected animals and most of the deaths were attributed to this parasite. At the time the nervous signs and liver pathology were regarded as complications due to the coincidental ingestion of some unknown poisonous plant.

During 1966, the State Veterinarian at Gobabis in Namibia reported mortalities in goats where almost 50 % of the annual crop of kids died on a farm in the district. The highest number of deaths occurred during the period February to March and involved mainly animals 1–6 months of age.

Clinical signs and gross pathology were similar to those seen during the earlier outbreaks. Marked hepatomegaly was again encountered and some animals had died as a result of a ruptured liver and subsequent haemoperitoneum. A small percentage of adult animals in the flock developed fairly pronounced atrophy of the muscles of the back and hindquarters, to such an extent that the croup assumed an almost vertical position in some individuals (Fig. 1).

A pathogenic strain of *Escherichia coli* was isolated from these kids. As this organism was suspected to have played a major role in the mortalities, an experimental vaccine against the particular strain was prepared by the Onderstepoort Veterinary Institute (OVI) and used, but to no avail.

No undue mortalities were experienced in this flock during 1967, but from March to May 1968, deaths again occurred. The disease also appeared in goats on another farm in the same district where kids died after gradually losing condition and eventual cachexia, or died suddenly as a result of ruptured livers. Animals forwarded to the laboratory at the OVI

from the latter farm were heavily infected with *S. papillosus*. These findings prompted this investigation into the pathogenicity of this parasite in goats.

MATERIALS AND METHODS

Experimental animals

The animals used were Boer goat crosses, bred at the OVI. They were housed in stables with concrete floors which were thoroughly cleaned twice daily to ensure that they were parasitologically as clean as possible. Kids were born and individually raised in cages with wire floors, which were cleaned daily. Pregnant females were dosed with thiabendazole and levamisole before being placed in these cages for parturition. All animals were fed a mixed grain concentrate and lucerne hay *ad lib.* For certain groups of goats this ration was supplemented monthly by dosing with 2,2% sodium selenite (approximately 5 mg/25 kg) and fortnightly with 100 IU vitamin E. The animals were examined clinically and their rectal temperatures taken daily, and they were weighed weekly. Sulphonamides were used to treat animals suffering form coccidiosis, whenever necessary.

Experimental design

Ninety-four goats were used in the experiment. They were divided into 13 different groups, one of which was the control group. *S. papillosus* infections of those that were affected were achieved by the percutaneous route (*vide infra*). The details of the goats in each of the groups, the treatments they received and the outcomes are provided in Tables 1–10.

GROUP 1: PRELIMINARY TRIALS TO DETERMINE THE PATHOGENICITY OF THE STRAIN OF *S. PAPILLOSUS* USED

Seven goats (goats 1–7) were infected repeatedly (*vide infra*) and the total dose that each animal received exceeded 400 000 larvae (Table 1).

GROUP 2: PRELIMINARY TRIALS WITH REPEATED DOSES TOTALLING FROM 220 000–650 000 LARVAE

Six animals (goats 8–13) were used (Table 2).

GROUP 3: REPEATED LOW LEVEL INFECTION WITH 10 000–75 000 LARVAE, SUPPLEMENTED WITH SELENIUM AND VITAMIN E

Five animals (goats 19–23) were used (Table 3).

GROUP 4: MULTIPLE INFECTION WITH 10 000–50 000 LARVAE, SUPPLEMENTED WITH SELENIUM AND VITAMIN E

Five animals (goats 24–28) were used. Most frequently a dose of 25 000 larvae was used but occasionally 10 000 or 50 000 larvae were given (Table 4).

GROUP 5: THREE TO FOUR DOSES OF 25 000 LARVAE OVER AN 11-D PERIOD

Three young kids, 4–5 months old (goats 77–79) were used (Table 5).

GROUP 6: TWO TO THREE INFECTIONS OF 2 000–5 000 LARVAE OVER A 14-D PERIOD

Thirteen goats, goats 39–51, varying from 4–12 months of age were used (Table 6).

GROUP 7: SINGLE OR DOUBLE HIGH LEVEL INFECTION WITH 100 000–500 000 LARVAE, SUPPLEMENTED WITH SELENIUM AND VITAMIN E

Sixteen highly susceptible animals, 2 months old to adult, were infected (goats 29–38 and 71–76). These goats were tested for susceptibility 10 d previously by judging their skin reaction to the application of 25 000 larvae. They were dosed twice with thiabendazole and once with levamisole prior to experimental infection (Table 7).

GROUP 8: SINGLE LOW LEVEL INFECTION WITH 28 000–50 000 LARVAE

Fifteen goats (goats 52–66) raised under worm-free conditions were used. The ages of these animals ranged from 8–15 months (Table 8).

GROUP 9: CHALLENGING OF GOATS WITH AN ACQUIRED IMMUNITY WITH REPEATED DOSES OF LARGE NUMBERS OF LARVAE. SUPPLEMENTED WITH SELENIUM AND VITAMIN E

Four adult animals (goats 67–70) were used. From 300 000–500 000 larvae were used at a time. Doses were spaced from 100–200 d apart. Prior to the experiment the goats were exposed to 25 000–100 000 larvae to ascertain their immune status. All the animals were treated with thiabendazole from days 160–169. Two goats (goats 67 and 68) were reinfected with 400 000 larvae on day 339 and day 330, respectively (Table 9).

GROUP 10: CHALLENGING OF GOATS WITH AN ACQUIRED IMMUNITY WITH 50 000 LARVAE APPLIED ON TEN CONSECUTIVE DAYS. SUPPLEMENTED WITH SELENIUM AND VITAMIN E

Five animals varying in age from 14 months to adult, were used (goats 14–18). The immune status of the goats was determined by the skin reaction evoked by the initial application of 25 000 larvae. In addition, goats 14, 15 and 18 received 500 000 larvae on days 12 and 13 (Table 10).

CONTROLS

Five goats (goats 83–87) were kept as controls. Faecal analyses and blood chemistry were done periodically.

TABLE 1 Group 1: Preliminary trials of infection, multiple high level doses

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
1	14	19–450 (days 0–71) Total: 1 119	7,5 (day 62) 33,7 (day 70)		61 (E, G) 68 (A, W) 78 (R)	75 (KE)		Muscles Lymph nodes Intestines Liver (scar)
2	14	30–500 (days 0–148) Total: 3 530	1,9 (day 49) 0,3 (day 62) 1,4 (day 70) 0,0 (day 173)	0,21	63 (E, G) Recovered	173 (K)		
4*	24	12–500 (days 0–133) Total: 3 337	0,0 (day 49) 0,2 (day 70) 0,6 (day 84) 0,0 (day 208)	Complicated by haemonchosis	63 (E) 210 (N)	208 (KE)		Muscles Brain Anaemia
5	6	200–400 x 3 (days 0, 11, 17) Total: 950	2,3 (day 12)	129,78	11 (E) 18 (A, F, G, cough) 21 (W)	21 (KE)		Lymph nodes Lungs Mesenterium
6	2	100–225 x 3 (days 0, 11, 17) Total: 445	0,0 (day 12) 37,0 (day 17)	44,49	19 (E, W, G)	20		Lungs Muscles Liver Lymph nodes Intestines
7	6	20–360 (days 0–32) Total: 930	0,0 (days 16, 30)	6,48 Complicated by haemonchosis	28 (E) 46 (W) 51 (Dr, G)	52 (K)		Lymph nodes Anaemia Liver

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea
A = Anorexia
R = Recumbent
G = Gnashing
* = Dosed on day 194 with thiabendazole
C = Chewing
M = Mild
W = Weakness
E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 2 Group 2: Preliminary trials of infection, 2–5 doses

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
8	1,5	180 (day 0) 50 day 7)	0,0 (day 11)	36,96	None	11,00		Gall bladder Intestines Mesenterium Lymph nodes
9	14	540 (day 0) 20 x 3 (days 7, 9, 11)		32,92	9 (E) 14 (A, W) 17 (R, F, G)	18,00	–4,5	Intestines Muscles Liver (M) Lymph nodes Mesenterium Kidneys Spleen
10	7	540 (day 0) 50 (day 7) 20 x 3 (days 11, 15, 17)	18,5 (day 16)	69,94	9 (E, A) 21 (R, A)	24,00 (KE)	–3,2 (day18)	Intestines Muscles Liver Lymph nodes
11	14	360 (day 0) 50 (day 7) 20 x 3 (days 11, 15, 17)	12,6 (day 16) 1,6 (day 30)	61,46	9 (E, A)	31,00	–2,0 (day 18)	Mesenterium Serosa Lymph nodes
12	1,5	180 (day 0) 20 x 2 (days 7, 9)		118,72	9 (E, W)	11	–0,45	Decomposed Muscle Lung Hydrothorax
13	1,5	180 (day 0) 20 x 2 (days 7, 9)	0,0 (day 11)	93,59	9 (E)	13,00		Intestines Muscle Lymph nodes Liver (M) Lungs

PI = Post infection
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F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 3 Group 3: Repeated low level infection, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
19	6	10–75 x 23 (days 0–49) Total: 1 085	100,6 (day 52)	48,09	20 (E) 52 (F, W)	52 (KE)	–2,7	Mesenteriums Intestines Lymph nodes Anaemia (M)
20	3	10–75 x 23 (days 0–49) Total: 1 135	0,4 (day 52)	54,1	34 (E) 49 (Cough)	52	–1,8	Intestines Lungs Anaemia (M)
21	3	10–75 x 21 (days 0–45) Total: 1 035	34,8 (day 40)	40,3	38 (E) 38 (E) 40 (Knuckling)	40 (KE)	–2,5	Intestines Muscles Lungs
22	28	10–75 x 25 (days 0–65) Total: 1 485	12,0 (day 57) 2,3 (day 70) 14,8 (day 78) 78,8 (day 127) 5,0 (day 134)	17,16	35 (E) 92 (Dr) Recovered	208 (K)	+11,8	Intestines
23	Adult	10–75 x 30 (days 0–64) Total: 1 410	0,8 (day 60) 1,1 (day 73) 2,0 (day 78) 1,4 (day 134)		38 (E) 45 (Dr) Recovered Dosed	Discharged (day 322)	+4,0	

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F = Foaming
Dr = Diarrhoea
A = Anorexia
R = Recumbent
G = Gnashing
C = Chewing
M = Mild
W = Weakness
E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 4 Group 4: Multiple infection, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
24	6	10–50 x 25 (days 0–55) Total: 660	2,6 (day 56)	49,00	34 (E) 56(Dr, N)	56 (KE)	–2,7	Intestines Muscles Mesenteriums Lymph nodes Anaemia (M)
25	3	10–5 x 18 (days 0–38) Total: 485	71,4 (day 41)	22,44	34 (E) 37 (Dr) 41 (R)	41 (KE)	–3,0	Muscles Liver Lymph nodes Lungs
26	Adult	10–50 x 30 (days 0–64) Total: 785	12,0 (day 63) 14,4 (day 84) 379,5 (day 127)	8,75	38 (E) 120 (W) 127 (G)	127 (K)	–5,9	Brain (S) Intestines Muscles
27	3	10–50 x 28 (days 0–58) Total: 735	114,4 (day 70)	16,88	37 (E) 67 (Dyspnoea) 70 (A)	70 (K)	+0,9	Intestines Brain Liver Lungs Muscles Kidneys Lymph nodes Anaemia
28	Adult	10–50 x 50 (days 0–73) Total: 785	4,4 (day 57) 5,4 (day 64) 4,4 (day 78)		41 (E) Lambed normally	Discharged (day 359)	+5,5	

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K = Killed
F = Foaming
Dr = Diarrhoea
A = Anorexia
R = Recumbent
G = Gnashing
C = Chewing
M = Mild
W = Weakness
E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 5 Group 5: Three to four doses of 25 000 infective larvae

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
77	4	25 x 3 (days 0, 4, 7)	493,2 (day 28)		28 (Dr)	28 (KE)	–1,4 (day 14)	Intestines
78	4	25 x 4 (days 0, 4, 7, 11)	43,5 (day 27)		22 (Lame in one hind leg)	27	–3,6 Intestines	Brain (oedema) Lung
79	5	25 x 4 (days 0, 4, 7, 11)	0 (day 36)		Progressive weakness	36 (KE)	–6,1	Intestines Liver

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Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 6 Group 6: Two to three very small infections

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
39	4	2 x 2 (day 0, 14)	1,8 (day 19) 2,5 (days 70, 110) 0,0 (days 91, 233, 399)		None	Discharged (day 455)	+23,0	
40	4	5 x 2 (days 0, 14)	15,5 (day 19) 10,2 (day 68) 0,0 (days 90, 233)		None	Discharged (day 455)	+22,0	
41	5	5 x 3 (days 0, 7, 14)	3,3 (day 19) 11,2 (day 70) 0,4 (day 91) 6,3 (day 11064) 0,0 (day 242)		None	Discharged (day 455)	+26,0	
42	8	2 x 3 (days 0, 7, 14)	6,0 (day 19) 14,1 (day 70) 0,0 (day 91) 13,5 (day 164)	9,64	Sudden death	164	+4,8	Liver rupture Kidneys Brain Anaemia
43	6	5 x 3 (days 0, 7, 14)	26,2 (day 19) 22,7 (day 71) 28,0 (day 111)	7,33	196 (R)	196 (K)	+1,6	Liver (scar) Brain (S) Cachexia

TABLE 6 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
44	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 2,5 (day 18) 2,1 (day 103) 0,0 (days 144–171) 2,4 (day 186)		111 (Abortion)	Discharged (day 245)	+9,3	
45	12	3 x 3 (days 0, 7, 14)	0,4 (day 11) 9,1 (day 38) 0,0 (days 117–164) 2,9 (day 354)		None	Discharged (day 354)	+17,0	
46	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 5,8 (day 38) 0,0 (days 117–171) 2,5 (day 354)		None	Discharged (day 354)	+16,6	
47	12	3 x 3 (days 0, 9, 14)	0,3 (day 11) 5,0 (day 38) 0,0 (days 117–171) 0,5 (day 217)		None	Discharged (day 217)	+10,2	
48	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 3,1 (day 80) 0,0 (days 111–354)		None	Discharged (day 354)	+26,0	
49	12	3 x 3 (days 0, 7, 14)	0,9 (day 11) 10,2 (day 38) 2,2 (day 111) 0,0 (days 217–354)		None	Discharged (day 354)	+15,0	
50	12	3 x 3 (days 0, 7, 14)	0,9 (day 11) 12,0 (day 38) 1,4 (day 179) 2,1 (day 269) 2,7 (day 288)		None	Discharged (day 354)	+21,1	
51	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 16,2 (day 38) 4,8 (day 107) 0,4 (day 354)		None	Discharged (day 354)	+14,5	

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R = Recumbent
G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 7 Group 7: Single or double high level infection, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
29	6	300,0 (day 0)	5,0 (day 53) 135,6 (day 59)	11,02	24 (E) 55 (Dr) 57 (A) 59 (W)	59 (K)	–1,10	Intestines Mucles, lungs Liver (M), kidney Anaemia
30	28	300,0 (day 0)	9,8 (day 53) 1,2 (day 97)	14,29	28 (E) 86 (A) 87 (N) 97 (N)	97 (K)	–1,80	Brian (S) Intestines Lymph nodes
31	Adult	300,0 (day 0)	2,4 (day 64) 8,0 (day 151)	0,78	95 (Knuckling)	151 (K)	–2,70 (day 42)	Brain (S) Intestines
32	2	300,0 (day 0)	1,2 (day 9)	72,3	9 (Dr)	9	0,00	Intestines, lungs
33	4	300,0 (day 0)	10,4 (day 27) 0,0 (day 43)	84,7	7 (Dr) 25 (A) 36,0 (W 41,0 (R)	43	0,00	Muscle, liver Intestines Brain (S) Lymph nodes Anaemia (M)
34	2	300,0 (day 0)	1,4 (day 18)	14,4	11 (E) 13 (A) 15 (Dr) 17 (W, F)	18	–1,10	Intestines (*E. coli*) Lungs, kidney Muscle (M) Lymph nodes
35	2	300,0 (day 0)	21,6 (day 16)	138,0	9 (Dr) 10 (A) 12 (E) 13 (R)	14	–0,40	Intestines (*E. coli*) Heart
36	12	500,0 (day 0)	259,6 (day 16)	158,3	12 (E, W) 15 (R)	16	+0,36	Lymph nodes Intestines Lungs, liver (S) Spleen, kidney
37	Adult	300,0 (day 0) 200,0 (day 142)	2,8 (day 56)		24 (E)	Discharged	+19,8 (day 206)	
38	Adult	300,0 (day 0) 200,0 (day 141)	4,0 (day 56)		23 (E)	Discharged	+12,0 (day 195)	
71	4	100 (day 0)	326,0 (day 29)		Weakness Emaciation	29 (KE)	–3,2	Intestines Lymph nodes Gall bladder Peritoneum Muscle Anaemia

TABLE 7 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
72	12	100 (day 0) 300 (day 12)	408,0 (day 29)		Weakness Emaciation	29	–6,6	Intestines Peritoneum Gall bladder Brain (oedema) Liver (M)
73	6	100 (day 0)	48,8 (day 21)		21 (Dr, E)	21	–3,6 (day 14)	Intestines Lymph nodes Liver
74	4	100 (day 0)	596,0 (day 27)		23 (C, F)	27 (KE)	–3,2 (day 22)	Intestines Muscles Anaemia (M)
75	5	100 (day 0) 300 (day 12)			23 (C, E, F)	23 (KE)	–5,2	Intestines Lungs Muscles Kidneys
76	4	100 (day 0) 300 (day 12)	78,8 (day 22)		Emaciation	22 (KE)		Intestines Cachexia

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G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 8 Group 8: Single low level infection of goats reared worm-free

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
52	14	8 (day 0) 20 (day 4)	5,4 (day 48) 5,6 (day 217)		152 (N, E, F) 216 (N) 217 (R)	217	–4,3	Brain (S) Liver (scar) Muscles
53	14	40 (day 0)	8,6 (day 164) 0,0 (day 201) 9,2 (day 223) 17,2 (day 250) 6,5 (day 271)		55 (Dr) 237 (N, E)	276 (KE)	–7,3	Brain (S) Liver (scar) Intestines
54	12	50 (day 0)	1,1 (day 11) 15,3 (day 18) 0,5 (day 38) 20,4 (day 53)		55 (W) 56 (F, N)	56 (KE)	–8,2	Kidneys Intestines Liver
55	14	40 (day 0)	0,4 (day 11 8,0 (day 18) 46,6 (day 117) 89,2 (day 147)		103 (N)	147	–7,7	Brain (S) Lungs
56	11	50 (day 0)	0,0 (day 11) 61,6 (day 18) 81,2 (day 41)		Progressive emaciation	82	–5,5	Brain Liver (S) Ascites Kidneys
57	12	50 (day 0)				Discharged	–3,9 until day 38 Gain after day 38	
58	12	30 (day 0)	19,8 (day 22)	17,1	Sudden death	22	–0,7	Liver rupture Intestine Anaemia
59	9	30 (day 0)	16,8 (day 28)		Emaciation	119	–4,5	Decomposed Liver (scar) Brain NA*
60	8	50 (day 0)			Emaciation	42	–6,6	Gall bladder Liver Other organs NA*
61	15	50 (day 0)	104,6 (day 80)		Emaciation	91	–4,8	Liver (scar) Muscle, heart Kidney

TABLE 8 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
61	15	50,0 (day 0)	104,6 (day 80)		Emaciation	91	–4,8	Liver (scar) Muscle, heart Kidney
62	12	50,0 (day 0)	11,0 (day 13) 0,0 (days 74–250)			Discharged	–2,0 (day 30)	
63	9	40,0 (day 0)	19,4 (day 70)		55 (W) Emaciation	70	–6,0	Brain, muscle Intestines Lungs, heart Lymph nodes Anaemia
64	12	40,0 (day 0)	6,1 (day 237)			Discharged	–2 (day 55)	
65	12	50,0 (day 0)	146,4 (day 144) 0,2 (day 150)		Emaciation	150	–8,4	Decomposed Brain, lungs Mesenterium
66	12	40,0 (day 0)	9,3 (day 27)		Sudden death	27	–1,0	Liver rupture Intestines Lungs, heart Lymph nodes Anaemia

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 9 Group 9: Challenging hypersensitive goats with repeated high level doses, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
67	Adult	25 (day 0) 300 (day 7) 500 (day 105) 400 (day 330)	0,0 (days2 66–85) 1,6 (day 133) 0,0 (days 290–350) 2,5 (day 351) 1,0 (day 374)	0,77	None Dosed with thiabendazole (day 160)	374 (K)	+16,4	None
68	Adult	50 (day 0) 300 (day 15) 500 (day 144) 400 (day 339)	0,1–0,2 (days 74–94) 0,9 (day 152) 0,0 (days 142, 299–360, 383) 2,9 (day 376)		None Dosed with thiabendazole (day 169)	383 (K)	+17,3	None
69	Adult	100 (day 0) 300 (day 14) 500 (day 115)	0,0 (days 74–93, 289, 650) 0,5 (day 152) 0,1–0,2 (day 629, 637)	None (day 169)	None Dosed with thiabendazole	Discharged	+9,1	
70	Adult	25 (day 0) 300 (day 8) 500 (day 106)	0,1 (day 67) 0,0 (days 71 and 86)	thiabendazole (day 161)	125 (A, E, Dr) Dosed with	Discharged	+14,5	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 9 Group 9: Challenging hypersensitive goats with repeated high level doses, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
67	Adult	25 (day 0) 300 (day 7) 500 (day 105) 400 (day 330)	0,0 (days2 66–85) 1,6 (day 133) 0,0 (days 290–350) 2,5 (day 351) 1,0 (day 374)	0,77	None Dosed with thiabendazole (day 160)	374 (K)	+16,4	None
68	Adult	50 (day 0) 300 (day 15) 500 (day 144) 400 (day 339)	0,1–0,2 (days 74–94) 0,9 (day 152) 0,0 (days 142, 299–360, 383) 2,9 (day 376)		None Dosed with thiabendazole (day 169)	383 (K)	+17,3	None
69	Adult	100 (day 0) 300 (day 14) 500 (day 115)	0,0 (days 74–93, 289, 650) 0,5 (day 152) 0,1–0,2 (day 629, 637)	None (day 169)	None Dosed with thiabendazole	Discharged	+9,1	
70	Adult	25 (day 0) 300 (day 8) 500 (day 106)	0,1 (day 67) 0,0 (days 71 and 86)	thiabendazole (day 161)	125 (A, E, Dr) Dosed with	Discharged	+14,5	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 10 Group 10: Challenging hypersensitive goats with mainly low doses

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
14	Adult	50 x 11 (days 0–10) 500 (day 13)	3,0 (day 42) 8,2 (day 50) 15,2 (day 63)	23,6	Progressive emaciation 104 (A) 128 (F, N)	135 (K)	–33,0	Osteoporosis Intestines Liver (scar) Brain (S) Lungs Lymph nodes Anaemia
15	14	50 x 11 (days 0–11) 500 (day 13)	6,7 (day 43)	30,69	29 (E, A) 43 (R, N)	43 (KE)	–5,2	Brain (S) Intestines Lymph nodes Muscles
16	20	50 x 11 (days 0–10)	4,2 (day 41) 27,6 (day 49) 174,0 (day 77)	23,6 56 (Dr)	30 (E) 37 (A) 51 (N)	77 (K)	–11,4	Intestines Brain (S) Heart Muscles Liver (S) Kidney Anaemia
17	Adult	50 x 11 (days 0–10)	0,0 (days 41, 49, 54, 62)		Lambed normally (day 266)	Discharged (day 339)	+18,2 (day 266)	
18	Adult	50 x 11 (days 0–10) 500 (day 12)	0,0 (days 42, 50, 55, 64)			Discharged (day 278)	+14,3	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 6 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
44	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 2,5 (day 18) 2,1 (day 103) 0,0 (days 144–171) 2,4 (day 186)		111 (Abortion)	Discharged (day 245)	+9,3	
45	12	3 x 3 (days 0, 7, 14)	0,4 (day 11) 9,1 (day 38) 0,0 (days 117–164) 2,9 (day 354)		None	Discharged (day 354)	+17,0	
46	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 5,8 (day 38) 0,0 (days 117–171) 2,5 (day 354)		None	Discharged (day 354)	+16,6	
47	12	3 x 3 (days 0, 9, 14)	0,3 (day 11) 5,0 (day 38) 0,0 (days 117–171) 0,5 (day 217)		None	Discharged (day 217)	+10,2	
48	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 3,1 (day 80) 0,0 (days 111–354)		None	Discharged (day 354)	+26,0	
49	12	3 x 3 (days 0, 7, 14)	0,9 (day 11) 10,2 (day 38) 2,2 (day 111) 0,0 (days 217–354)		None	Discharged (day 354)	+15,0	
50	12	3 x 3 (days 0, 7, 14)	0,9 (day 11) 12,0 (day 38) 1,4 (day 179) 2,1 (day 269) 2,7 (day 288)		None	Discharged (day 354)	+21,1	
51	12	3 x 3 (days 0, 7, 14)	0,3 (day 11) 16,2 (day 38) 4,8 (day 107) 0,4 (day 354)		None	Discharged (day 354)	+14,5	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 7 Group 7: Single or double high level infection, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
29	6	300,0 (day 0)	5,0 (day 53) 135,6 (day 59)	11,02	24 (E) 55 (Dr) 57 (A) 59 (W)	59 (K)	–1,10	Intestines Mucles, lungs Liver (M), kidney Anaemia
30	28	300,0 (day 0)	9,8 (day 53) 1,2 (day 97)	14,29	28 (E) 86 (A) 87 (N) 97 (N)	97 (K)	–1,80	Brian (S) Intestines Lymph nodes
31	Adult	300,0 (day 0)	2,4 (day 64) 8,0 (day 151)	0,78	95 (Knuckling)	151 (K)	–2,70 (day 42)	Brain (S) Intestines
32	2	300,0 (day 0)	1,2 (day 9)	72,3	9 (Dr)	9	0,00	Intestines, lungs
33	4	300,0 (day 0)	10,4 (day 27) 0,0 (day 43)	84,7	7 (Dr) 25 (A) 36,0 (W 41,0 (R)	43	0,00	Muscle, liver Intestines Brain (S) Lymph nodes Anaemia (M)
34	2	300,0 (day 0)	1,4 (day 18)	14,4	11 (E) 13 (A) 15 (Dr) 17 (W, F)	18	–1,10	Intestines (*E. coli*) Lungs, kidney Muscle (M) Lymph nodes
35	2	300,0 (day 0)	21,6 (day 16)	138,0	9 (Dr) 10 (A) 12 (E) 13 (R)	14	–0,40	Intestines (*E. coli*) Heart
36	12	500,0 (day 0)	259,6 (day 16)	158,3	12 (E, W) 15 (R)	16	+0,36	Lymph nodes Intestines Lungs, liver (S) Spleen, kidney
37	Adult	300,0 (day 0) 200,0 (day 142)	2,8 (day 56)		24 (E)	Discharged	+19,8 (day 206)	
38	Adult	300,0 (day 0) 200,0 (day 141)	4,0 (day 56)		23 (E)	Discharged	+12,0 (day 195)	
71	4	100 (day 0)	326,0 (day 29)		Weakness Emaciation	29 (KE)	–3,2	Intestines Lymph nodes Gall bladder Peritoneum Muscle Anaemia

TABLE 7 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
72	12	100 (day 0) 300 (day 12)	408,0 (day 29)		Weakness Emaciation	29	–6,6	Intestines Peritoneum Gall bladder Brain (oedema) Liver (M)
73	6	100 (day 0)	48,8 (day 21)		21 (Dr, E)	21	–3,6 (day 14)	Intestines Lymph nodes Liver
74	4	100 (day 0)	596,0 (day 27)		23 (C, F)	27 (KE)	–3,2 (day 22)	Intestines Muscles Anaemia (M)
75	5	100 (day 0) 300 (day 12)			23 (C, E, F)	23 (KE)	–5,2	Intestines Lungs Muscles Kidneys
76	4	100 (day 0) 300 (day 12)	78,8 (day 22)		Emaciation	22 (KE)		Intestines Cachexia

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 8 Group 8: Single low level infection of goats reared worm-free

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
52	14	8 (day 0) 20 (day 4)	5,4 (day 48) 5,6 (day 217)		152 (N, E, F) 216 (N) 217 (R)	217	–4,3	Brain (S) Liver (scar) Muscles
53	14	40 (day 0)	8,6 (day 164) 0,0 (day 201) 9,2 (day 223) 17,2 (day 250) 6,5 (day 271)		55 (Dr) 237 (N, E)	276 (KE)	–7,3	Brain (S) Liver (scar) Intestines
54	12	50 (day 0)	1,1 (day 11) 15,3 (day 18) 0,5 (day 38) 20,4 (day 53)		55 (W) 56 (F, N)	56 (KE)	–8,2	Kidneys Intestines Liver
55	14	40 (day 0)	0,4 (day 11 8,0 (day 18) 46,6 (day 117) 89,2 (day 147)		103 (N)	147	–7,7	Brain (S) Lungs
56	11	50 (day 0)	0,0 (day 11) 61,6 (day 18) 81,2 (day 41)		Progressive emaciation	82	–5,5	Brain Liver (S) Ascites Kidneys
57	12	50 (day 0)				Discharged	–3,9 until day 38 Gain after day 38	
58	12	30 (day 0)	19,8 (day 22)	17,1	Sudden death	22	–0,7	Liver rupture Intestine Anaemia
59	9	30 (day 0)	16,8 (day 28)		Emaciation	119	–4,5	Decomposed Liver (scar) Brain NA*
60	8	50 (day 0)			Emaciation	42	–6,6	Gall bladder Liver Other organs NA*
61	15	50 (day 0)	104,6 (day 80)		Emaciation	91	–4,8	Liver (scar) Muscle, heart Kidney

TABLE 8 *(continued)*

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
61	15	50,0 (day 0)	104,6 (day 80)		Emaciation	91	–4,8	Liver (scar) Muscle, heart Kidney
62	12	50,0 (day 0)	11,0 (day 13) 0,0 (days 74–250)			Discharged	–2,0 (day 30)	
63	9	40,0 (day 0)	19,4 (day 70)		55 (W) Emaciation	70	–6,0	Brain, muscle Intestines Lungs, heart Lymph nodes Anaemia
64	12	40,0 (day 0)	6,1 (day 237)			Discharged	–2 (day 55)	
65	12	50,0 (day 0)	146,4 (day 144) 0,2 (day 150)		Emaciation	150	–8,4	Decomposed Brain, lungs Mesenterium
66	12	40,0 (day 0)	9,3 (day 27)		Sudden death	27	–1,0	Liver rupture Intestines Lungs, heart Lymph nodes Anaemia

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 9 Group 9: Challenging hypersensitive goats with repeated high level doses, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
67	Adult	25 (day 0) 300 (day 7) 500 (day 105) 400 (day 330)	0,0 (days2 66–85) 1,6 (day 133) 0,0 (days 290–350) 2,5 (day 351) 1,0 (day 374)	0,77	None Dosed with thiabendazole (day 160)	374 (K)	+16,4	None
68	Adult	50 (day 0) 300 (day 15) 500 (day 144) 400 (day 339)	0,1–0,2 (days 74–94) 0,9 (day 152) 0,0 (days 142, 299–360, 383) 2,9 (day 376)		None Dosed with thiabendazole (day 169)	383 (K)	+17,3	None
69	Adult	100 (day 0) 300 (day 14) 500 (day 115)	0,0 (days 74–93, 289, 650) 0,5 (day 152) 0,1–0,2 (day 629, 637)	None (day 169)	None Dosed with thiabendazole	Discharged	+9,1	
70	Adult	25 (day 0) 300 (day 8) 500 (day 106)	0,1 (day 67) 0,0 (days 71 and 86)	thiabendazole (day 161)	125 (A, E, Dr) Dosed with	Discharged	+14,5	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 9 Group 9: Challenging hypersensitive goats with repeated high level doses, supplemented with selenium and vitamin E

Goat no.	Age (months)	Number of larvae (x 10^3) and exposures	Some nematode egg counts (x 10^3)	Nematode count (x 10^3)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
67	Adult	25 (day 0) 300 (day 7) 500 (day 105) 400 (day 330)	0,0 (days2 66–85) 1,6 (day 133) 0,0 (days 290–350) 2,5 (day 351) 1,0 (day 374)	0,77	None Dosed with thiabendazole (day 160)	374 (K)	+16,4	None
68	Adult	50 (day 0) 300 (day 15) 500 (day 144) 400 (day 339)	0,1–0,2 (days 74–94) 0,9 (day 152) 0,0 (days 142, 299–360, 383) 2,9 (day 376)		None Dosed with thiabendazole (day 169)	383 (K)	+17,3	None
69	Adult	100 (day 0) 300 (day 14) 500 (day 115)	0,0 (days 74–93, 289, 650) 0,5 (day 152) 0,1–0,2 (day 629, 637)	None (day 169)	None Dosed with thiabendazole	Discharged	+9,1	
70	Adult	25 (day 0) 300 (day 8) 500 (day 106)	0,1 (day 67) 0,0 (days 71 and 86)	thiabendazole (day 161)	125 (A, E, Dr) Dosed with	Discharged	+14,5	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 10 Group 10: Challenging hypersensitive goats with mainly low doses

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
14	Adult	50 x 11 (days 0–10) 500 (day 13)	3,0 (day 42) 8,2 (day 50) 15,2 (day 63)	23,6	Progressive emaciation 104 (A) 128 (F, N)	135 (K)	–33,0	Osteoporosis Intestines Liver (scar) Brain (S) Lungs Lymph nodes Anaemia
15	14	50 x 11 (days 0–11) 500 (day 13)	6,7 (day 43)	30,69	29 (E, A) 43 (R, N)	43 (KE)	–5,2	Brain (S) Intestines Lymph nodes Muscles
16	20	50 x 11 (days 0–10)	4,2 (day 41) 27,6 (day 49) 174,0 (day 77)	23,6 56 (Dr)	30 (E) 37 (A) 51 (N)	77 (K)	–11,4	Intestines Brain (S) Heart Muscles Liver (S) Kidney Anaemia
17	Adult	50 x 11 (days 0–10)	0,0 (days 41, 49, 54, 62)		Lambed normally (day 266)	Discharged (day 339)	+18,2 (day 266)	
18	Adult	50 x 11 (days 0–10) 500 (day 12)	0,0 (days 42, 50, 55, 64)			Discharged (day 278)	+14,3	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

TABLE 10 Group 10: Challenging hypersensitive goats with mainly low doses

Goat no.	Age (months)	Number of larvae ($\times 10^3$) and exposures	Some nematode egg counts ($\times 10^3$)	Nematode count ($\times 10^3$)	Onset of clinical signs (days PI)	Day of death (PI)	Mass gain/loss (kg)	Main lesions
14	Adult	50 x 11 (days 0–10) 500 (day 13)	3,0 (day 42) 8,2 (day 50) 15,2 (day 63)	23,6	Progressive emaciation 104 (A) 128 (F, N)	135 (K)	–33,0	Osteoporosis Intestines Liver (scar) Brain (S) Lungs Lymph nodes Anaemia
15	14	50 x 11 (days 0–11) 500 (day 13)	6,7 (day 43)	30,69	29 (E, A) 43 (R, N)	43 (KE)	–5,2	Brain (S) Intestines Lymph nodes Muscles
16	20	50 x 11 (days 0–10)	4,2 (day 41) 27,6 (day 49) 174,0 (day 77)	23,6 56 (Dr)	30 (E) 37 (A) 51 (N)	77 (K)	–11,4	Intestines Brain (S) Heart Muscles Liver (S) Kidney Anaemia
17	Adult	50 x 11 (days 0–10)	0,0 (days 41, 49, 54, 62)		Lambed normally (day 266)	Discharged (day 339)	+18,2 (day 266)	
18	Adult	50 x 11 (days 0–10) 500 (day 12)	0,0 (days 42, 50, 55, 64)			Discharged (day 278)	+14,3	

PI = Post infection
K = Killed
F = Foaming
Dr = Diarrhoea

A = Anorexia
R = Recumbent
G = Gnashing
* = Not available

C = Chewing
M = Mild
W = Weakness

E = Sunken eyes
KE = Killed in extremis
S = Severe

Helminthology

To obtain large numbers of larvae for experimental work, faecal cultures were initially made from natural cases from Namibia and later from experimentally produced donor animals. A modification of the technique of Roberts & O'Sullivan (1950) was used (Reinecke 1968).

After 72 h of incubation the jars containing the faeces were removed from the incubator and exposed to indirect light. Larvae that migrated up the inner surface of the jars were gently rinsed with de-ionized water into 100 mℓ measuring cylinders. This process was repeated after 1 h.

A few drops of the larval suspension were transferred to a glass slide and examined microscopically for motility. The ratio between live and dead larvae was established and used to determine the number of live larvae in the final dilutions. A drop of Lugol's iodine was then added to kill the larvae, which were closely examined to confirm the purity of the culture.

The volume of the harvested larval suspension was increased to 100 mℓ by the addition of de-ionized water. After thorough mixing of this suspension, 4–6 aliquots of 0,5 mℓ were withdrawn and mixed with Lugol's iodine in order to kill and count the larvae, and the total number of infective larvae estimated. Where very large numbers of larvae had to be counted, 1 mℓ of the larval suspension was diluted to 10 mℓ with de-ionized water. Aliquots were then withdrawn from the dilution for counting and final estimation of the number of infective larvae.

Approximately 1 h prior to infection, the desired numbers of infective larvae were pipetted into centrifuge tubes which were placed in a beaker containing water at 30 °C. The skin at the selected site of infection (about 100 mm^2) was shaved free of hairs. Immediately before infection the site was thoroughly washed with warm water and dried with cotton wool swabs. No soap, detergents or other chemicals were used to clean the skin. The supernatant of the larval suspension was discarded and the remaining fluid containing the larvae was gradually transferred to the infection site, which was kept moist while the animal was restrained for 15–20 min to allow maximal penetration of the larvae. A specific skin site was used once only.

Faecal nematode egg counts were done according to Reinecke's (1968) modification of the McMaster method of Gordon & Whitlock (1939).

The intestinal tracts and some of the gall bladders were processed for helminth recovery as described by Reinecke (1961).

Specimens of the liver, kidney, brain, lung, muscle, spleen and heart were collected from two goats (group 1: goats 3 and 6) and processed in a similar manner as that used for the mucosae of the intestinal tract.

The specimens recovered by the methods described above, were diluted with water to a volume of 2 ℓ, vigorously stirred and aliquots of one-tenth of the original volume withdrawn.

In many cases it was possible to count the worms that were recovered from the intestinal tracts macroscopically by pouring the respective aliquot into a shallow black tray of which the bottom had been divided into squares. Each square was carefully examined and all the worms counted with a hand tally-counter. A number of the worms were subsequently removed for verification of the species. In cases where the parasites could not be seen with the unaided eye or where total counts were less than 1 000 the entire specimen was examined under a stereoscopic microscope using a counting chamber, and all the worms were removed, identified and counted.

Pathology

Natural cases

Paraffin embedded specimens of liver, kidney and small intestine were available from a single kid that had died during one of the initial outbreaks in 1960 in the Gibeon district. Grossly, marked hepatomegaly with haemorrhages in the liver was present in this animal.

During the period 1966/68 formalin fixed organ specimens were received by the OVI from six fatal cases in kids from one of the farms concerned in the Gobabis district. Three of these kids had died as a result of ruptured livers.

Eight adult goats and two kids were obtained from the same farm for observation and necropsy at the OVI during 1966. Histopathological specimens from three goats on each of the two affected farms in the Gobabis district were collected during an *in situ* investigation in 1968. A further 20 animals of varying age were again acquired in 1968 and sent by rail to the OVI. Three of the goats died *en route* to the laboratory and two shortly after arrival, one of which showed hepatomegaly and rupture of the liver. The remainder was used as donors of worm larvae or for specific experimental procedures.

Experimental cases

GROSS PATHOLOGY

All the animals that died or were killed *in extremis* or those slaughtered were necropsied and the lesions studied.

MATERIAL FOR LIGHT MICROSCOPY

Specimens from various organs were collected at necropsy and fixed in 10 % buffered formalin. Mus-

cle specimens were excised, placed on cardboard strips, left to dry for 1–5 min, labelled and placed in the fixative. All tissue blocks were embedded in paraffin, and sections were cut at 5 µm thickness and stained with haematoxylin-eosin (HE) using standard procedures.

MATERIAL FOR ELECTRON MICROSCOPY

Brain specimens were collected immediately after the animals had been euthanized and were fixed in 4% glutaraldehyde in Millonig's buffer (Millonig 1961) at pH 7,2–7,4, postfixed in buffered 2% osmium tetroxide, dehydrated in graded ethyl alcohol and embedded in Araldite (Luft 1961). From the Araldite blocks sections 1–2 µm thick were cut, stained with toluidine blue pyronin (Ito & Winchester 1963) and examined under a light microscope. Suitable blocks were selected for ultra-thin sectioning. The thin sections for electron microscopy were stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963).

Skin reactions

The capacity of helminths to stimulate immediate (Type I) hypersensitivity reactions has found widespread application in immunodiagnosis (Ogilvie & Worms 1976). For more than half a century, wheal and flare reactions have been described in humans with the presumptive diagnosis of various helminthic conditions including strongyloidosis (Fülleborn 1926).

Based on the above and evidence of skin reactions of an allergic nature in goats that showed some resistance to infection in group 1 (see above) a further two trails, Experiments 11 and 12, were conducted.

EXPERIMENT 11

In this trial, skin reactions caused by larval penetration were measured. Six adult animals (group 11: goats 88–93), which were obtained from one of the original farms in Namibia, were used. These animals had been exposed to natural infection by *S. papillosus*.

A single application of 50 000 larvae was made on a shaven area on the side of the neck in a similar way as for the previous experimental groups. The thickness of the skin was measured by means of calipers prior to and 1½, 4, 6, 22, 30, 48 and 96 h after larval application (Table 14).

In addition, five adult goats from the Institute at Onderstepoort were used. They received 50 000 larvae daily for ten consecutive days (group 10). Each successive dose was applied to a new site on the skin over various parts of the body. Three goats received a final challenge of 500 000 larvae on day 12 or day 13.

EXPERIMENT 12

In this experiment the skin reaction caused by larval metabolites was determined. A number of larvae collected in sterilized glass tubes (± 7 500 per tube) were transferred to sterile tubes containing 5 mℓ deionized water and stored at 4 °C for 3 d. The supernatant containing metabolites was withdrawn and injected intradermally on the side of the neck into four animals (group 12: goats 94–97) which were regarded as immune, as they were adult animals not kept under worm-free conditions. Skin sites were prepared in the same manner as for the larvae.

Three injection sites were used in every goat. The volumes of the inoculum used in two goats were 0,1 mℓ, 0,125 mℓ and 0,25 mℓ. The other two goats each received 0,05 mℓ and 0,1 mℓ of the inoculum in two different sites. Tap water (0,05 mℓ) was injected intradermally at another site as a control measure in the latter two animals.

In two of the above-mentioned goats a few drops of the larval suspension used for infection were placed on the conjunctiva of one eye.

Clinical-pathological analysis

Determinations were done on five goats from group 3, five from group 4, ten out of 16 goats from group 7, five goats form group 10 and a control group, comprising five goats ranging in age from six months to adult.

Blood was collected both in heparin and for serum by venipuncture from the jugular vein at approximately weekly intervals in the initial phase of each experiment and then intermittently, and eventually irregularly, up to death or until it was certain that the individual would survive. The control group was bled at weekly intervals over a period of 12 weeks. The latter values were used as a standard against which those of the treatment groups were compared.

Haematological and clinicopathological tests were performed using the standard techniques employed at this laboratory at the time (1969–1972). Haematocrits (PCV) were performed using the Wintrobe tube method (Wintrobe 1967).

Chemical determinations of the various blood constituents were done according to the following methods:

- Blood urea (Brown 1957)
- Creatinine by the Jaffe reaction and glucose by the Benedict reaction as adapted by Hawk, Oser & Summerson (1954)
- Aspartate and alanine transaminases as detailed by King & Wootton (1959)
- Alkaline phosphatase (Bessey, Lowry & Brock 1946)

- Bilirubin (Malloy & Evelyn 1937)
- Total plasma protein by the biuret reaction according to King & Wootton (1959)
- The zinc sulphate turbidity test (Kunkel 1947)
- Calcium (Ferro & Ham 1957)
- Magnesium (Neil & Neely 1956)
- Sodium and potassium by flame photometry
- Inorganic phosphate by the method of Fiske & Subbarow (1925)
- Bicarbonate (Van Slyke, Stillman & Cullen 1919)

A total of 3 500 data points for these laboratory measurements were evaluated and trends in group means over time were compared.

Mineral analyses

The mineral levels analyzed were copper, manganese, zinc, cobalt, iron, magnesium and calcium. Selenium analyses were conducted in selected cases only.

At necropsy a 30–60 g liver sample was taken from the edge of the organ and placed in 10% formalin in which it was preserved until analyzed. A Beckman 979 atomic absorption spectrophotometer with a turbulent flow burner and wet ashing of the sample were used (Boyazoglu, Barrett, Young & Ebedes 1972). To the solution obtained by the above method, 20 mℓ of a 5 % lanthanum oxide solution and 79 mℓ water were added to give a 1:100 dilution. This sample was used for direct readings of iron, copper, manganese, zinc and cobalt. Five millilitres of this solution were placed in a 25 mℓ volumetric flask with a further 5 mℓ 5 % lanthanum oxide and made up to volume. This 1:500 dilution was used for determinations of magnesium and calcium.

The selenium analyses were conducted according to the method of Allaway & Vary (1964).

RESULTS

Helminths

The complete data collected on faecal nematode egg counts are not listed in Tables 1–10 because many animals had lengthy lists of counts. In these animals only the first and final counts are given when available and selected intermediate counts to indicate the wide variation that was encountered.

At the earliest nematode eggs appeared in the faeces between days 9 and 11. Very high counts were obtained within 3–4 weeks after infection but highest peaks were also reached after 5–35 weeks. Often no eggs were found in between fairly high counts. Some animals died or had to be destroyed with high (100–600 x 10^3 epg), medium (10–90 x 10^3 epg) and low counts (200–1 600 epg) (e.g. group 2: goat 11 on day 31, group 3: goat 20 on day 52, group 7: goat 30 on day 97 and group 8: goat 65 on day 150), or even no worm eggs in the faeces (e.g. group 2: goat 8 on day 11, group 5: goat 79 on day 36 and group 7: goat 33 on day 43). There was no consistent positive correlation between the larval dose and faecal nematode egg count or the larval dose, faecal nematode egg count and severity of symptoms and/or lesions.

Nematode counts were not done in all the animals and the numbers given include larvae and immature stages from the intestines, abomasum and gall bladder. The percentages of successful infection were calculated and found to vary from 0,006 % in immune goats (group 1: goat 2 and group 9: goat 67), 2,3–4,7 % in severely challenged hypersensitive goats (group 10), 0,26–1,1 % in others that probably also had an immunity (group 3: goat 22, group 4: goat 26, group 7: goat 31) and 3,7–54 % in susceptible animals, the highest being in very young susceptible goats 1,5–8 months of age (group 2: goat 12, group 6: goats 42 and 43, group 7: goat 35). One goat (group 6: goat 42) had an unexpectedly high infection rate at 164 d of 161 %, probably due to incorrect initial or final counts.

Low nematode numbers with high faecal egg counts were found in group 4: goat 26 on day 127 and group 7: goat 29 on day 59. Inversely, high nematode numbers with low faecal egg counts were encountered in group 3: goat 20 on day 52 and group 7: goat 33 on day 43. It was also evident that certain animals had some innate resistance to the parasite (group 6: goat 57 and group 8: goat 64). Faecal nematode egg counts were consistently low in these animals as well as in those which had high levels of immunity to the parasite (groups 9 and 10: goats 17 and 18).

Severe pathological lesions such as status spongiosus of the brain occurred even in animals with a low nematode count (group 4: goat 26, 8 750 worms on day 127; group 6: goat 43, 7 330 worms on day 196 and group 7: goat 31, 780 worms on day 151). Ruptured livers with haemoperitoneum were encountered in young animals with relatively low burdens, such as 17 100 (group 8: goat 58 on day 22) and 9 640 (group 6: goat 42 on day 164).

The nematodes were often recovered from the abomasum (range 10–1 500) and gall bladder (range 105–490) and also from various other tissues. Thus, 449 worms were recovered from 74 g muscle, 35 from 19,5 g lymph node, 199 from 20 g diaphragm, three from 5 g spleen, five from 10 g myocardium, 16 from one lung, two from 10 g brain tissue and one larva in a small piece of brain from another goat, ten from a kidney and four from a liver. In both kidney

and liver the pieces blended were very small and the mass undetermined. A few females were distinguishable in histological sections of the lung, brain, muscles and lymph nodes.

The animals

Clinical signs and mortality

The clinical signs recorded in the majority of the experimental animals were similar to those usually associated with parasitism, namely subnormal mass gains, inappetence, lethargy, abnormal stools, progressive weakness, dehydration, sunken eyes and eventually emaciation and death. A single animal in each of groups 1, 7 and 10, as well as four goats in group 8 showed nervous signs, mainly postural imbalances. Three goats, one in group 6 (goat 42) and two in group 8 (goats 58 and 66) died suddenly without any preceding clinical signs as a result of a ruptured liver associated with hepatomegaly. The clinical signs as noticed in the various groups were as follows:

GROUP 1

One animal (goat 3) died of rumenal bloat of undetermined cause 21 d after receiving a single application of 19 000 larvae. It was consequently disregarded for the purpose of this experiment.

Of the remaining six animals, three were killed *in extremis*, one recovered, another unexpectedly developed nervous signs on day 201 after an apparent recovery and the last goat was sacrificed 20 d after infection.

Goats 1, 2 and 4 received small numbers of larvae (12 000–30 000) initially and only 37 d later were they again exposed to repeated large numbers varying from 50 000–500 000. All three the animals showed clinical signs between days 61 and 63, namely sunken eyes accompanied by a slight mucopurulent discharge, intermittent gnashing of teeth and a slight loss of bodily condition.

The bodily condition of goat 1 continued to deteriorate and it became progressively weaker, with anorexia, occasional gnashing of teeth and sunken eyes. It became recumbent on day 73 and was killed for necropsy 2 d later.

Goats 2 and 4 recovered within a few days of showing clinical signs and started to gain mass. Goat 2 was slaughtered in good condition on day 173. Goat 4 suddenly developed signs of imbalance on day 201. It had a wide-base stance, an extended neck with lowered head and a stiff, ataxic gait (Fig. 2). It was still feeding but disinclined to move and was killed 7 d later (day 208).

Experimental animals 5, 6 and 7 repeatedly received large numbers of larvae. Sunken eyes, gnashing of teeth and progressive loss of condition, anorexia and eventual death occurred in all three cases. In addition, goat 5 continuously salivated and coughed intermittently from days 18–21 when it died.

GROUP 2

Of the six goats, five died and the remaining animal was killed *in extremis*. The three 6-week-old kids succumbed sooner (days 11–13) while the older animals survived somewhat longer (days 18–31).

As in the previous group, the first clinical sign noticed was slightly sunken eyes on day 9 accompanied by a mild mucopurulent ocular discharge in five of the animals. A dramatic loss of body mass occurred and the goats became progressively weaker and eventually recumbent 1–2 d before death. Intermittent gnashing of teeth and in one animal foaming at the mouth were noticed shortly before death. Another goat had very soft faeces terminally.

GROUP 3

The three younger animals in this group (3–6 months old) died or were killed *in extremis* from days 46–52. Transient clinical signs of strongyloidosis similar to those of the previous groups were seen in the two adult goats after which they remained clinically normal for the duration of the experiment. However, the latent period was longer. Sunken eyes were seen from day 20 in one case and from days 34–38 in the other four animals. In the three kids that succumbed a progressive loss of body mass was recorded from the second to the third week after infection. Goat 21 knuckled over on one hind leg on day 40 when it was killed and goat 19 foamed at the mouth terminally (day 52). The older goats also showed a temporary loss of mass two weeks after first exposure to larvae.

GROUP 4

Clinical signs were noted between days 34 and 38 in one adult goat and three young kids which were killed *in extremis* between days 41 and 127. The remaining adult developed slightly sunken eyes on day 41.

GROUP 5

Of the three kids in this group, one died on day 27 after showing lameness and two were killed *in extremis* on days 28 and 36, respectively. Progressive weakness and diarrhoea were the only signs noticeable.

GROUP 6

Only two of the 13 goats used in this experiment succumbed. One died unexpectedly on day 164 as a result of a ruptured liver and the other animal became

recumbent and was killed *in extremis* on day 196. No clinical signs were recorded in the remaining animals.

GROUP 7

A total of 16 animals were included in this group. The three 2-month-old kids died between days 9 and 18, seven animals of 4 and 6 months between days 21 and 59, and the two 12-month-old goats died on days 16 and 29, respectively. Two of the three adult animals recovered after transient symptoms and goat 31 was killed on day 151. All three the two-month-old group kids developed diarrhoea, goats 32 and 35 on day 9 and goat 34 on day 15. Goat 32 died rapidly on the same day without showing any further clinical signs.

Anorexia, sunken eyes, lethargy, salivation (in goat 34) with eventual recumbency and death were recorded in the young kids. Similar clinical signs were observed in the 4 to 6-month and 12-month age groups. In addition, two animals in the former group showed chewing movements with foaming at the mouth on day 23. Abdominal pain was evident in one of these animals as shown by spasmodic contraction of the abdominal muscles.

Sunken eyes were noted in the three adult goats between days 23 and 28. Two of these animals showed no further clinical signs and recovered. Goat 30 exhibited anorexia on day 86 and on day 87 it wandered aimlessly before going down in sternal recumbency. The neck was arched and the head continuously swayed from side to side. It showed increasing stupor and was slaughtered on the same day.

GROUP 8

Of the 15 goats, 12 died or were slaughtered *in extremis*. Three animals (goats 57, 62 and 64) survived the infection with a temporary loss of mass being the only clinical sign. Two 12-month-old animals (goats 58 and 66) died on days 22 and 27, respectively, from ruptured livers without any previous clinical signs. Four goats in this group exhibited nervous signs. Goat 52, apart from having sunken eyes and slight foaming at the mouth on day 152, also had a staggering gait, a wide-based stance of the hindquarters and pushed against solid objects (Fig. 3). It made an apparent recovery until day 216 when the staggering gait reappeared. On day 217 the animal was recumbent with nystagmus and was killed for necropsy.

From day 237 until day 276 when it was slaughtered *in extremis*, goat 53 showed periodic ataxia, postural imbalance, a wide-base stance with sideways swaying, circling, star-gazing and pushing against objects.

On day 55, goat 54 developed an unsteady gait and the following day it was staggering, bumped into objects and wandered aimlessly. The animal also showed polypnoea and mild foaming at the mouth. It was killed *in extremis* on day 56. Continuous tremors of the head and neck with occasional tremors involving the whole body were seen on day 103 in goat 55. These signs disappeared but the animal gradually deteriorated and died on day 147. The remaining six animals in this group showed signs associated with gradual emaciation and eventual death.

GROUP 9

The four goats in this group showed marked to very marked local reactions at the initial infection site. It was consequently assumed that these animals had an acquired immunity and hence large numbers of larvae were re-applied 101–102 d later. Only goat 70 developed transient clinical signs of anorexia, sunken eyes and diarrhoea, accompanied by a loss of mass from day 125 onwards. These signs disappeared after the animal was treated with thiabendazole on day 161. Apart from the initial skin reaction the other three goats showed no clinical signs at any stage of the experiment.

Goats 69 and 70 were discharged while goats 67 and 68 were again challenged with large numbers of larvae 225 d after the previous application. No clinical signs were seen and the two animals were slaughtered on days 374 and 383 for the collection of specimens.

GROUP 10

Similar to those in the previous group, the goats used in this experiment possessed an acquired immunity as evidenced by the local skin reaction after the first application of larvae. They were challenged daily with 50 000 larvae for ten consecutive days. In addition, goats 14, 15 and 18 received a further 500 000 larvae 2–3 d after the last daily dose of 50 000 larvae. Two animals (goats 17 and 18) showed no untoward effects while the other three animals revealed various clinical signs and were killed.

Goat 14 gradually lost mass and became emaciated. It was killed for necropsy on day 135. Terminally, this goat became apathetic and very weak with foam at the mouth. It adopted a wide-base stance, moved sluggishly and lost 33 kg during this time. On day 29 the eyes of goat 15 were sunken and it was feeding poorly. Dry, misshapen faecal pellets (Fig. 4), covered with mucus were observed from day 40, the animal weakened progressively, developed a wide-base stance, became recumbent and was killed for necropsy on day 43.

Goat 16 was first noticed with sunken eyes on day 30. This became more noticeable and from day 37 this animal was feeding poorly. From day 51 it periodically pushed against solid objects. Diarrhoea appeared on day 56 and was present until day 76 when the faeces changed to hard lumps. It was killed for necropsy on day 77.

CONTROLS

All these animals were eventually slaughtered and specimens for microscopic studies and micro-element analysis collected at necropsy.

Pathology

Gross pathology

The incidences of the various gross pathological changes noted in the experimental goats are listed in Table 11. Lesions in both natural cases and the different experimental groups, were not constant as far as severity and occurrence is concerned, but did not vary to such an extent as to justify a separate description for each group. When applicable, certain aspects of the lesions in the various groups are commented upon. A short summary of the affected organs and principal macroscopic and microscopic lesions are given in Tables 1–11. Cachexia is reflected in the second last column of Tables 1–10 under "Mass".

In 58 % of the goats necropsied, cachexia was a prominent feature, with complete depletion of the body fat depots and severe wasting of the skeletal muscles. The condition of the carcasses in the remaining animals varied from fair to poor. In goats that died relatively soon after exposure to larvae, animals that developed nervous signs after apparent recovery and cases which died unexpectedly as a result of hepatic rupture, the condition of the carcasses was usually fair to good. Anaemia was present in approximately 30 % of the cases, including the two cases in group 1 (goats 4 and 7) which were complicated by haemonchosis, and those with ruptured livers (group 6: goat 42, group 8: goats 58 and 66). Goats in groups 1–5 and 7, which received massive or multiple large doses and the majority of which died before day 50 generally revealed the following changes: pulmonary oedema, oedema of the mesenterium and mediastinum, splenomegaly, enlargement and oedema of the mesenteric lymph nodes and peritonitis.

TABLE 11 Incidence of gross lesions in the experimental goats

Lesion	Percentage
Bile stasis	64
Cachexia	58
Enteritis	57
Mesenteric lymphadenopathy	55
Nephrosis	51
Myopathy	47
Hydropericardium	45
Interstitial pneumonia	42
Oedema of lungs	40
Oedema of mesenterium	38
Anaemia	30
Petechiae in intermuscular fascia	26
Myocardial degeneration	25
Hydrothorax	21
Peritonitis	17
Hepatomegaly	15
Ascites	15
Typhlitis	15
Cholecystitis	13
Splenomegaly	13
Hepatitis (haemorrhages and scars)	11
Nephritis	8
Oedema of mediastinum	8
Rupture of liver and haemoperitoneum	6
Congestion of lungs	4
Perforating cholecystitis and peritonitis	4
Colitis	2
Haemorrhage in skeletal muscles	2

Some of these animals also exhibited hydrothorax, hydropericardium, ascites, interstitial pneumonia, bile stasis, cholecystitis, enteritis, typhlitis, nephrosis, muscular and myocardial degeneration. Rarely, hepatomegaly (in two animals), congestion of the lungs and haemorrhages in the abomasum were recorded.

Goats in the above-mentioned groups that survived for longer periods, up to day 173, usually showed more pronounced cachexia and bile stasis but fluid accumulations in the body cavities were seldom noted.

The pathological changes that were recorded in the various organs and tissues of the experimental animals are described below.

LIVER AND GALL BLADDER

Goat 42 (group 6) which received 3 x 2000 larvae over a 14-d period died of a ruptured liver on day 164. The external surface of the liver of this animal had a slate-blue tinge. It was prominently enlarged with an irregular, slightly mottled appearance and a mildly increased consistency (Fig. 5). Haemoperitoneum was present as well as moderate hydrothorax and hydropericardium, prominent pulmonary oedema, emphysema of the lungs and mild enlargement of the lymph nodes.

The second goat (group 6: goat 43) which died in this group revealed hepatic scars, which could have been related to previous ruptures. However, this case was also complicated by pediculosis and ear mange.

Liver lesions were encountered most frequently in group 8. Two goats (16,7 %) had ruptured livers (Fig. 6) and 33 % revealed hepatic scars (Fig. 7). Goat 66 had a light brown liver with a mosaic pattern and a localized area of small haemorrhages, which appeared to be mainly centrilobular in distribution. It was mildly enlarged and a rupture was found near the umbilical fissure. The rupture occurred in the same locality in the other animal, goat 58 (group 8). The gross appearance of the latter's liver differed in several aspects. As for goat 42 (group 6) the liver had

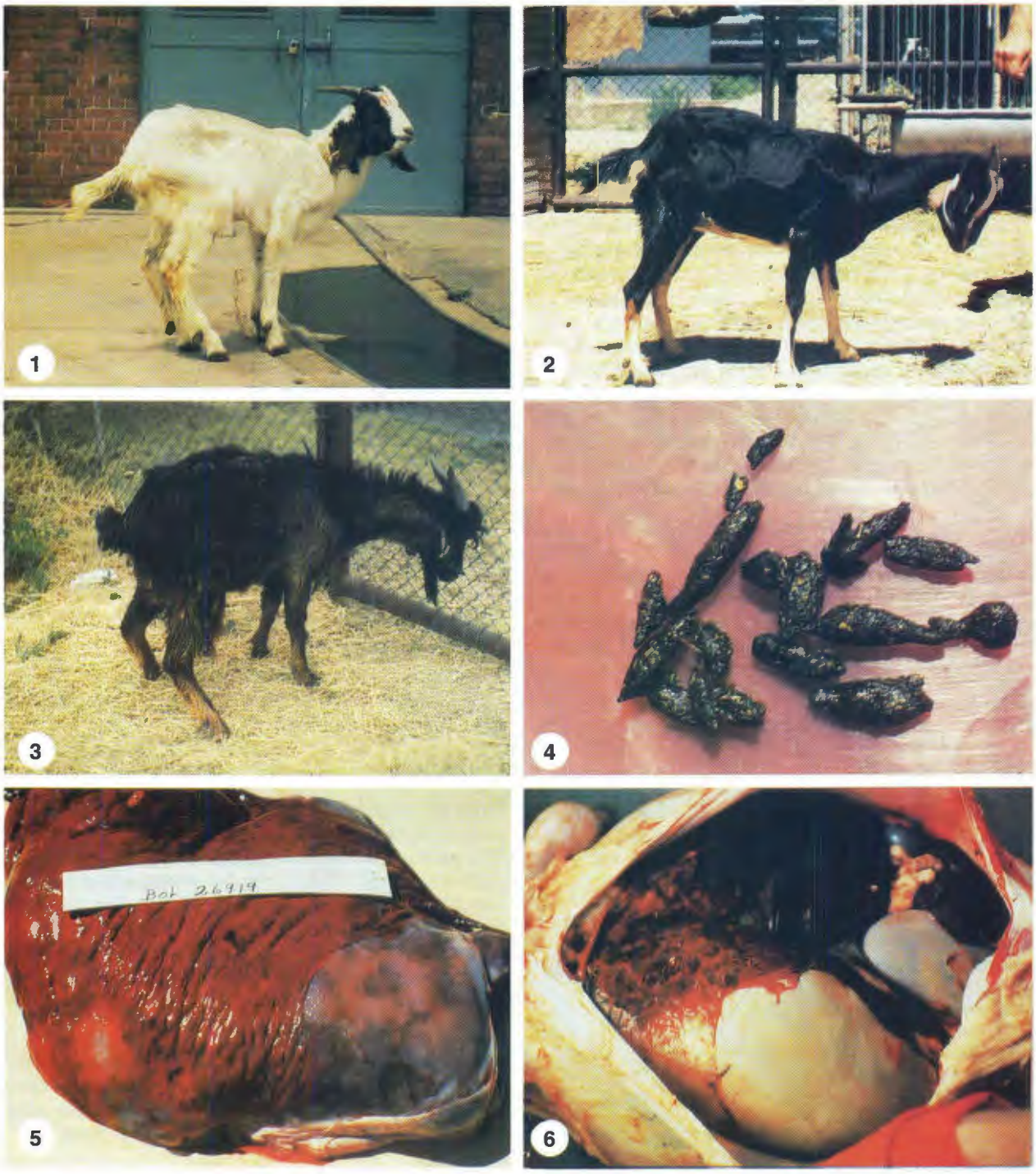


FIG. 1 Goat naturally infected with *Strongyloides papillosus*. Note emaciation and an almost vertical croup

FIG. 2 Goat infected with *S. papillosus* (goat 4). Clinical signs include a wide-based stance, and an extended and lowered neck

FIG. 3 Note the wide-based stance of the hind quarters, extended neck and pushing syndrome in goat 52

FIG. 4 The faecal pellets of goat 15 were dry and elongated

FIG. 5 Goat 42 that died following rupture of the liver. The liver is partially covered by a blood clot and the parenchyma bluish and mottled

FIG. 6 Opened abdomen of a goat infected with *S. papillosus* (group 8). Note the haemoperitonium following rupture of the liver

a slate-blue tinge and was distinctly enlarged. A localized area with foci of subcapsular haemorrhages, stretching for some distance into the parenchyma, occurred on the dorsal aspect of the parietal surface. Two of the other cases with outspoken hepatomegaly (group 8: goats 54 and 56) revealed a similar slate-blue tinge with localized areas of haemorrhage. On cut surface these livers had a variegated reddish-brown colour and in one, the consistency was slightly increased.

No obvious enlargement was present in the livers which revealed scars, namely group 1: goat 1, group 6: goat 43, group 8: goats 52, 53, 59 and 61, and group 10: goat 14. These scars were in most cases present on the diaphragmatic surface of the organ and consisted of irregular linear or focal sunken areas.

Except for the animals mentioned above, group 1: goat 6, group 7: goats 33 and 36 and group 10: goats 14 and 16) revealed moderate to severe gross liver pathology such as hepatomegaly, degeneration and haemorrhages.

Mild to very marked extrahepatic bile stasis of the gall bladder (Fig. 8) was seen in 64 % of experimental animals. This feature was seen in all groups, except group 6. In some goats the gall bladder contained up to 700 mℓ bile. Due to the considerably distended gall bladders, the overlaying hepatic tissue developed localized pressure atrophy and in a few goats a complete, localized disappearance of liver tissue (in a circular fashion) was seen so that the gall bladder visible from the parietal surface of the liver.

Cholecystitis associated with foul smelling bile was noted in 13 % of goats. Erosions and ulcerations were present in the mucous membrane of the gall bladder in some animals, with resultant perforation and bile seepage in two cases. A localized peritonitis with adhesions between the gall bladder and the intestine was present in some cases, whilst a few developed severe bile stasis, with hepatic haemorrhages in the area of attachment of the gall bladder to the liver (Fig. 9).

DIGESTIVE TRACT

A suspected mild to moderate enteritis was present in 57 % of cases. This frequently occurred in association with mesenteric oedema (Fig. 10). Dilation of Brünner's glands was grossly detectable in some goats. A pseudomembranous ileitis and typhlitis, presumably of a secondary nature, occurred in two kids, group 4: goat 27 and group 7: goat 34, and colibacillosis was confirmed in the latter. Erosions were evident on the ileo-caecal valves of a few goats. The caecum and colon sometimes revealed atony and contained semi-solid lumpy ingesta. Erosive typhlitis was present in 15 % of the animals and appeared more frequently in individuals that died within 6–8 weeks after infection.

Stasis of the forestomachs and dilation of the initial portion of the duodenum were recorded in a few cases. Misshapen, shrunken faecal pellets, containing more undigested fibrous material than normal, were frequently seen.

SPLEEN AND LYMPH NODES

Splenomegaly which varied from mild to pronounced was noticed in 13 % of the goats but mainly in those that died between days 16 and 27, and only rarely later (group 10: goat 15 which died on day 43 and group 4: goat 24 on day 56). Although the splenic corpuscles were prominent in some cases, they were more frequently small and associated with a fleshy appearing red pulp.

The mesenteric lymph nodes were enlarged and frequently oedematous in 55 % of the animals that died from days 11–27, but also in some up to day 75, which received multiple doses of larvae.

Enlargement of mediastinal lymph nodes was always associated with pulmonary oedema and/or interstitial pneumonia and occurred during the same period. The exceptions were single animals in each of groups 6, 7, 8 and 10 that died on days 164, 181, 217 and 135, respectively.

KIDNEYS

Nephrosis was present in 51 % of the goats. It was seen as varying degrees of swelling, sometimes marked, with an evenly speckled light yellowish-brown colour. The incidence of this lesion was more or less evenly spread throughout the various groups and occurred in goats that died from day 11 up to day 276. In two cases, group 2: goat 9 and group 7: goat 75, petechial haemorrhages were present in the cortex.

LUNGS

Pulmonary oedema, mostly alveolar but sometimes septal, was found in 40 % of the animals. It occurred in all the various groups but was more commonly present in animals dying more acutely. Approximately 11 % of the cases of lung oedema occurred in goats that died or were killed between days 41 and 164.

Areas of atelectasis, pneumonia and suspected interstitial pneumonia (Fig. 11) were noticed in 42 % of the animals from all the groups and in animals dying at different stages of the disease. These lesions occurred mainly in the cranial and medial lobes but were also occasionally present in the caudal lobes. The ventral parts of the lobes were involved and the lesions were small and localized in nature.

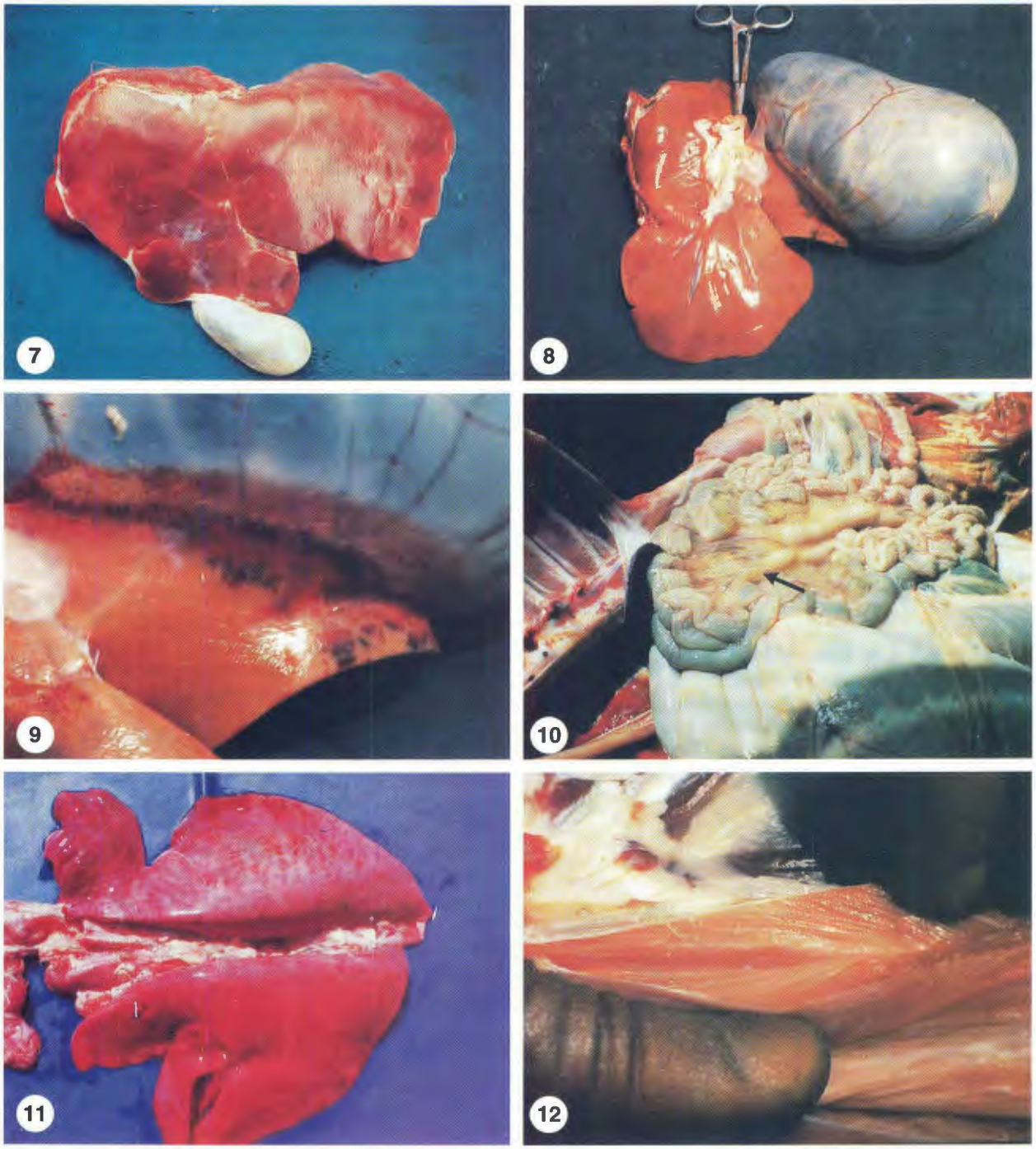


FIG. 7 Liver from a goat that was slaughtered (group 8). The surface is irregular due to scar formation

FIG. 8 Note the severe distention of the gall bladder in an infected goat

FIG. 9 The liver showing bile stasis and hepatic haemorrhages, associated with peritonitis in an infected goat

FIG. 10 Mesenteric oedema (arrow) in a goat infected with *S. papillosus*

FIG. 11 Congestion and interstitial pneumonia as evidenced by a mottled appearance and failure of the lungs to collapse

FIG. 12 Pale areas of degeneration of skeletal muscle in an infected animal

Numerous petechial haemorrhages were present in some of the lungs of goats, which died in the acute phase of the disease.

HEART

Suspected degeneration of the myocardium was grossly detectable in 25% of goats, occurring in all the groups with the exception of groups 5 and 6. Generally the myocardium had a lighter brown colour and in some animals a few paler areas were seen in and adjacent to the papillary muscles or within the septum and wall of the right ventricle.

BONE

Mild osteoporosis was evident only in goat 14 (group 10).

BONE MARROW

The femoral bone marrow of 19 goats, which included cases from groups 1, 3, 4, 5, 7, 8 and 10 as well as three controls were examined. In seven out of ten cases where anaemia was recorded, the bone marrow appeared hypoplastic and was predominantly fatty, semi-gelatinous or mildly haematopoietic. However, in three cases there appeared to be adequate red bone marrow. Seven of the remaining nine experimental cases also had predominantly hypoplastic marrows. In three of the goats in group 4 (goats 24, 26 and 27), and two in group 10 (goats 14 and 16) decreased erythropoiesis was evident.

SKELETAL MUSCLES

Except for generalized atrophy of the skeletal muscles in the cachectic carcasses, areas of suspected degeneration were visible in 47% of the goats (Fig. 12). However, this change was usually mild and less prominent than in the field cases. The subscapularis, longissimus dorsi, triceps, biceps brachii, quadriceps group, biceps femoris, extensor carpi radialis, semitendinosus, semimembranosus, gastrocnemius, infraspinatus, supraspinatus, vastus lateralis and flexor muscles were usually affected. Occasionally were petechial haemorrhages encountered in the intermuscular fascia and less frequently in the muscles.

Histopathology

INTESTINES

The earliest stage studied was day 9 when larvae, worms and eggs were already present in the duodenum and jejunum. Small numbers of eggs were seen in the ileum on day 14 and both worms and eggs were also noticeable in this region as early as day 18. However, worms and eggs were generally most numerous within the jejunum.

The parasites and eggs were localized within the mucosa and were never found beyond the muscularis mucosa. Although their exact location could not always be determined accurately, the overall impression was that they principally parasitize the subepithelial layer of the lamina propria (Fig. 13, 14). An impression was gained that the epithelial layer could also be parasitized but this could not be proven. The eggs occurred as strings in channels within the lamina propria, frequently surrounded by some mucoid matter and bacteria. The latter feature was on occasion very striking and accompanied by an eosinophil infiltration, as well as lymphocytes and plasma cells (Fig. 15). Parasites and eggs were also regularly found free in the lumen of the intestines.

The reaction caused by the larvae seemed to be initiated by their penetration into the mucosa and subsequently aggravated by further migration and the production of eggs in the migratory channels. Secondary bacterial infection, e.g. colibacillosis followed which aggravated the primary lesions.

Mitotic figures in the epithelium of the intestines of the experimental goats appeared to be more numerous than in the controls. Mild haemorrhages were noticeable in the mucosa (Fig. 16) and the crypts were frequently distended by cellular debris containing epithelial cells, neutrophils and mucus. Epithelial necrosis of the tips of a small number of villi leading to small erosions was occasionally seen in well preserved specimens during the early stages of infection. The underlying lamina propria of these villi was slightly haemorrhagic and contained inflammatory cells.

Inflammatory oedema was often noticeable in all the layers of the intestinal wall. This led to a mushroom appearance of the villi, which was characterized by swelling, widening and flattening of the tips. Such an effect, however, was at times also caused by the cell reaction, parasites and eggs. Oedematous fluid and distended lymphatics were frequently seen in the lamina propria. Lymphangitis, even occasional arteritis (group 2: goat 9) and peritonitis were occasionally present. Mild oedema was noticeable up to day 59 in goats that had received a single dose.

The cell reaction was usually of a mixed type, but purulent foci were very frequently present. A mild eosinophillic reaction occurred in ten cases only. Secondary bacterial infection provoked severe necrotic, pseudomembranous or ulcerative lesions especially in the lower portion of the jejunum, ileum and caecum of six cases. The ileo-caecal valve was frequently involved.

Peyer's patches were either subnormal, hyperplastic or atrophic and karyorrhexis was present in one animal. Small numbers of globular leucocytes were noticeable in some animals especially in the lower

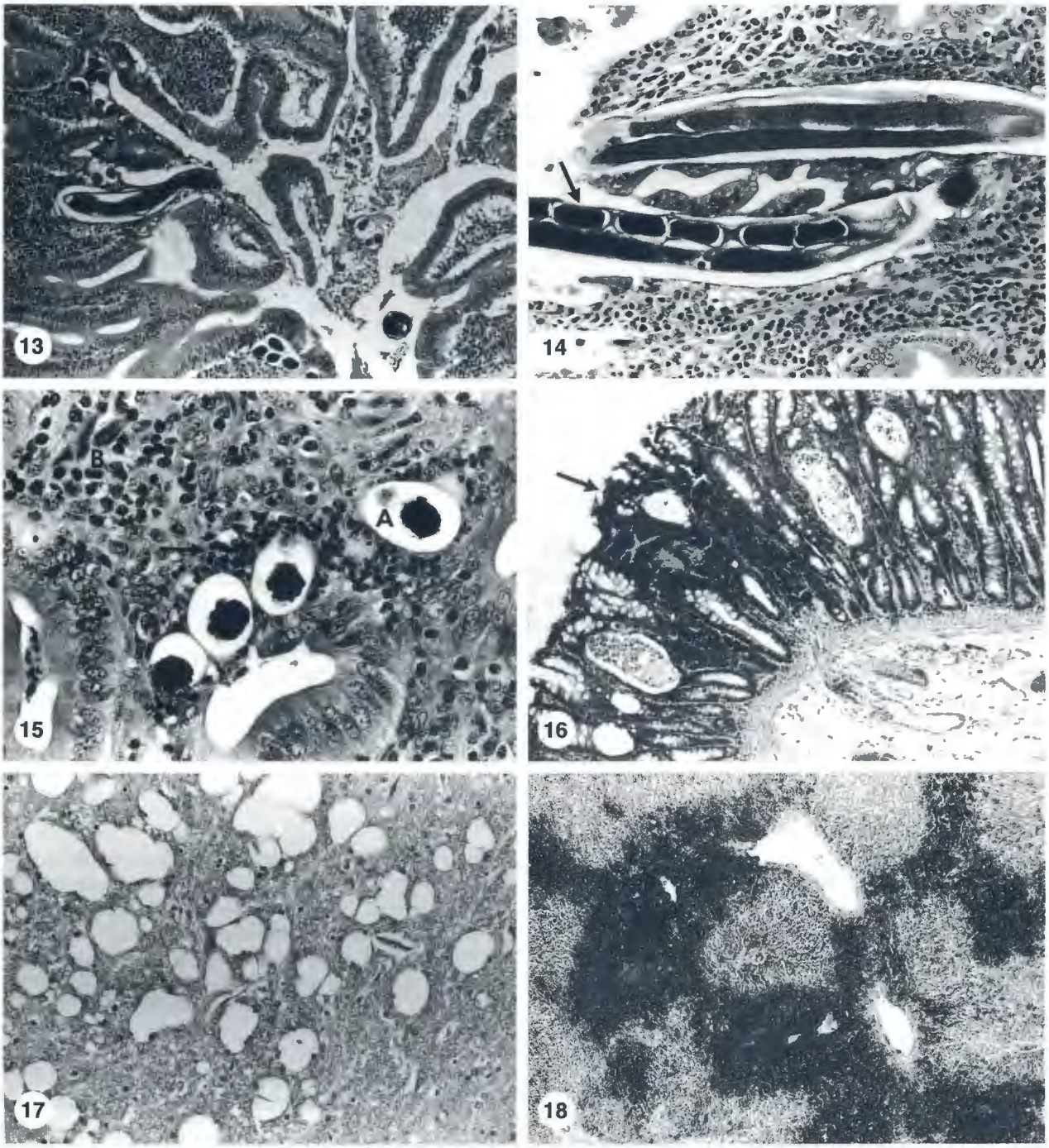


FIG. 13 Adult females and eggs superficially in the mucosa of the small intestine. HE, X 100

FIG. 14 Higher magnification of adult *Strongyloides*. Eggs (arrow) are visible in the uterus. HE, X 200

FIG. 15 Higher magnification of eggs (A) in the lamina propria surounded by eosinophils (arrow) as well as lymphocytes and plasma cells (B). HE, X 400

FIG. 16 Histologic section of the large intestine. Note distention of crypts by inflammatory cells, focal mucosal haemorrhage (arrow) and submucosal oedema. HE, X 100

FIG. 17 Status spongiosus in the cerebral white matter of an infected goat. The vacuoles varied in size and were empty. HE, X 100

FIG. 18 Section of liver to show centrilobular necrosis and haemorrhage with bridging between adjacent lobules. HE, X 40

portion of the small intestine. Dilation of Brünner's glands was seen in five cases. Mucocatarrhal colitis was present in one animal. In group 8 (goat 54) a superficial necro-purulent reaction with prominent epithelial vacuoliation was noticed.

CENTRAL NERVOUS SYSTEM

A status spongiosus mainly of the white matter but also in the grey matter and some nuclei, was the most common and most prominent lesion in the CNS in the majority of goats (Fig. 17). The severity of this lesion was closely correlated with the occurrence of nervous signs. In those animals with lesions that did not show pronounced nervous signs the following were recorded terminally: recumbency, gnashing of teeth, lethargy and marked weakness. A total of 23 cases (43 %) necropsied revealed various degrees of status spongiosus. With the exception of group 2, brain lesions occurred in all the groups in which animals reacted to larval exposure.

The vacuoles varied in size and shape and both small and large ones could be present in the same animal (Fig. 17). Usually they were large and oblong, often multilocular and traversed by delicate filaments. Eosinophilic debris was at times present within the vacuoles. The vacuoles could also be elongated as if following the course of nerve fibres. Vacuoliation of astrocytes was noticeable in some cases and Vacuoliation of neurons occurred in two goats (group 7: goat 72 and group 3: goat 20). Neurons compressed by vacuoles occurred in three cases (group 4: goat 26, group 7: goat 72 and group 10: goat 16). Endothelial hypertrophy was occasionally noted.

The incidence of lesions in the central nervous system in the various groups is given in Table 12.

The most common sites affected and the most severe lesions occurred in the white matter of the roof nuclear area of the cerebellum, especially in the periventricular zone. The corpus striatum, particularly the inner capsule, thalamus, midbrain and medulla oblongata were more or less equally involved. These areas were followed in frequency of occurrence of lesions by the periventricular area of the cerebrum, the granular layer and the folia of the cerebellum, the spinal cord and the optic tracts. The cerebral cortex, subcortical cerebral white matter and the cerebral peduncles were usually only moderately or mildly affected. Only mild lesions were seen in the optic fasciculus and the molecular layer of the cerebellum. The incidence and degree of status spongiosus in some major areas of the CNS are reflected in Table 13.

Very mild to moderate oedema and glial swelling were present in 56 % of cases (group 1: goat 1, group 2: goat 13, group 4: goats 24 and 25, group 5: goat 78, group 7: goats 34, 36 and 72, group 8: goats 56, 63 and 65) usually from days 13–29 but also on days 41–82 and in one animal on day 150. One of the control goats, however, also showed swelling of glial cells after being killed by exsanguination.

TABLE 12 The incidence of lesions in the central nervous system of experimental goats

Experimental group	Number of cases with lesions	Experimental procedure
1	2 (33 %)	Multiple doses : Total 400 x 10^3 larvae
2	–	Multiple doses : 20–180 x 10^3 larvae
3	2 (50 %)	Multiple doses : 10–50 x 10^3 larvae
4	3 (75 %)	Multiple doses : 25 x 10^3 larvae
5	–	Multiple doses : 25 x 10^3 larvae, 3–4 doses
6	2 (100 %)	Multiple doses : 25 x 10^3 larvae, 2–3 doses
7	4 (29 %)	Multiple doses : 100–500 x 10^3 larvae, 1–2 doses
8	7 (58 %)	Single dose : 28–50 x 10^3 larvae
9	–	Multiple doses : 300–500 x 10^3 larvae, 3 doses to immune animals
10	3 (100 %)	Multiple doses : 50 x 10^3 larvae, 10 doses to immune animals

( ) Percentage of affected goats in the group

Severe to very severe status spongiosus of the brain was encountered in the brain from days 43–276, moderate cases from days 43–208 and mild cases from days 27–70. In the mild cases the status spongiosus was sometimes accompanied by brain oedema.

It was evident that status spongiosus was caused by single, multiple high as well as low level doses of larvae and that severe lesions could be encountered even 9 months after a single low to medium dose.

Multifocal gliosis, mild mononuclear perivasculitis and very mild meningitis were present in two animals (group 7: goat 31 on day 151 and group 4: goat 26 on day 127).

LIVER

- Ruptured livers

Three cases (6 %) were encountered. The most striking changes seen were marked oedema and centrilobular necrosis followed by haemorrhages (Fig. 18, 19). The spaces of Disse were markedly distended with proteinaceous fluid sometimes containing eosinophilic globules. In some cases evidence of hepatocytic exocytosis was seen.

Streaks of necrosis and haemorrhage were frequently present, stretching from one central vein to another (Fig. 18). The areas surrounding some of the hepatic veins were also necrotic and haemorrhagic. The hepatocytes adjacent to the areas of necrosis showed fatty changes.

TABLE 13 The incidence and degree of status spongiosus in the major areas of the central nervous system of experimental goats

Area of CNS affected	Degree of severity and percentage of goats affected				
	Very severe	Severe	Moderate	Mild	Total
Cerebrum					
Cortex	–	–	4	43	47
Subcortical white matter	–	–	9	35	43
Periventricular white matter	–	4	17	–	21
Corpus striatum	17	4	26	30	77
Thalamus	22	17	4	8	51
Optic tracts	–	4	4	26	31
Midbrain	17	–	17	35	69
Cerebral peduncles	–	–	–	17	17
Cerebellum					
Molecular layer	–	–	–	9	9
Granular layer	–	4	4	39	47
Folia	–	–	–	35	35
Roof nuclear area	43	–	17	30	90
Medulla oblongata	17	9	4	22	52
Optic fasciculus	–	–	–	13	13
Spinal cord	–	4	–	35	39

Goat 42 (group 6) had recent haemorrhages within the wall of some hepatic veins in conjunction with localized early segmental vascular fibrosis of the wall. Early intimal fibrosis was also present in some portal veins. Large infarcts and haemorrhages were seen in goat 58 (group 8), accompanied by extensive areas of fibroplasia and bile duct proliferation on the edges, and interspersed among the necrotic parts and haemorrhages. Portal fibroplasia and a mild mononuclear cell infiltration were evident. The walls of some of the hepatic veins, especially the intima, were markedly thickened due to oedema and the presence of a fibrinoid-like material, causing the intima to bulge into the lumen, often in a polypoid fashion. No vascular changes were found in goat 66 (group 8) but severe oedema, particularly around the central veins and haemorrhages were present. Many of the hepatocytes in the oedematous centrilobular areas showed coagulative necrosis. Throughout the organ, many hepatocytes contained small cholesterol-like clefts within the cytoplasm. Glisson's capsule was markedly thickened in all three these cases.

In those cases where hepatic scars were recognized macroscopically (13%) (Fig. 7), fibrous thickening of Glisson's capsule, sometimes with haemorrhages, was found. Fibroplasia was also present within the portal areas. Coagulative centrilobular or irregular necrosis with concomitant haemorrhages was frequently seen (Fig. 18, 19). Marked hyalin globular degeneration was a striking feature in one case (group 8: goat 61). It is noteworthy that in goat 14 (group 10) localized necrotic and haemorrhagic lesions with semi-purulent vasculitis of some hepatic and central veins were observed. These lesions resembled parasitic migratory tracts. Aberrant larval migration in this case could be excluded because of the extended interval of 122 d between infection and death.

- Livers with marked hepatomegaly

In these cases a prominent oedema, frequently in association with congestion was present. Oedema involved the spaces of Disse as well as the portal areas and the walls of some central, portal and hepatic veins. Haemorrhages and endothelial hypertrophy and proliferation were also noticeable in several veins. Centrilobular necrosis and haemorrhages which occurred in the ruptured livers, as well as the thickening of Glisson's capsule were also present in a number of the enlarged livers.

- Non-enlarged livers

Of the remaining goats approximately 40% had mild to severe fatty changes which occurred centrilobularly, peripherally or throughout the entire lobule. Cloudy swelling was noticed in 22% of animals. In three goats hyaline droplet degeneration was noticeable in the hepatocytes. One of these animals had a ruptured liver. Apart from those animals which had prominently enlarged livers (*vide supra*) in which oedema was present, the livers of seven goats in this group also showed various degrees of oedema (24%). One goat (group 2: goat 9) had small fibrinoid thrombi in some of the hepatic veins.

Localized necrosis, sometimes in association with a neutrophil infiltration without a distinct pattern of distribution was present in 13 % of the cases. Proliferation of reticulo-endothelial (RE) cells was noticed in 22 % and bile duct proliferation along with some mononuclear reaction in 13 % of the livers examined.

Numerous bacteria were found in the bile ducts of an exsanguinated case (group 10: goat 14). Necrosis and oedema of the smaller bile ducts was evident. Bacteria resembling to those in the bile ducts were seen in the portal blood vessels as were isolated thrombi.

KIDNEY

Fatty change of the epithelial cells of the cortical tubuli was the most common lesion (64 %). Oedema, hyaline droplet degeneration, tubular necrosis and dilatation were noted in a smaller number of goats. The capsular spaces of the glomeruli of approximately 20 % of cases that died in the earlier stages of the disease (days 11–27) contained abnormal amounts of a proteinaceous fluid. This was often associated with a similar excessive quantity of material in the cortical tubuli.

Two goats, (group 2: goat 9 with multiple larval doses and group 7: goat 75 with two doses on day 0 and day 12 that died on days 18 and 23, respectively), had vascular changes in medium-sized arteries. These lesions consisted of a fibrinohaemorrhagic vasculitis associated with endothelial cell hypertrophy and proliferation, accompanied by a very mild mixed-cell reaction and karyorrhexis (Fig. 20). Thrombosis was seen in one of these cases which resulted in multiple infarction.

Two other goats, group 7: goats 36 and 33, with single large doses of larvae and that died on days 16 and 43, respectively, also showed hypertrophy and hyperplasia of the endothelial cells of some of the small arteries with haemorrhages and mild karyorrhexis in the walls. In one of these goats haemorrhages were also present around the larger arteries.

SPLEEN

The most common and striking microscopic feature was the activation and proliferation of the monocyte:macrophage system (MM) which occurred in animals of all the experimental groups, except group 5. This phenomenon was most obvious in goats in groups 3, 4 and 7. The red pulp appeared very compact in a few cases (group 4: goat 26 and group 10: goats 15 and 16) with an abundance of irregular, interwoven islets of plasmocytes and more eosinophilic patches containing a paucity of red cells. A few large multinucleated MM cells were present in group 10: goat 16. The infiltration of varying numbers of neutrophils, mainly in the marginal zone, was the following common change. It occurred most frequently in group 7, in two animals in each of groups 3 and 10 and in one animal in each of groups 4 and 8. Four animals in group 7 and one goat in each of groups 2 and 6 revealed lymphoid atrophy while karyorrhexis of the lymph follicles was found in one animal in group 2. The lymph follicles were usually well developed except in the young animals.

LYMPH NODES

Lymphoid hyperplasia and oedema were present in 44 % of cases and purulent lymphadenitis in approximately 34 %. MM system hyperplasia was commonly observed. Karyorrhexis was present in the lymph follicles of a few cases, mostly in animals dying in the early stages of the condition. Large cells with vesicular nuclei, often multinucleated were seen scattered in the medulla in two goats (group 2: goat 9 and group 5: goat 77). Fibrinoid inflammation of the capsular sinuses was present in group 1 (1). Russell body plasmocytes were seen in some lymph nodes.

MYOCARDIUM

Oedema was the microscopic lesion most frequently present in the heart, followed by vacuolar changes in the sarcoplasm, probably hydropic in nature. Fatty changes, focal Zenker's hyaline necrosis, haemorrhages and cloudy swelling, in this order of frequency also occurred. Mineralisation of necrotic foci was seen in three instances. Multifocal replacement fibrosis was recorded in four goats and a mild interstitial fibrosis with an accompanying leukocyte infiltration in two cases. These lesions were usually not pronounced and were most common in goats of groups 7 and 8.

Large multiple well-defined clear vacuoles were observed in the cytoplasm of the Purkinje cells in three cases. Associated with these vacuoles were suspected mineralized cytoplasmic debris in a perinuclear situation. In some of these cells the nuclei were pyknotic.

SKELETAL MUSCLES

Lesions were found in the skeletal musculature of 55 % of the necropsies performed and in all the groups except group 6. These varied from very mild to severe in nature. The mild lesions consisted of swollen fibres, which stained less eosinophilic due to the myofibrils being pushed apart by a foamy oedematous substance. This was followed by rarefaction, vesiculation and vacuoliation of muscle cells. Accumulation of a proteinaceous, oedematous fluid in the interstitium was recorded in 20 % of the animals. Migratory larvae were seen in only one case.

The more severe lesions were Zenker's degeneration, necrosis (44 %) and fragmentation (Fig. 21)

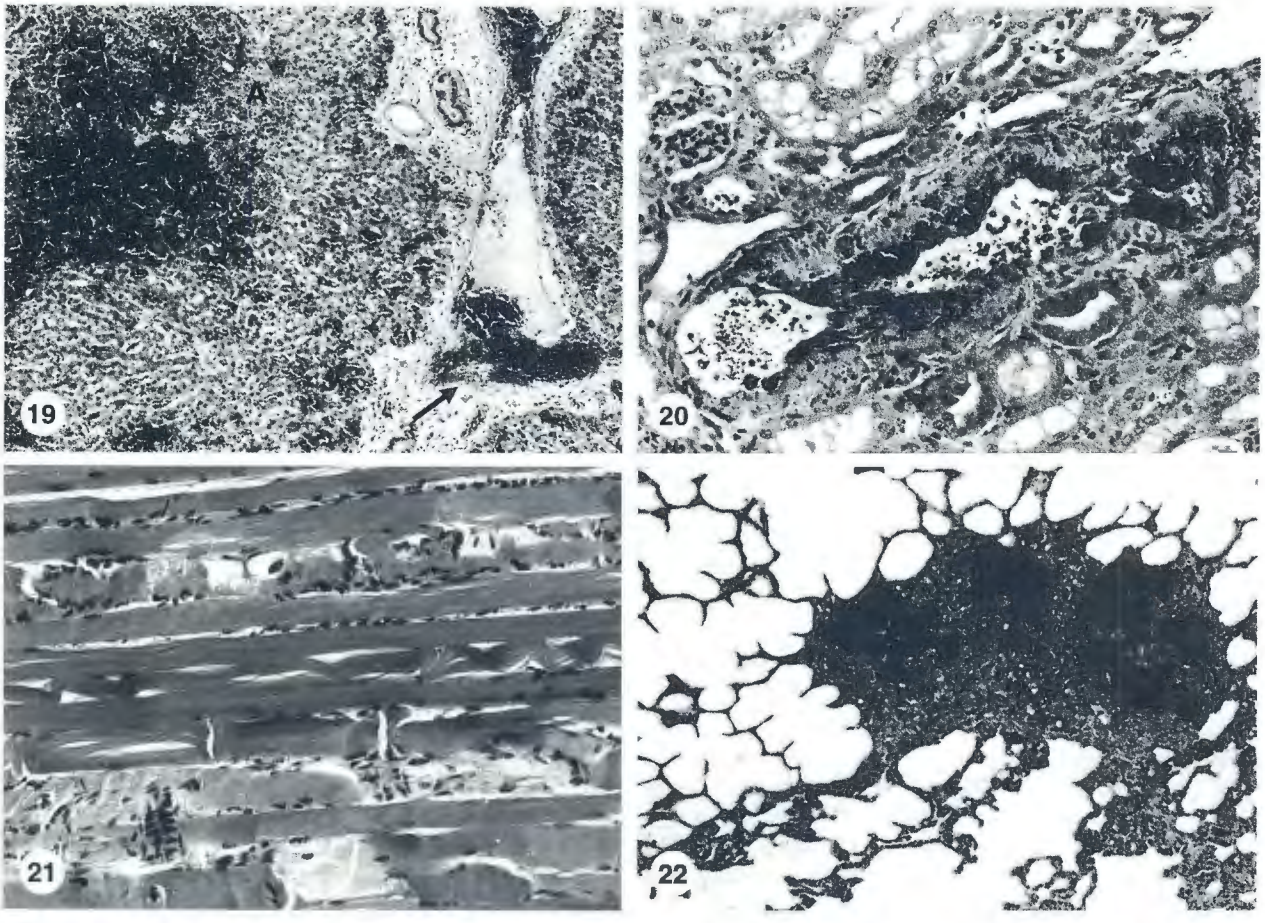


FIG. 19 Liver of an infected goat. Note necrosis and haemorrhage in the lobule (A), and subendothelial haemorrhage (arrow) in a portal vein. HE, X 100

FIG. 20 Histologic section of kidney. There is diffuse fibrinohaemorrhagic vasculitis with surrounding mild inflammation. HE, X 200

FIG. 21 Necrosis and fragmentation of muscle fibres. HE, X 200

FIG. 22 Multifocal haemorrhage and interstitial inflammation in the lung. HE, X 100

followed by mineralization (13 %). Regenerative changes as evidenced by sarcolemmal proliferation and basophilia of the cytoplasm were frequently observed. Atrophy of muscle fibres was recorded in 20 % of animals. A localized mixed cell interstitial reaction and mild haemorrhages were occasionally present especially in the more acute cases. Muscular lesions were noticed in groups receiving selenium and vitamin E as well as in the unsupplemented groups.

The lesions were seen in goats dying from days 11–217. In 55 % of the goats, lesions were identified in 22 different muscles. The muscles most frequently affected were: longissimus dorsi, gastrocnemius, semimembranosus, vastus lateralis and semitendinosus. These were followed by the lower flexors and extensors, infraspinatus, subscapularis, triceps brachii, supraspinatus, biceps brachii, vastus medialis, biceps femoris, diaphragm and intercostal muscles. In a few animals, the vastus intermedius, gluteus muscles, adductor and neck muscles were also affected.

LUNGS

Nineteen goats (35 %) had interstitial pneumonia (Fig. 22) and five cases (9,2 %) developed pneumonia. Oedema was present in 22 % of cases but haemorrhages and fibrinohaemorrhagic plugs were only noticed in very acute cases (days 9 and 23). Lymphoid proliferation was seen in a few cases between days 43 and 135.

CAVERNOUS SINUS AND RETE MIRABILE

Localized phlebitis and fibrinoid thrombosis with leukostasis were observed in group 2: goat 9.

EYE

One goat (group 10: goat 14) had eye lesions characterized by a mild kerato-conjunctivitis, iritis and mild

TABLE 14 Skin thickness, in millimeters, after a single application of 50 000 larvae to the neck of goats with a naturally acquired immunity

Goat no.	Skin thickness before application	Skin thickness after application						
		1,5 h	4 h	6 h	22 h	30 h	48 h	96 h
88	5,0	16	14	19	42	Slaughtered		
89	4,0	11	13	21	39	39	28	19
90	3,0	12	13	11	29	39	26	18
91	3,0	14	19	26	40	41	27	17
92	4,0	10	11	12	21	Slaughtered		
93	4,5	16	19	24	36	35	21	16

mononuclear perivasculitis of scattered retinal vessels. Sero-fibrinous material was present in the anterior eye chamber.

SKIN

Skin biopsies were never collected and specimens were taken from two animals at necropsy (group 1: goat 1 and group 2: goat 9). Multiple purulent foci in the dermal papillae containing larvae as well as a mononuclear reaction were present in the former and a semi-purulent reaction with hyperkeratosis in the latter.

Electron microscopy

Electron microscopy of brain specimens revealed that the vacuoles seen with the light microscope were mainly associated with oligodendrocytes. It was evident that the vacuoles resulted from the splitting of the intraperiod line and the accumulation of fluid in the intermyelinic clefts.

Skin reactions

Reactions caused by larval penetration (Table 14)

Within 1–3 min after placing the larvae on the skin, irritation was evidenced by twitching of the skin. After release, the goats were restless and attempted to scratch the affected area. Patchy or diffuse irregular red areas appeared after 3–5 min and turned to a reddish purple within 5–10 min (Fig. 23). This purplish colour remained noticeable for up to 20 h. Swelling commenced within 5 min and rapidly increased to approximately three times the original skin thickness after 1 h. The maximum swelling was reached at 24–30 h. Thereafter it gradually subsided in approximately 8 d (Fig. 24).

In the majority of cases the swelling was diffuse (Fig. 25) but in a few goats irregular lumpy swellings occurred. Swellings were painful and warm to the touch within 20 min after larval application. Papules and pustules were noticeable at 20–24 h while at 44–48 h multiple pustules were present in the majority of goats. In about 30 % a sero-sanguineous exudate, which formed a crust after approximately 48 h, exuded from the skin.

A diminishing skin reaction occurred after the application of 50 000 larvae on ten successive days (Fig. 26). However, a large dose of 500 000 larvae given 2–3 d after the last dose of the series evoked just as severe a reaction as with the initial dose of 50 000 larvae.

One of the goats that received a few drops of the larval suspension on the conjunctiva had a swollen eye with a muco-purulent exudate 40 h later. The other animal showed no reaction except for a few petechiae on the conjunctiva.

Reactions caused by larval metabolites (Table 15)

Reactions were caused by the metabolites but were not as severe as with the larval application. Maximum swelling (± 2–4 fold) occurred at approximately the same time (21–28,5 h) and subsequently subsided gradually. With the exception of the first animal, the reactions were directly correlated with the volume of metabolite administered. The control sites showed insignificant increases in thickness of 0,50–1,0 mm.

Clinical pathology

Group mean values over time

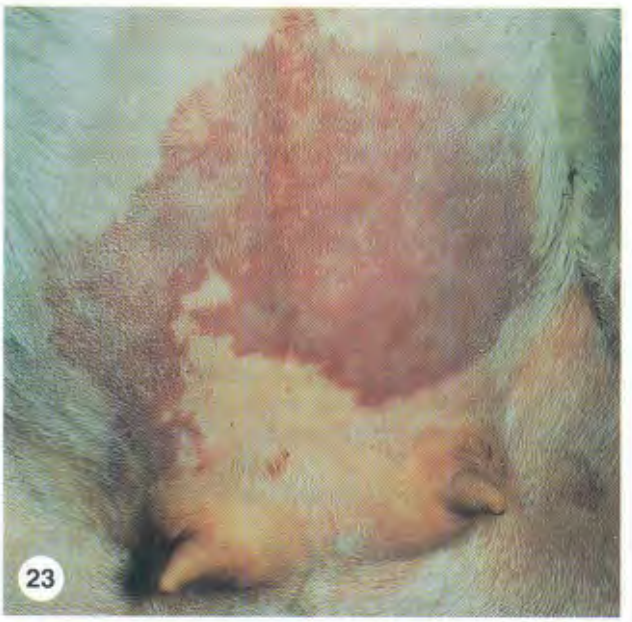
HAEMATOCRIT (FIG. 27–29)

Changes in haematocrit (packed cell volume—PCV) were the most profound of all the parameters determined. The PCV fell from starting values *c.* 37 % in all groups sampled, including the control group, up to day 28, reaching values of 23–26 %. This trend, however, could not be confirmed for group 10 animals

TABLE 15 Skin thickness, in millimeters, after intradermal injection of larval metabolites into the neck of goats with a naturally acquired immunity

Goat no.	Injection site and quantity injected		Skin thickness before injection		Skin thickness after injection				
			1 h	1,5 h	2,5 h	3 h	4,5 h	21 h	28,5 h
94	Anterior 0,100 mℓ	4,3	–	5,8	–	–	7,0	14,0	12,5
	Middle 0,125 mℓ	4,1	–	7,1	–	–	8,7	13,6	12,0
	Posterior 0,250 mℓ	4,2	–	8,6	9,5	–	10,4	13,3	12,8
95	Anterior 0,100 mℓ	4,0	–	6,0	–	–	6,0	7,0	7,4
	Middle 0,125 mℓ	4,2	–	7,3	–	–	8,0	10,4	10,0
	Posterior 0,250 mℓ	4,0	–	8,2	10,0	–	10,0	11,6	12,0
96	Anterior 0,050 mℓ	4,0	6,2	–	–	7,2	–	17,0	–
	*Control 0,050 mℓ	4,5	4,5	–	–	5,1	–	5,0	–
	Posterior 0,100 mℓ	4,7	6,3	–	–	7,5	–	20,5	–
97	Anterior 0,050 mℓ	5,0	7,6	–	–	7,5	–	11,0	–
	*Control 0,050 mℓ	5,0	5,0	–	–	5,0	–	6,0	–
	Posterior 0,100 mℓ	5,0	8,4	–	–	8,5	–	17,0	–

* Tap water injected in control sites



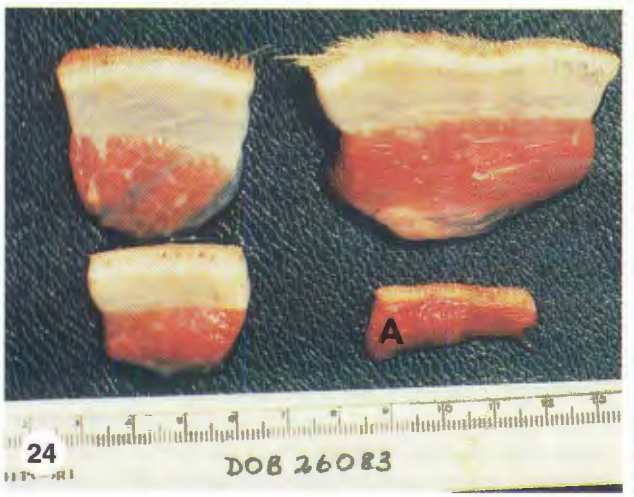
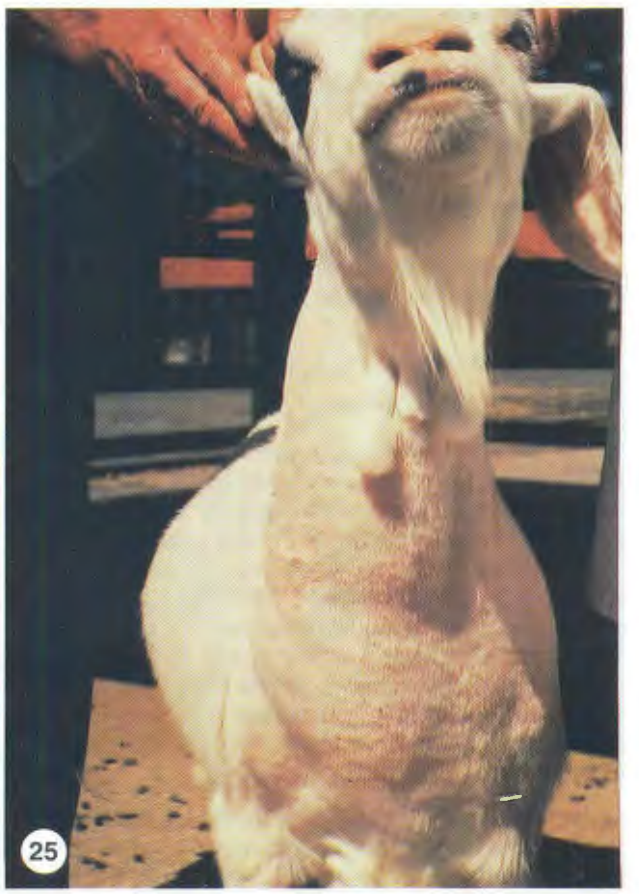


FIG. 23 Extensive cutaneous erythema of the udder after infection with *S. papillosus*

FIG. 24 Cross section of skin, indicating severe subcutaneous oedema. Compare with normal skin (A)

FIG. 25 Skin of neck and anterior thorax of an immune goat showing erythema and swelling



as they were sampled only once (on day 14) during this period. The decline in PCV varied from 13 units (Controls, groups 3 and 4) to as little as seven units (group 7).

Between days 28 and 63 the PCV of animals from the control group recovered to five units below starting value (32 %).

The PCV in group 4 continued to decline reaching 16 % (a drop of 22 units) by day 77 (11 weeks) post infestation (p.i.). The PCV of group 7 animals also continued to decline but more gradually, reaching 19 % by day 77. Group 3 PCV values declined further until day 49 (reaching 17 %) but then rose again to 28–29 % by 12 weeks p.i. (day 84).

Group 10 PCV values dropped only slightly to 29 % by day 49, then dipped to 22 % on day 56 but recovered to 27 % by day 63.

However, in the single remaining animal the PCV was recorded at only 14 % on day 70—the lowest recorded PCV value for the entire trial.

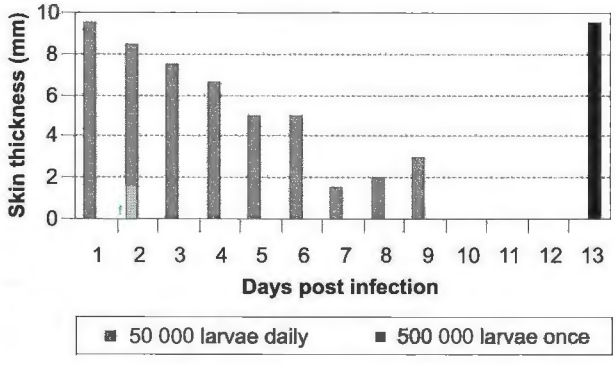


FIG. 26 Average increase in skin thickness after successive daily and single application of larvae in immune goats (group 10)

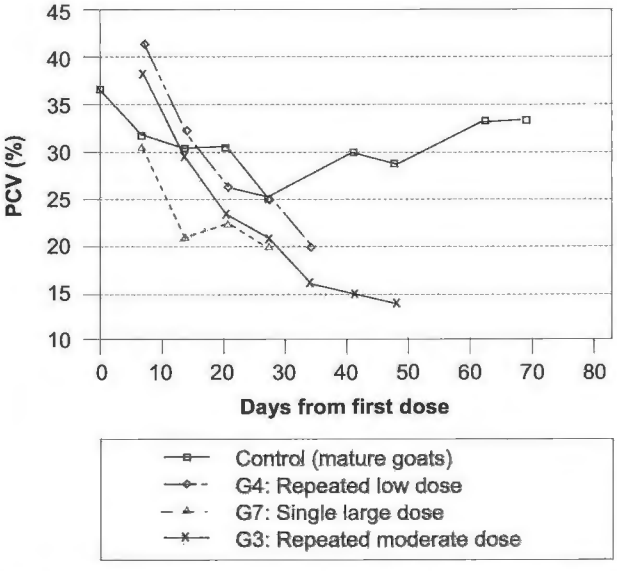


FIG. 28 Weekly group means of the packed cell volume of goats six months old or younger

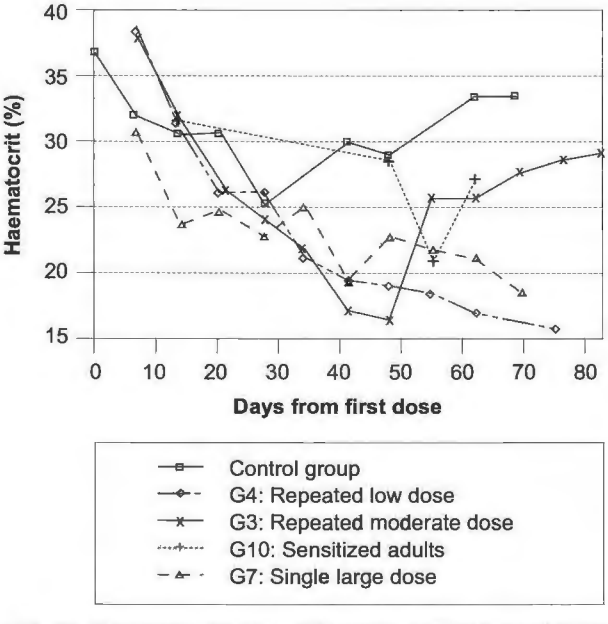


FIG. 27 Weekly group means of the packed cell volume of all the sampled goats during the experiment

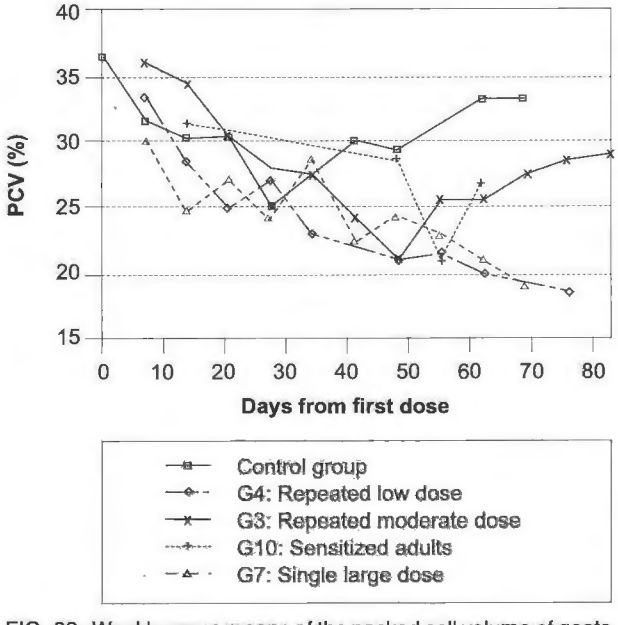


FIG. 29 Weekly group means of the packed cell volume of goats over six months old

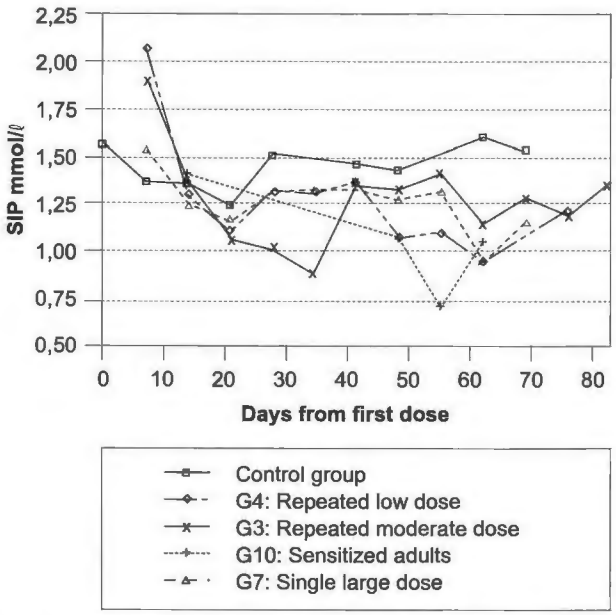


FIG. 30 Weekly group means of the serum inorganic phosphate of all goats sampled during the experiment

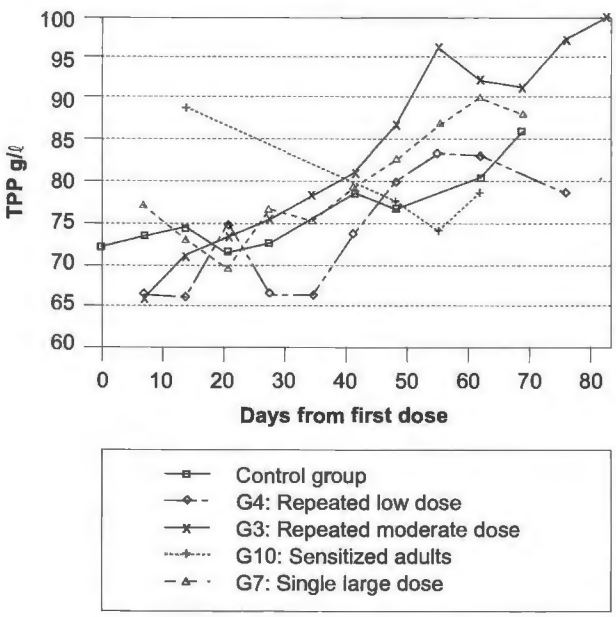


FIG. 31 Weekly group means of the total plasma protein of all the goats sampled during the experiment

SERUM INORGANIC PHOSPHATE (FIG. 30)

Serum inorganic phosphate (SIP) group mean values exhibited profound changes over time, second only to those recorded for the PCV (*vide supra*).

In group 3, these values dropped from 1,9 mmol/ℓ (day 7) to 0,9 mmol/ℓ (day 35) and then rose to settle at 1,3 mmol/ℓ. In group 4, the drop was from 2,05 mmol/ℓ (day 7) to 1,1 mmol/ℓ (day 21) without any further significant changes. In group 7, the drop was gradual from 1,55 mmol/ℓ (day 7) to around 1 mmol/ℓ by day 70 p.i. The change in group 10 was from 1,4 mmol/ℓ (day 14) to as low as 0,7 mmol/ℓ (day 56) but then the values increased sharply to 1,05 mmol/ℓ by day 63 p.i. The single remaining animal registered 1,7 mmol/ℓ by day 70.

ASPARTATE TRANSAMINASE (AsT = SGOT)

There was a moderate rise in serum AsT activity (from initial values of < 65 I.U/ℓ) in all groups (except group 10) up to day 49 (mean values *c.* 75 I.U/ℓ). From day 49 the mean serum activities continued to rise for the next 3 weeks in groups 4 and 7, reaching values in excess of 100 I.U/ℓ.

The control group's activities remained at around 70 I.U/ℓ and group 3 activity fell to 35 I.U/ℓ by day 63 (9 weeks) but then rose steeply to 86 I.U/ℓ by day 84 p.i. In group 10, the AsT activity declined only marginally from 60 I.U/ℓ (day 14) to 43 I.U/ℓ (day 49). Thereafter there was a steep rise to 68 I.U/ℓ by day 63 p.i. The single remaining animal's value rose to 87 I.U/ℓ on day 70.

ALANINE TRANSAMINASE AND ALKALINE PHOSPHATASE

There was no evidence of a clearly discernable pattern of changes in these serum enzyme activities for any of the sampled groups when compared with the controls.

TOTAL PLASMA PROTEIN (FIG. 31)

There was a slight rise in the total plasma protein (TPP) of control animals (from 72–86 g/ℓ). The patterns in groups 4 and 7 were similar to those of the control groups. Changes in some of the other infected groups were noted. In group 3, the mean TPP commenced at 66 g/ℓ (day 7), and rose to 100 g/ℓ by day 84 p.i. In contrast, however, group 10 TPP values declined from 89 g/ℓ (day 14) to 79 g/ℓ (day 63). The single remaining animal in this group registered a value of 66 g/ℓ on day 70.

ZINC SULPHATE TURBIDITY

In groups 7 and 10 the mean group values increased significantly (doubled) between days 63 and 70. The single remaining animal (group 10: goat 16) registered a low reading on day 70. It should be noted that in control animals the zinc sulphate turbidity values more than doubled from days 63–70 having shown no significant change before day 63. With the exception of these noted changes, there were no discernable time-related patterns in this parameter.

GLUCOSE AND CREATININE

There was no evidence of a clearly discernable pattern of change over the trial period for any of the sampled groups.

UREA

There was a moderate upward trend from 6–8 mmol/ℓ over the trial period in all group means. Individual animals accounted for spikes in group mean values on day 49 (group 3), day 56 (group 7) and day 70 (group 4). No such spikes were recorded for creatinine.

ELECTROLYTES

Too few values were obtained from the treatment groups for sodium and potassium to be able to identify any trends.

Calcium, magnesium and blood bicarbonate showed no evidence of a discernable pattern of change in the group mean values. The inorganic phosphate, however, showed significant changes (*vide supra*).

Mineral analyses

The individual values and means are given in Tables 16 and 17. In comparison with the control group, Cu was very low in many of the experimental animals, followed to a lesser degree by Mn. Although the values of Co, Fe and Zn were also lower than the controls, deficiencies of these elements are questionable. A Se deficiency which was suspected on initial histopathological evidence in some natural and experimental cases, could not be verified by liver analyses as one of the controls also revealed a deficiency (<0,13 ppm) but this could be attributable to a Se deficiency in the lucerne hay on which the animals were raised. The Se values in the field cases were considerably higher than in the controls and experimental cases.

DISCUSSION

The primary object of this study was to establish the pathogenicity of a strain of *S. papillosus* recovered from goats in Namibia that died in natural outbreaks and to describe the lesions in the liver, brain and musculature.

Turner (1955, 1959a, b) reported that lambs and kids exposed to 100 000 or more larvae usually died whereas others exposed to fewer than 100 000 larvae developed non-fatal clinical infections. Acquired resistance developed in lambs infected percutaneously with 10 000–30 000 larvae per exposure at 2-d intervals for 20 d. Stankiewicz (1971) substantiated Turner's (1959b) observations. This, however, is not in agreement with the result of the present studies where some kids died or had to be destroyed after experimental infection with three doses of 2 000–5 000 larvae per exposure (group 6) and where 80 % from another group given a single dose of 30 000–50 000 larvae succumbed (group 8). These results indicated that the strain obtained from Namibia was more pathogenic than the strain used by Turner (1955, 1959a, b) and Stankiewicz (1971).

The prepatent period of 9 d and peak egg production within 3–4 weeks was in accordance with the findings of Vegors (1954) and Turner (1959a). However, peak egg production also occurred between 5 and 35 weeks after infection. The egg counts fluctuated frequently as stated by Turner (1959b).

Sandground (1928) found that less than 10 % of the larvae applied to the skin developed to adults in the intestines. In the present experimental infections, the recovery rate varied from 3,7–54 % in susceptible goats and from 0,26–4,7 % in animals with an acquired immunity. The susceptibility of the latter group, however, depended on the size and number of the challenges. The period of maximum patency was not determined but one goat with severe skin lesions was destroyed on day 276.

The most susceptible age, as determined experimentally, varied from 6 weeks to 6 months followed by a second age group, between 6 and 12 months. Under natural circumstances it would appear that older animals could also be susceptible, due either to lack of previous exposure or an overwhelming challenge. Young animals usually died within 9–30 d after doses exceeding 75 000 larvae. Others died or had to be destroyed *in extremis* up to 70 d post-infection.

Many of the clinical signs recorded such as dehydration (sunken eyes), inappetence, emaciation, weakness, cachexia, diarrhoea, anaemia, respiratory distress and abnormal stools were in accordance with those reported by Woodhouse (1948), Turner (1959a) and Round (1963). Fever was never recorded. Polydypsia was noticed in sheep only (unpublished observations). Nervous signs exhibited from day 43, however, have never previously been reported. In all, about 22 % of the experimental goats revealed clinical signs that could histopathologically be related to lesions in the brain and spinal cord. The incidence was 33 % in clinically affected animals exposed to single percutaneous doses of 30 000–50 000 larvae (group 8) and 100 % in hypersensitive goats in which the acquired resistance was broken down by repeated exposures to medium or large doses of larvae.

Sudden death due to hepatic rupture in 6 % of the experimental goats has also not previously been reported in the literature. It is an important feature of strongyloidosis in goats which could cause considerable confusion in unenlightened diagnosticians. The phenomenon was almost exclusively seen in young goats up to 12 months of age. Grinding of the teeth, foaming at the mouth, and misshapen, dehydrated faecal pellets were other additional noteworthy clinical signs.

TABLE 16 Macro- and micro-element analysis (ppm on a wet basis)

	Age (months)	Goat no.	Mg	Ca	Cu	Mn	Se (wet basis)	Se (dry basis)	Co	Fe	Zn	Day of death
Natural cases		a	64,0	–	12,0	10,0	0,29	–	12,0	79,0	149,0	
		b	153,0	–	100,0	14,0	0,32	–	15,0	257,0	218,0	
		c	43,0	–	159,0	13,0	0,24	–	18,0	88,0	299,0	
		d	188,0	–	176,0	14,0	0,28	–	12,0	–	250,0	
		e	197,0	–	16,0	16,0	0,18	–	6,0	–	165,0	
		f	207,0	–	10,0	12,0	0,24	–	11,0	–	192,0	
		g	217,0	–	135,0	6,0	0,20	–	22,0	–	184,0	
		h	238,0	–	35,0	12,0	0,28	–	11,0	–	203,0	
		i	218,0	–	131,0	12,0	0,31	–	10,0	–	174,0	
Group 1	14	1	183,0	–	20,7	1,8	–	–	9,2	–	209,0	75
	14	2	119,0	145,0	32,2	4,7	0,16	0,48	22,0	198,0	176,0	173
	24	4	99,0	119,0	22,3	6,0	0,20	0,60	10,5	212,0	99,2	208
	6	5	345,0	–	10,6	7,6	–	–	2,1	–	121,0	21
	2	6	356,0	–	10,6	4,3	–	–	20,1	–	107,0	20
	6	7	99,0	137,0	8,9	4,9	–	–	13,0	202,0	101,0	52
Group 2	14	8	181,0	–	26,3	2,1	–	–	15,4	–	138,0	11
	7	9	91,0	138,0	14,1	3,4	–	–	22,9	285,0	193,0	18
	14	10	121,0	151,0	17,5	8,1	0,16	0,48	12,9	230,0	203,0	24
	1,5	11	126,0	96,0	7,9	4,3	–	–	17,5	204,0	293,0	31
	1,5	12	311,0	–	8,4	4,4	–	–	14,2	–	103,0	11
		13	186,0	–	8,0	4,8	–	–	8,1	–	115,0	13
Group 3 (Selenium and vitamin E supplemented)	6	19	113,0	101,0	19,2	5,8	–	–	16,1	212,0	212,0	52
	3	20	132,0	138,0	12,9	3,9	–	–	12,3	249,0	208,0	52
	3	21	100,0	125,0	31,1	5,2	0,14	0,42	17,5	300,0	177,0	40
	28	22	106,0	143,0	14,7	4,2	0,10	0,30	19,1	234,0	241,0	208
Group 4 (Selenium and vitamin E supplemented)	6	24	112,0	132,0	20,6	4,0	–	–	11,0	215,0	148,0	56
	3	25	122,0	150,0	37,0	3,6	0,09	0,27	14,3	249,0	208,0	41
	Adult	26	132,0	133,0	18,9	4,8	–	–	18,1	202,0	247,0	127
	3	27	123,0	101,0	9,0	5,8	0,30	0,39	17,5	192,0	324,0	70
Group 5	4	77	–	–	–	–	–	–	–	–	–	–
	4	78	78,0	132,0	37,9	6,3	–	–	10,4	354,0	150,0	27
	5	79	105,0	82,0	39,4	6,5	–	–	13,5	500,0	402,0	36
Group 6	8	42	61,7	137,0	21,4	2,7	0,19	0,57	13,8	152,0	154,0	164

TABLE 16 *(continued)*

	Age (months)	Goat no.	Mg	Ca	Cu	Mn	Se (wet basis)	Se (dry basis)	Co	Fe	Zn	Day of death
Group 7	6	29	100,0	119,0	7,6	6,0	0,11	0,33	19,6	333,0	201,0	59
(Selenium and	28	30	100,0	119,0	30,5	6,0	0,10	0,30	10,7	340,0	103,0	97
vitamin E	Adult	31	125,0	138,0	9,2	3,6	–	–	17,5	201,0	250,0	151
supplemented)	2	32	113,0	114,0	21,0	4,9	–	–	12,7	292,0	143,0	9
	4	33	120,0	134,0	30,0	4,3	–	–	15,9	260,0	203,0	43
	2	34	120,0	134,0	5,2	4,9	0,17	–	12,9	213,0	101,0	18
	2	35	112,0	125,0	4,3	3,6	–	–	13,0	277,0	203,0	14
	12	36	83,0	114,0	5,0	5,6	–	0,51	10,0	212,0	93,0	16
	4	71	90,0	145,0	26,2	8,3	–	–	13,2	282,0	145,0	29
	12	72	111,0	220,0	30,8	7,8	–	–	13,5	310,0	133,0	29
	6	73	71,0	140,0	19,3	9,0	–	–	12,9	189,0	203,0	21
	4	74	91,0	128,0	16,8	8,1	–	–	10,5	234,0	119,0	27
	5	75	119,0	138,0	16,8	6,2	–	–	17,5	186,0	204,0	23
	4	76	88,0	106,0	52,3	3,7	–	–	13,9	415,0	193,0	22
Group 8	14	52	149,0	–	38,1	6,0	–	–	19,4	119,0	195,0	27
	14	55	224,0	–	42,6	6,1	–	–	22,9	177,0	206,0	147
	11	56	78,0	95,0	8,4	5,7	0,19	–	16,2	321,0	171,0	82
	15	61	404,0	253,0	28,0	8,1	–	–	18,2	159,0	247,0	91
	9	63	292,0	454,0	62,9	5,8	–	–	24,0	111,0	210,0	70
	12	66	453,0	488,0	33,3	6,3	–	–	25,9	187,0	127,0	70
Group 10	Adult	14	113,0	134,0	9,8	8,0	0,12	0,36	17,5	394,0	177,0	135
(Selenium and	14	15	100,0	143,0	6,8	5,8	–	–	22,7	260,0	193,0	43
vitamin E	20	16	117,0	140,0	8,0	4,7	–	–	20,1	291,0	291,0	77
supplemented)												
Controls	6–adult	83	109,0	101,0	69	7,2	0,15	0,13	22,0	404,0	268,0	–
		84	100,0	141,0	37	7,1	0,15	0,45	19,2	303,0	258,0	–
		85	98,0	151,0	62	7,1	–	–	11,6	415,0	229,0	–
		86	112,0	123,0	52	7,3	0,09	0,27	18,4	361,0	281,0	–
		87	106,0	83,0	41	7,2	0,13	0,39	22,0	256,0	305,0	–

TABLE 17 Group mean values of macro- and micro-element analyses

	Mg		Ca		Cu		Mn		Se (wet basis)		Se (dry basis)		Co		Fe		Zn	
Natural cases	(9)	169,6	(9)	–		86,0	(9)	12,1	(9)	0,26	(9)	–		13,0	(9)	141,3	(3)	203,7	(9)
Group 1	(6)	200,2	(6)	133,6	(3)	17,6	(6)	4,8	(6)	0,18	(2)	0,54	(2)	16,8	(6)	204,0	(3)	135,5	(6)
Group 2	(6)	169,3	(6)	128,3	(3)	13,7	(6)	4,5	(6)	0,16	(1)	0,48	(1)	15,1	(6)	239,6	(3)	174,1	(6)
Group 3	(4)	112,8	(4)	126,7	(4)	19,4	(4)	4,7	(4)	0,12	(2)	0,36	(2)	16,2	(4)	248,7	(4)	209,5	(4)
Group 4	(4)	122,3	(4)	129,0	(4)	21,3	(4)	4,5	(4)	0,19	(2)	0,33	(2)	15,2	(4)	214,5	(4)	231,7	(4)
Group 5	(2)	91,5	(2)	107,0	(2)	38,6	(2)	6,4	(2)	–		–		11,9	(2)	427,0	(2)	276,0	(2)
Group 6	(2)	97,9	(2)	142,5	(2)	39,6	(2)	5,5	(2)	0,19	(2)	0,57	(1)	14,8	(2)	189,5	(2)	168,5	(2)
Group 7	(14)	102,5	(14)	133,8	(14)	19,6	(14)	5,8	(14)	0,19	(3)	0,38	(3)	13,8	(14)	267,4	(14)	163,8	(14)
Group 8	(6)	266,7	(6)	265,5	(4)	35,5	(6)	6,3	(6)	0,19	(1)	–		21,1	(6)	179,0	(6)	192,6	(6)
Group 10	(3)	110,0	(3)	139,0	(3)	8,2	(3)	4,6	(3)	0,12	(1)	0,36	(1)	20,1	(3)	315,0	(3)	186,0	(3)
Controls	(5)	105,0	(5)	119,8	(5)	52,0	(5)	7,1	(5)	0,13	(4)	0,39	(4)	18,6	(5)	347,8	(5)	268,2	(5)

( ) Number of goats in a group/specimens analyzed

Evidence of acquired resistance or immunity to infection with *Strongyloides* spp. as experienced in this study, has been recorded in the past in various animal species.

Sandground (1928) found that once an infection of *S. stercoralis* was established in dogs an effective and lasting immunity resulted. With *S. ratti*, Sheldon (1937a, b, c) produced immunity in rats after serial infections with small doses of larvae. He observed a sudden loss of parasites from rats 30 d after infection, which he regarded as indicative of acquired resistance. Chandler (1947) believed that helminths which feed on the intestinal tissues, such as *Strongyloides* spp., produce a localized rapidly developing immunity. According to Turner (1959a), Chandler (1947) also stated that in the case of nematodes which employ a parenteral phase of migration, immunization may also be of the parenteral type. Stewart (1955) proposed a "self-cure" phenomenon in helminth infections, which apparently represents gastro-intestinal hypersensitivity caused by previous infections. Enigk (1952a) reported that an acquired immunity prevented reinfection of sows with *S. ransomi*. In rabbits, Jaron (1964) established immunity with repeated sublethal doses of a sheep and a rabbit strain of *S. papillosus*. The absence of changes in the leukocytic pattern of goats which have been immunized previously with multiple larval doses of *S. papillosus* in comparison with marked changes in animals receiving large single doses, was ascribed by Ratynska-Prill (1975) as indirect proof of the production of immunity.

Spontaneous cessation of deaths due to strongyloidosis in lambs was ascribed in part to the development of an acquired resistance (Turner & Wilson 1958).

Turner (1959b) showed that a strong resistance to infection with *S. papillosus* developed in the majority of 9-week-old lambs exposed to immunizing infections of larvae by experimental infection or by lambs and kids grazing for a period of about 2 months on pastures that had been contaminated with eggs and larvae of this parasite.

Bezubik (1972) succeeded to produce some degree of immunity in 1-month-old lambs by exposing them to one or two sublethal doses of irradiated larvae of *S. papillosus*. He found a lesser degree of immunity in such young lambs with identical doses of non-irradiated larvae. Stankiewicz (1969a, b) and Stankiewicz & Brzozowska (1972) showed that doses of 50 000 and 100 000 non-irradiated larvae of *S. papillosus* produced a high degree of immunity. Stankiewicz (1971) dosed various groups of 6-month-old sheep with 30 000 larvae once or twice weekly, using either sheep or a rabbit strain of *S. papillosus*. All the animals immunized in this way survived challenging doses of 300 000 larvae while five out of six control sheep succumbed.

In goats and sheep infected with *S. papillosus*, serum antibodies were demonstrated by means of the indirect fluorescent antibody (IFA) technique (Du Plessis, Pienaar & Basson 1970). The immunoglobulins were adsorbed by the larval cuticles of the parasite and proved to be highly specific. Although a correlation was present between the IFA and complement fixation (CF) titres, the CF test lacked specificity. Low titres with IFA were also determined in post colostral sera of kids from immune dams.

The degree of immunity that develops in *Strongyloides* indicates that immunization could be attempted during outbreaks or seasons of high risk. The feasibility, however, of using larvae is questionable and the possibility of using larval metabolites as an inoculum as indicated by the present studies needs further investigation.

In man, Fülleborn (1926) developed a skin test to show up immunity against *S. stercoralis*. Brannon & Faust (1949) detected in 23 out of 25 individuals a "positive" skin reaction with an extract made from larvae. Woodhouse (1948) in a single sheep and a steer, and Marotel *et al.* (1949, cited by Enigk 1952a) and Vegors & Porter (1950) in calves, observed an allergic type of skin reaction after the initial infection with *S. papillosus*. Varju (1968) noted a similar reaction in pigs with strongyloidosis. Enigk (1952a) described an allergic reaction in the skin and subcutis with repeated infections 10 d after the initial infection in pigs and nutria. A slight to marked skin reaction at the site of larval application first appeared in lambs on the fourth exposure to larvae, 6 d after the initial exposure (Turner 1959b). Wheals measuring up to 30 mm in diameter were accompanied by many pustules. This sensitivity according to Turner (1959b) was seen as a generalized erythema and/or urticaria and appeared in both new and old exposure sites of infected animals.

Similar skin reactions were encountered in the present study. These reactions, however, were absent in parasite-free control lambs or lambs exposed to infection for the first time (Turner 1959a), but slight local inflammation was recorded by Vegors & Porter (1950) and also noticed in the present studies. Furthermore, the acquired immunity in the present studies was broken down by repeated exposures to large numbers of larvae (group 10) and was not persistent as indicated by workers such as Turner (1959a).

Immunological imbalance is regarded as important in auto-infection and hyper-infection with *S. stercoralis* (De Paoli & Johnsen 1978). These authors regarded the increased incidence of auto-infection in the gibbon with this parasite as indicative of an immunological deficiency in this species. Fatalities in man have occurred due to strongyloidosis when infected patients received immunosuppressive therapy for other diseases (Cruz, Reboucas & Rocha 1966).

Turner (1959a, b) and Turner *et al.* (1960) recorded the pathological changes caused by *S. papillosus* and Enigk (1952a, b) reported extensively on the pathogenesis of *S. ransomi* in pigs and nutria. In a review, Varju (1963) described lesions of strongyloidosis as reported by various other research workers, such as intestinal catarrh, oedema and epithelial necrosis. He also mentioned a suspected toxic effect by some parasitic metabolites. Fülleborn (1929) described meningeal haemorrhages in strongyloidosis, but no mention was made in the literature of lesions in the brain, which was a unique feature in 43 % of the experimental goats in the present study. Moderate to severe status spongiosus, observed from days 43–276, was not correlated with any trace element deficiencies or advanced hepatic lesions and unrelated to single, multiple or severe larval exposures.

The very low incidence of parasites encountered in the few brains sampled for the presence of worms, as well as the bilateral symmetry of the lesions and the absence of haemorrhages and migratory routes, excluded a possible direct larval origin of such lesions, except in two cases where mild gliosis, and vascular and meningeal lesions could be ascribed to a possible larval impact.

The study of ultrathin sections revealed that the vacuoles originated from the accumulation of fluid in the intermyelinic clefts of mainly oligodendrogliocytes. These findings indicated that the status spongiosus was caused by oedema which was also frequently present in other organs. It could be related either to a possible toxic substance of the parasite or an electrolyte imbalance, or both.

Widening of the spaces of Disse (synonymous with hepatic oedema) accompanied by hepatic degeneration, was described by Enigk (1952a) in cases of *S. ransomi* infection.

In the present study, liver lesions were very frequently encountered but their nature and pattern differed considerably. Oedema, degeneration, necrosis and haemorrhages were frequently seen. Ruptured livers occurred in the age group 8–12 months exposed to multiple low level or single low level larval applications. The lesions in the livers of some of these goats and a few from other groups were reminiscent of migratory tracts seen in other parasites. Ruptured livers were, however, also encountered in animals with a latent period of up to 150 d in which case larval migration could be excluded as a possible cause for rupture. Unless adult parasites from the gall bladder would start wandering into the liver, such a possibility is therefore highly unlikely. On the other hand, the presence of oedema and vascular lesions in both liver and kidneys as well as hepatic degeneration could be important factors in the pathogenesis of hepatic ruptures. Low to almost normal levels of Cu were encountered in cases with ruptured livers (21,4–33,3 ppm), cases with moderate to severe hepatosis (5–10,6 ppm) and scarred livers (20,7–57,8 ppm). A copper deficiency was consequently not a constant feature and, at the most could only facilitate hepatic ruptures through its effect in cross-linking in collagen and elastin. The hepatic scars probably indicate non-fatal healed ruptures and were encountered in 13 % of cases in both single low level as well as multiple low or high level recipients of various ages. They either died or had to be destroyed between 119 and 276 d post-infection. Furthermore, about 64 % of these goats with either ruptured livers or scars originated from single low-level larval recipients which were highly susceptible (6–14 months). These observations, therefore, excluded a hypersensitivity reaction as a possible enhancing mechanism for rupture.

Cholecystitis due to strongyloidosis as described in man (Enigk 1952a) was present in several goats together with severe gall bladder distension. It is evidently caused by the parasites which were frequently encountered in the gall bladder and by anorexia.

Enigk (1952a) mentioned initial muscular lesions simulating trichinosis caused by the penetration of the fibres by larvae of *S. ransomi*, but did not describe it in detail. The severity of muscular lesions in subacute to chronic cases of *S. papillosus* infections encountered in the present studies has not previously been reported. It occurred in both selenium and vitamin E supplemented and unsupplemented goats and is consequently not associated with such deficiencies *per se*, although it is thought to have complicated the lesions in the unsupplemented groups. Myocardial lesions have also not been reported previously in the literature. As in the other tissues, oedema probably played an important role in the pathogenesis but it is possible that a Cu deficiency could have been a contributory factor.

The splenic MM proliferation and abundance of plasmacytes in the spleen and lymph nodes of some animals confirmed, as indicated previously by Chandler (1953, cited by Varju 1963), Varju (1968) and Du Plessis *et al.* (1970), that, apart from cellular antibodies, humoral responses to the parasite play an important role in acquired resistance.

Endothelial hypertrophy and proliferation could be due either to anoxia or a toxic effect or both. The fibrino-haemorrhagic arterial lesions in the kidneys of two animals and arterial haemorrhages in two others, however, were strange and probably not immune-mediated, based on a lack of cellular response and the fact that some of the animals had had only one exposure to larvae. Three animals with these renal vascular lesions had low copper values (5,0, 14,1 and 16,8 ppm) and the fourth 30,0 ppm, indi-

cating that besides a toxic agent, copper deficiency could have played a role in the development of these lesions. Renal lesions, apart from haemorrhages (Enigk 1952a) have not previously been reported in the literature.

As reported by Turner (1959a) and Enigk (1952a) in cases of *S. papillosus* and *S. ransomi*, respectively, an interstitial and alveolar pneumonia also developed in a number of cases in the present study in goats. The presence of such lesions in the field in outbreaks of strongyloidosis, has given rise to erroneous diagnoses of pasteurellosis by farmers.

A few cases were complicated by haemonchosis, pediculosis, coccidiosis and secondary *E. coli* infection, but these did not in any way seriously complicate the lesions or cloud the valid issues discussed.

Skin biopsies were not collected for microscopic studies and consequently such studies could not be done. Only two samples were collected at necropsy in which acute and subacute lesions were observed.

Enigk (1952a) concluded that after a single exposure to *S. ransomi*, microscopic changes were only found at the sites of larval application, lymph nodes, lungs and intestines and that these lesions would heal completely within 3 weeks. In the present studies, single exposures to *S. papillosus* resulted in serious lesions in remote organs such as the liver and brain.

Lesions in the intestines were in accordance with those described by the various researchers already mentioned. Parasites and strings of eggs were found very frequently in the lamina propria in association with oedema and mild inflammatory changes. Ulceration and pseudomembranous lesions were seen in six cases, mainly in the ileum and jejunum but also in the caecum and colon. This finding is in disagreement with Turner (1959a) who described the most severe lesions in the duodenum and proximal portion of the jejunum. Moczon (1972) suggested that the changes seen in Brünner's glands could be due to the increased production of serotonin to ease the effects of histamine activity.

The cause of the anaemia, except in cases of hepatic rupture, was not determined. There was no indication as suggested by Moczon (1972), that extravasation in the lungs could play an important role. Toxic products as well as deficiencies (e.g. Cu) are more likely causes.

The results of the clinical pathological studies should be interpreted with caution as only 31,6% of the experimental animals were sampled. Some of the goats with severe hepatic lesions and hepatic rupture, amongst others, were not included.

Despite the fact that the control group became moderately anaemic by day 28, the anaemia in groups 3, 4 and 7 by day 28 should not be ignored. The pattern subsequent to day 28 clearly demonstrates that the production of anaemia is a significant pathophysiological mechanism in *Strongyloides* infection in naive animals regardless of the dose or route of administration. The development of anaemia in sensitized animals (group 10) appears to be less profound and less consistent although severe anaemia was recorded in individual sensitized animals.

The anaemia was very profound in animals less than 12 months old. Most of these animals died or were slaughtered *in extremis*. Consequently, the group mean values recorded after day 35 tend to reflect the PCV of the more mature animals which were not as severely affected by the parasites. In several animals the anaemia could be correlated with hypoplasia of the red bone marrow.

Development of anaemia in strongyloidosis has been recorded in goats by Bezubic & Turner (1964), in sheep by Stankiewicz & Brzozowska (1972) and in rabbits by Chromicz (1967).

SIP levels are particularly high in young animals (Bogin, Shimshony, Avidar & Israeli, 1981). As the age composition of the groups differed, and especially from the control group, the control group mean values over the experimental period can only be used to reflect the stability of the experimental conditions i.e. there was no imbalance in phosphate intake.

All the infected groups showed a decline in SIP values during the experimental period. This decline was most profound in the groups 3 and 4 during the first 3 weeks p.i. The fact that subsequent to that there was an apparent stabilization of SIP values (especially for groups 4 and 7) may simply reflect the demise of a larger proportion of the younger experimental animals.

Group 10, consisting of sensitized adults, is much more comparable with the controls with respect to age. Like the other treatment groups, this group also showed a progressive development of hypophosphataemia over the experimental period. Goat 14 of group 10 became severely hypophosphataemic in contrast to the other animals in its group which showed only moderate declines in SIP. This correlates well with the osteoporosis seen macroscopically.

The gradual rise in serum aspartate transaminase (AsT) activity during the first 49 d of the trial in infected groups paralleled that in the control group. Therefore, only alterations subsequent to day 49 can be regarded as significant. The release of increased amounts of AsT into circulation in groups 4 and 7 may reflect a general tissue hypoxia during the last 4 weeks of the trial. However, equally, this may reflect the hepatocellular damage attributable to causes other than hypoxia such as toxic products and deficiencies.

The drop in serum AsT activity in group 3, by contrast, in conjunction with the recovery of PCV values over the same period, supports the suggestion that the serum AsT rise can, at least in part, be ascribed to hypoxia.

Serum albumin was not determined and could have been expected to have dropped due to the parasitic enteritis. This would then have been expected to bring about a decline in measured total plasma protein (TPP). In contrast, however, groups 3 and 7 in particular but also group 4, recorded an increase in TPP. This would suggest that there was a very marked increase in the non-albumin fraction of the TPP. In light of the lack of rise in zinc sulphate turbidity (reflecting immunoglobulin), this would suggest that this was due to an increase in fibrinogen which would reflect an inflammatory state (Jain 1986).

The contrasting pattern of mean TPP values recorded for group 10 can, however, not be explained by the same rationale. The fact that this group recorded a high initial mean value on day 14 could be attributed to haemoconcentration but neither the PCV nor the creatinine values (an index of glomerular filtration rate) supports this supposition. The levels encountered subsequent to this first recorded mean are not particularly different from that of the control group.

The recorded spikes in group means of urea for groups 3, 4 and 7 on days 49, 77 and 56, respectively, were produced by individual animals recording very high values, some 3–5 times higher than that recorded by the other experimental animals. (These individual readings were from group 3: goats 19 and 20, 26,9 mmol/ℓ and 19,1 mmol/ℓ, respectively; group 7: goat 29, 37,4 mmol/ℓ; and group 4: goat 27, 20,4 mmol/ℓ).

The fact that these changes were not accompanied by a concomitant rise in creatinine leaves only two possible explanations. First, these animals might have experienced severe ammonia loading either from tissue catabolism or from dietary origin at or around the time of sampling. Second, that these were spurious readings due to laboratory error. The latter, however, seems unlikely in light of the fact that there were no additional abnormal urea readings amongst the other 188 values.

The micro- and macro-element analyses of liver specimens from the experimental and natural cases in comparison with local controls, indicated that deficiencies of Cu, Mn and possibly of Se could develop in *S. papillosus* infections. A few animals also had low values for Fe and Zn and another animal suffered from osteoporosis due either to aphosphorosis or Cu deficiency. This could probably either be explained by a malabsorption syndrome or that the parasites are absorbing some of these nutrients. Inappetence is believed to play a contributory role. Supplementation of Se in certain groups did not appear to be effective according to the analyses. This could be due to inadequate amounts, poor availability of sodium selenite or malabsorption. In all, however, it became evident that supplementation of at least Cu, Se and Mn is indicated in strongyloidosis and that systemic administration would be preferable for immediate effect, whereas dietary correction via licks is indicated for long term benefit.

The variation in susceptibility of animals from the same age is noteworthy. It emphasizes that such experimental studies, albeit with parasites, toxic plants or other pathogens, could be very useful tools in selecting animal lines with either innate resistance or superior immune system mechanisms. This is a neglected avenue which needs more investigation and implementation.

In conclusion it should be re-emphasized that apart from the intestines, various organs and tissues such as liver, brain, lungs, muscles, lympho-reticular system, heart and kidneys are affected in goats infected with *S. papillosus*. This helminth, whose pathogenicity has been debated for several decades, is unique as an intestinal parasite in goats in producing such a variation of lesions in so many organs even remote ones such as the brain.

In a short pilot experiment using five sheep of various ages and the same goat strain, similar lesions were produced in the intestines and lungs, mild to moderate degeneration in the skeletal muscles and myocardium and a mild status spongiosus of the brain in some animals (J.G. Pienaar & P.A. Basson, unpublished observations 1972). However, severe liver lesions and rupture were not encountered. The brain lesions have also been noticed in natural cases of ovine strongyloidosis (P.A. Basson, unpublished observations 1972). Inoculation of live worms into the duodenum of susceptible lambs produced continuous sinus tachycardia immediately after inoculation and death due to sudden cardiac arrest (Nakamura, Tsuji, Taira & Hirose 1994). These authors conclude that live parasitic females are responsible for cardiac dysfunction regardless of the presence or absence of migratory larvae.

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