Schistosoma mattheei infection in cattle: The course of the intestinal syndrome, and an estimate of the lethal dose of cercariae

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ABSTRACT

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Three groups of young oxen were infected percutaneously with cercariae of *Schistosoma mattheei*. Three of five oxen infected with 248 cercariae kg^{-1} mass died or were killed *in extremis* 58–70 d after infection, a fourth survived extremely severe clinical schistosomosis and the fifth was only slightly affected. None of seven calves infected with 187 cercariae kg^{-1} died, while one of seven exposed to 119 cercariae kg^{-1} was *in extremis* (possibly not from schistosomosis) when killed after 378 d. The LD_{50} appears to be in the region of the highest dose tested (248 cercariae kg^{-1}), but depends on variations in the viability of the cercariae used.

The clinical syndrome was characterized by a drastic, rapid loss in body mass; a severe diarrhoea containing blood clots; straining, gnashing of the teeth, occasional groaning, and other signs of abdominal pain; and markedly sunken eyes. Lethally infected oxen did not become recumbent until shortly before death. Some severely affected animals made remarkable, but slow, recoveries without treatment.

Schistosomes, in close association with granulomata, are described—apparently for the first time—in the omental veins of cattle.

Mean worm development in three calves that died or were killed *in extremis* in the acute stage of the disease, was 55,5 %. In contrast to most previous findings with *S. mattheei*, in two of these animals, more female than male worms developed.

The worms were recovered by perfusion and, in one animal, a large number of intestinal veins were dissected open to estimate the efficiency of the perfusion method. Only 1,9 % of the total worm burden had not been removed by perfusion in this animal.

Keywords: Body mass, cattle, cercariae, clinical syndrome, granulomata, intestinal syndrome, LD_{50}, lethal dose, omental veins, *Schistosoma mattheei*

INTRODUCTION

Schistosoma mattheei is the dominant schistosome species in animals in southern Africa, and it occurs together with *Schistosoma leiperi* and *Schistosoma margrebowiei* (Pitchford 1977; Michelson 1989; De Bont, Vercruysse, Southgate, Rollinson & Kaukas

1994). Further north, it overlaps with, and is later replaced by, *Schistosoma bovis* as the dominant species (Michelson 1989).

Surveys reviewed by Lawrence (1978) and De Bont *et al.* (1994) indicate a prevalence range of *S.mattheei* in cattle, of from 20, up to as high as 92%, in areas that favour transmission of this parasite, but most of the infections are subclinical in intensity. Serious outbreaks of clinical schistosomosis do, however, occur, at least in Zimbabwe and South Af-

rica (Strydom 1963; Lawrence 1978; Van Wyk, Bartch, Van Rensburg, Heitmann & Goosen 1974). They have apparently never been reported in Zambia (De Bont *et al.* 1994).

A number of workers have described the clinical syndrome (termed the intestinal syndrome by Lawrence 1978) which develops after exposure of fully susceptible cattle to *Schistosoma mattheei* cercariae (Hussein 1968, 1971; McCully & Kruger 1969; Van Wyk & Bartsch 1971; Lawrence 1976, 1977a, 1978).

Van Wyk & Bartsch (1971) showed, however, that a different syndrome develops if the immunity of infected cattle is broken down by challenge with large numbers of cercariae. Subsequently, this reinfection syndrome, referred to by Lawrence (1978) as the hepatic syndrome, was described in field outbreaks in cattle in Northern Province (Van Wyk, Bartsch, Van Rensburg, Heitmann & Goosen 1974) and Zimbabwe (Lawrence 1977b).

The experiment described in this paper was designed to estimate the lethal dose of *S. mattheei* cercariae for unsensitized cattle, with the aim of later testing the resistance of cattle carrying varying worm burdens from a primary infection, to a challenge that is lethal to naive cattle.

MATERIALS AND METHODS

The parasite

The strain of *S. mattheei* used and its maintenance in the laboratory have been described by Van Wyk (1973). A minimum of 25 *Bulinus (Physopsis)* spp. snails were used for collection of the required number of cercariae.

The calves

Twenty-eight young oxen in good condition and originating from the Onderstepoort Veterinary Institute, were used in the experiment. They comprised various breeds (Table 1) and at the commencement of the trial they were about 10 months old, and had a mean mass of 307 kg.

On introduction to the laboratory from the Onderstepoort experimental farm, which is free from *S. mattheei* infection, they were dewormed with thiabendazole [Thibenzole (Merck, Sharp & Dohme)] at a dosage rate of 100 mg kg^{-1} live mass, and were then stabled on concrete, under trematode-free conditions, with negligible exposure to roundworms. They were fed good-quality lucerne hay *ad lib.* and about 3 kg of concentrate each per day.

The available animals for the three infection groups were ranked by live mass and randomized into the groups by the use of tables of random numbers.

Before infection, and during the course of the experiment, the calves were weighed at frequent, but irregular intervals. For comparisons between groups, the mass of each animal at each occasion was expressed as a percentage change over the initial live mass before infection.

The calves were infected over a period lasting more than a month (see below) but, for analysis, the changes in mass of individual animals were synchronized and expressed graphically in relation to the time of infection, which was designated as day 0 (Fig. 1, 2 and 3). For comparison, the masses of the uninfected controls were synchronized with those of the calves infected first (Fig. 4). Values for changes in mass of each animal were read from these graphs during the course of the experiment. These values were compared statistically and the mean change per experimental group was used to draw Fig. 5.

Infection

Calves 15 and 21 were infected during March 1970 and the rest, from 7–27 April 1970. As far as possible, consecutive doses of cercariae were allocated to the three experimental groups of calves in turn. However, the numbers of cercariae available were extremely limited, and at times calves were selected according to the cercarial doses assigned to them (and hence without regard to the groups) so that maximum use could be made of the available cercariae.

The cercarial suspensions were prepared and the animals were infected by dangling their tails in a measuring cylinder containing cercarial suspension for 25 min, as described by Van Wyk & Groeneveld (1973). The calves were infected in the shade.

For most infections, the cercarial suspension was placed in translucent 2-ℓ plastic cylinders (Van Wyk & Groeneveld 1973). In the case of calf 9, however, the cercarial suspension exceeded 2 ℓ and the cercariae were placed in a flat-sided glass cylinder of 4-ℓ capacity, which made it possible to observe them during infection. After infection, the tails were not stripped of cercarial suspension, but were gently lifted from the cylinders. The cercariae remaining in the cylinders were counted and these counts were regarded as the numbers which had failed to penetrate (Van Wyk, Heitmann & Van Rensburg 1975).

Calves 6 and 7 were restrained by ropes during infection, but they struggled violently, spilling a large portion of the cercarial suspension. After that, he calves were allowed to stand unfettered in the crush. Two calves (2 and 12) were tranquilized with chlorpromazine (Largactil, May-Baker) before infection.

In calves 3, 4, 6, 9, 12, 16 and 19, the initial dose of cercariae was lower than required, and each animal was infected on a second occasion, 7–15 d (mean:

TABLE 1 Infection of animals

Calf	Breed[a]	No. of cercarial doses	Interval between doses (d)	Second dose as % of total dose	Total dose of cercariae	Dose/kg biomass	% cercariae that failed to penetrate
Group A							
1	Afr x Sd	1	—[b]	—	42 840	240	1,6
2	Afr x Aa	1	—	—	45 782	240	2,0
3	Afr x Sd	2	7	14,1	46 402	258	5,4
4	Afr x Sd	2	8	12,2	40 538	266	9,4
5	Afr x Sd	1	—	—	41 364	253	2,6
6	Afr x Sd	2	10	9,1	53 693	244	0,2
7	Afr x Sd	1	—	—	49 388	237	0,3
Mean	—	—	—	—	—	248	3,1
S.D.[c]	—	—	—	—	—	± 11	± 3,3
C.V. (%)	—	—	—	—	—	4	107
Group B							
8	Afr x Sd	1	—	—	41 052	187	3,4
9	Afr x Sd	2	13	7,7	34 703	212	16,8
10	Afr x Sd	1	—	—	31 968	176	2,1
11	Afr x Sd	1	—	—	27 645	180	2,3
12	Afr x Sd	2	10	11,2	41 976	185	4,3
13	Afr x Sd	1	—	—	30 806	178	3,3
14	Afr x Sd	1	—	—	35 448	189	5,5
Mean	—	—	—	—	—	187	5,4
S.D.	—	—	—	—	—	± 12	± 5,2
C.V. (%)	—	—	—	—	—	7	95,9
Group C							
15	Afr x Sd	1	—	—	17 978	121	3,9
16	Afr x Sd	2	15	18,4	25 906	118	3,5
17	Hereford	1	—	—	21 688	124	3,1
18	Afr x Sd	1	—	—	18 540	111	1,3
19	Afr x Sd	2	13	10,1	26 676	124	11,0
20	Afr x Sd	1	—	—	20 128	111	1,3
21	Afr x Sd	1	—	—	14 490	122	2,5
Mean	—	—	—	—	—	119	3,8
S.D.	—	—	—	—	—	± 6	± 3,3
C.V. (%)	—	—	—	—	—	5	87,7

[a] Afr: Afrikaner; Sd: South Devon; Aa: Aberdeen Angus
[b] Not calculated or not applicable
[c] S.D.: Standard deviation; C.V.: Coefficient of variation

TABLE 2 Worm-recovery data: calves that died of their own accord or were killed *in extremis*

Calf (Group)	Total No. of worms	% worm development from cercariae	Worm distribution (%)			Sex ratio		Days between infection and death
			Mesentery	Liver	Lungs	Males	Females	
1 (A)	19 921	46,5	90,1	8,9	1,0	48,5	51,5	58
2 (A)	27 820	60,8	88,4	10,3	1,3	52,5	47,5	66
3 (A)	27 467	59,2	93,3	5,6	1,1	47,7	52,3	70
15(C)	6 946	38,6	92,2	7,7	0,1	48,8	51,2	378
Mean	25 069[a]	55,5[a]	91,0	8,2	0,9	49,4	50,6	65[a]

[a] Excluding Calf 15, which was killed *in extremis* > 300 d after the other animals

TABLE 3   Contingency table for the death rate (from schistosomosis) in each infected group of calves

No. of calves	Dose (cercariae)			Total No. of calves
	A (248 kg⁻¹)	B (187 kg⁻¹)	C (119 kg⁻¹)	
That survived	2	7	7[a]	16
That died	3	0	0	3
Total	5	7	7	19

[a]   Including calf 15, as it is considered that, although affected by schistosomosis, this condition was not primarily responsible for the extreme reaction observed after more than 300 d

10,9 d) later (Table 1). The additional dose for each calf was small (mean: 11,8 % of the total dose) (Table 1).

Autopsy and worm recovery

Calf 1 was autopsied immediately after it had died "naturally", before the blood had coagulated; calves 2 and 3 were euthanased in the terminal stages of the disease, after intravenous injection of 200 000 IU of heparin [Heparin, 10 000 IU/mℓ (Medical & Hospital Supplies)].

At autopsy, the worms were recovered by modifications of the perfusion techniques described for the mesenteric and gastric radicles of the portal vein (mesentery) and the hepatic portal vein (liver) of monkeys (Foster, Cheetham & Mesmer 1968) and for the mesentery, liver and the pulmonary arterial system (lungs) of sheep (McCully & Kruger 1969).

The pancreas was carefully dissected away to expose the vena porta, and the blood supply of a closely associated hepatic lymph node was carefully ligated before removal of the node.

The perfusion fluid consisted of Earle's saline containing pentobarbital sodium [Sagatal or Euthatal (May-Baker)] (900 mg ℓ⁻¹ fluid).

During perfusion, as many of the worms as possible of those remaining in the small blood vessels on the intestines, were massaged into the larger vessels (Van Wyk, Van Rensburg & Heitmann 1976). About 40 ℓ of fluid was required for perfusing the mesentery of each calf.

In order to determine the number of worms that had remained in the mesenteric veins after perfusion, the mesenteric venous system of calf 2 was opened *in toto* to the level of the smallest arcuate veins. In addition, about 20% of the connecting intestinal veins between the intestines and the arcuate mesenteric veins were opened. The proximal portions of the intestinal veins (on the walls of the intestine) were also

inspected for the presence of worms and, when detected, they were removed from the veins and added to the total not removed by perfusion. The same procedure was followed with the veins on the forestomachs. This time-consuming dissection was done over a period of 5 d.

After they had been recovered, the worms were relaxed and killed, and the sexes were separated by vigorous shaking. The worms were fixed as described by Van Wyk *et al.* (1974). Total counts were made of the schistosomes recovered.

Statistical evaluation

Between-group differences in the number of cercariae which had failed to penetrate, and in changes in live mass of the calves, were tested for significance at the 95% confidence level by the Kruskal-Wallis Analysis of Variance by Ranks Test (Siegel 1956) and the Kruskal-Wallis Multiple Comparisons Test (Miller 1966).

In order to test whether significant differences (95 % confidence limit) exist for the percentage death rates between the three levels of infection, the Loglikelihood Ratio Test, G, of Kullback, Kupperman & Ku (1962) for a 2 x 3 contingency table was computed.

RESULTS

Infection

Because calves 6 and 7 in group A (Table 1) spilt a large volume of cercarial suspension during infection, they were excluded from the experiment; calves 10, 12, 18 and 20 spilt less than 10 mℓ of cercarial suspension during the first 15 min of exposure, a period which is regarded as critical for large-scale penetration of cercariae (Hitchcock 1949; Stirewalt 1956; Warren & Peters 1967). The rest did not appear to have spilt any of the suspension.

The cercariae observed during infection of calf 9 concentrated at the surface of the suspension within a few minutes of the onset of exposure.

Course of the disease

Calves 1, 2 and 3 in group A (248 cercariae kg⁻¹) either died or were euthanased terminally in the acute stages of the disease (58–70 d after infection) (Table 2). Calf 4, from the same group, developed what appeared to be a lethal reaction and was expected to succumb, but made a complete clinical recovery without treatment. Calf 15 (group C), which was killed *in extremis* 378 d after infection, had a

large fibrous lesion of 200 x 75 mm in the myocardium, and although it had numerous polypoid granulomas in the caecum and colon, its death was probably not due to schistosomosis. On the other hand, the cause of the myocardial infarction could not be determined, and it is not impossible that this could have been caused by aberrant migration of the parasite.

No differences were observed in the clinical course or clinical signs in calves infected with one cercarial dose, compared with those which had received additional doses after initial infection (Table 1).

Death rate of infected calves (Table 3)

By the Loglikelihood Ratio Test, G, for a 2 x 3 contingency table (Table 3) it was computed that G = 9,84. This denotes that significant differences at the 1% level of significance (0,006 < P < 0,008) exist for the percentage death rate in the heavily infected group and that in the other two groups, provided that calf 15 is regarded as not having died from schistosomosis. Furthermore, even if the death of calf 15 were to be attributed to schistosomosis, G = 7,085, and this is significant at the 5% level of significance (0,026 < P < 0,034).

Because of the small numbers of animals involved, the expected values in the cells (Table 3) are slightly smaller than would be required for strict validity of the test. However, in view of the high level of significance, this requirement can probably be disregarded.

Worm recovery

The perfusion process is exacting in cattle, and, in our hands, required about 3 h per beast.

The mean percentage worm development in the three calves which died in the acute stage of the disease, varied from 46,5–60,8% (Table 2). In calf 15, which was killed in extermis after 378 d, the development was 38,6%.

The worms' sex ratio was 49,4% males to 50,6% females (Table 2). A mean of 91% of the worms was recovered from the mesentery, 8% from the liver and 1% from the lungs.

In calf 2, 1,9% of the total worm burden was recovered from the opened mesenteric veins after perfusion. Very few worms were encountered in the 20% connecting intestinal veins that were opened. Furthermore, even when in copula, the female worms were visible in the unopened portions of the intestinal veins situated on the walls of the intestine, as the blood had been washed out by perfusion and calf 2 had little peri-intestinal fat. On the other hand, single males contained little pigment and could seldom be observed without the veins being opened. Nevertheless, even though male worms were in the ma-

jority in this animal, few of these males should have occurred in the mesentery unaccompanied by females, as there was only a small difference in the numbers of males and females in this animal. Therefore it is probably a reasonable estimate that more than 90% of the worms remaining in the unopened intestinal veins after perfusion were recovered.

When some of the larger mesenteric blood vessels of calf 2 were opened after storage at about 4–8 °C for 5 d, the worms were still alive, despite marked decomposition of the intestinal tissues.

Clinical signs

Clinical signs of disease were first seen 45 d after infection. The most prominent clinical sign was severe foetid diarrhoea containing blood clots, accompanied by intense intermittent straining, gnashing of the teeth, and occasional groaning. During straining bouts, faeces slowly oozed from the anus, continually wetting and soiling the perineum and caking around the tail (Fig. 6), causing local dermatitis. There was a rapid loss in body mass and the eyes sank deep into the orbits (Fig. 7). Milder cases also developed diarrhoea and faecal accumulations on their tails (Fig. 8). Examination of squash preparations of the blood clots revealed numerous schistosome eggs (Van Wyk 1971 and Fig. 9).

Ataxia and anorexia developed soon after the onset of clinical signs, the anorexia becoming complete before recovery, or persisting until death. The loss of body mass that occurred in most calves, began either near day + 40 (severe cases) or near day + 60, in which case it was usually of short duration (Fig. 1, 2 and 3). Calves 1 and 2 with live masses of 209 and 223 kg before the development of clinical signs, lost 20,1% and 20,2% mass, respectively, during 20 d after the onset of clinical signs. The eyes became so markedly sunken that the eyelids did not shut properly, leading to an accumulation of fluid between the eyeballs and eyelids. This was also observed in field cases, in which a purulent conjunctivitis developed in time (Van Wyk et al. 1974).

Affected calves remained standing until shortly before death and, consequently, the time of death was difficult to predict. Calf 1, which died of its own accord, was standing when inspected for the last time, 4 h before death. Calf 2 was recumbent prior to euthanasia, but rose unaided when stimulated; it soon subsided and lay either with its head on its flank, or extended anteriorly with the chin resting on the ground. In a subsequent experiment (Van Wyk & Bartsch 1971), it was seen that terminal cases lay flat on their sides.

Calf 4 (group A) developed signs similar to, but less severe than, those of the three calves that died. Initially, there was a rapid decline in condition, followed by a gradual decline to extreme emaciation over a
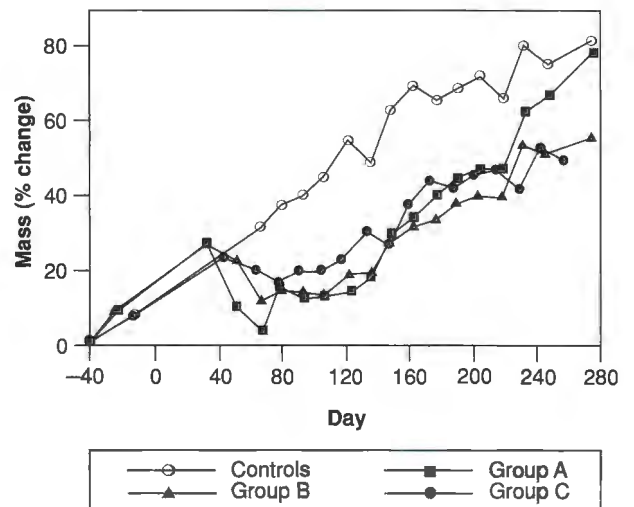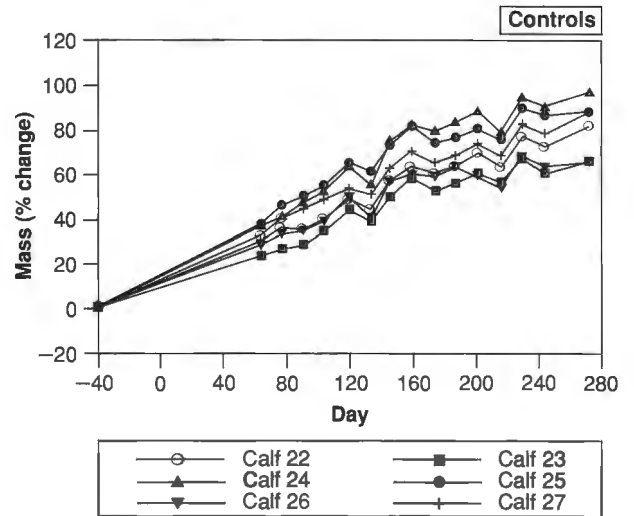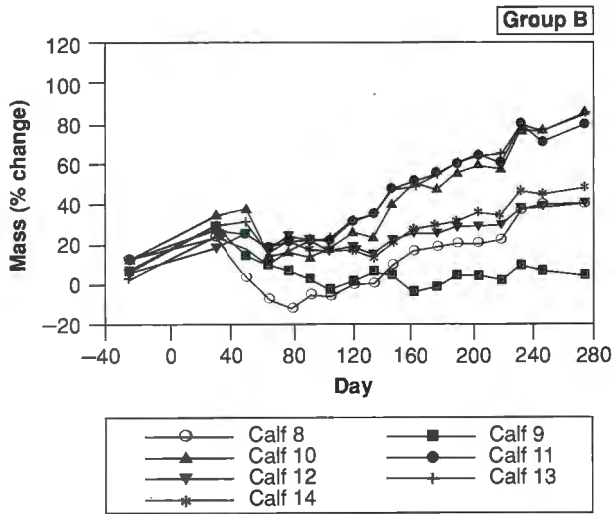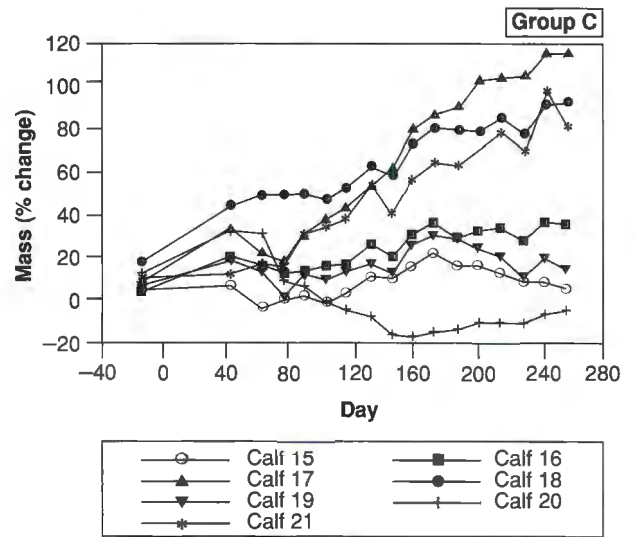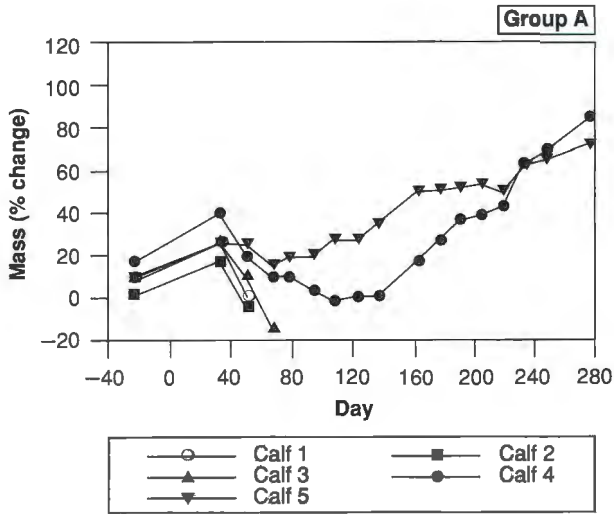
FIG. 1 Changes in mass: Group A calves, infected on day 0

FIG. 2 Changes in mass: Group B calves, infected on day 0

FIG. 3 Changes in mass: Group C calves, infected on day 0

FIG. 4 Changes in mass: Uninfected control calves. The "days" are synchronized as far as possible with infection of the calves in groups A–C

FIG. 5 Changes in mass: Mean per group of calves

FIG. 6

Badly affected calf 4 showing circum-caudal caking of faeces, caused by slowly oozing faeces during straining bouts

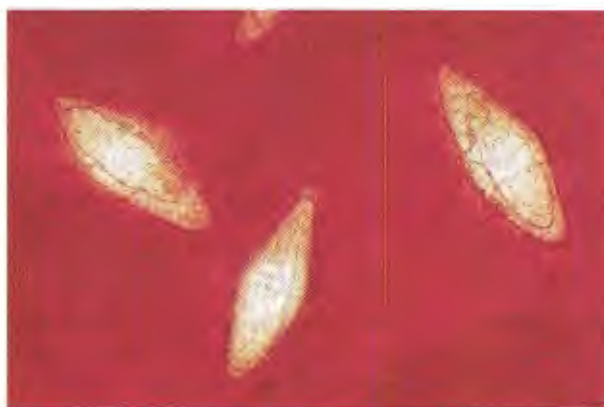FIG. 7 During the acute phase of the disease there was rapid loss in body mass and the eyes sank deep into the orbits

FIG. 8

Even in less severely affected animals faeces accumulated around the tail, from chronic diarrhoea and some straining

FIG. 9 Squash preparations of blood clots in the faeces revealed numerous *Schistosoma mattheei* eggs

[With permission of *Journal of the South African Veterinary Association*]
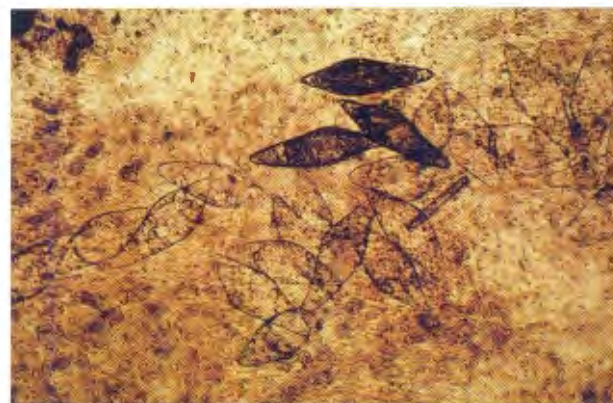

FIG. 10 Numerous *Schistosoma mattheei* eggs in squash preparations of intestinal mucosal scrapings

2½-month period and, finally, a rapid rate of mass gain greater than that of the control animals (Fig.1). Overall, 3½ months elapsed from the onset of the acute disease until all the mass lost by this calf had been regained.

The remaining animal in group A (calf 5) developed only a slight reaction to infection, and after recovery this animal also gained mass more quickly than did the uninfected controls (Fig. 1).

Calves 8 and 9 (group B) and three animals in group C (calves 15, 19 and 20) were also severely affected (Fig. 2 and 3), and remained thin, weak and with dull, staring hair coats. Calf 20, especially, had a prolonged reaction and did not regain the mass lost during the acute reaction before the experiment was terminated. After the turning point in the loss of mass, the calves in group B and C gained mass at a rate similar to that of the controls (Fig. 5).

The calves in group C showed the greatest variations in individual reactions to infection (Fig. 3). The

changes in mass did not, however, differ significantly (Kruskal-Wallis test; Siegel 1956) between groups A, B and C when these groups were tested at 28 occasions between day + 20 and day + 260 after infection (0,7 > *P* > 0,5 at day + 50 and day + 55, the times at which the largest differences were found).

Changes in mass were similar in the uninfected control calves (Fig. 4).

The three groups of infected calves differed significantly (0,01 < *P* < 0,05) from the control group in body-mass changes on days + 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 130, 140, 150, 160 and day + 170. The differences between the three infected and the single control group were highly significant at day + 70 and day + 120 (0,001 < *P* < 0,05), while they were not significant (*P* > 0,05) before day + 50 and after day + 170.

Autopsy

The macroscopic changes found on autopsy of calves 1, 2 and 3, resembled those described previously for single infections with *S. mattheei* (McCully & Kruger 1969; Van Wyk & Bartsch 1971; Van Wyk *et al.* 1974; Lawrence 1976, 1977a, 1978) and *S. bovis* (Hussein 1968; Saad, Hussein, Dargie, Taylor & Nelson 1980).

The predominant changes were dehydration, muscular atrophy, numerous granulomata and disseminated petechiae and ecchymoses in the intestines. Squash preparations of mucosal scrapings from the intestines revealed many ova (Fig. 10). Tumor hepatis and fatty degeneration of the liver were seen in one animal, and bronchitis, together with oedema, emphysema and atelectasis of the lungs were found in another. Macroscopically, the spleens and kidneys did not appear abnormal and no ascites, hydrothorax or hydropericardium were found in any of these animals.

In calf 3, worms were encountered in association with granulomata (which were up to about 0,5 cm in size) in the veins of the omentum.

DISCUSSION

Details of worm recoveries and their distributions in the calves that had survived the experiment and were killed after 18 months, will (DV) be published elsewhere.

Van Wyk & Bartsch (1971) and Lawrence (1977b) reported differences in the disease syndrome that develops after single infection of cattle with *S. mattheei*, compared with that in cattle challenged with massive doses of cercariae some months after an immunizing infection. In the present study, some of the calves received two doses of cercariae, while others were infected only once. However, the supplementary

doses were small and they were administered shortly after initial infection, when sensitization was probably negligible (Moore 1967; Foster & Broomfield 1971; Vernes, Biguet & Floc'h 1972a; Vernes, Floc'h & Biguet 1972b). Furthermore, the clinical disease which developed in these animals resembled the syndrome described after single exposure of cattle to *S. mattheei* (McCully & Kruger 1969; Van Wyk & Bartsch 1971; Lawrence 1976, 1977a, 1978) and *S. bovis* (Hussein 1971, 1973; Saad *et al.* 1980). Consequently, for the purpose of this investigation, all the calves infected in this trial are regarded as having been exposed to a single infection with cercariae of *S. mattheei*.

The clinical syndrome that developed in the present investigation does not appear to be peculiar to cattle exposed only once (or twice at short intervals) to cercariae, as similar clinical signs were reported in a field outbreak of schistosomosis, where single exposure was highly unlikely (Van Wyk *et al.* 1974; Lawrence 1977b). Lawrence (1976, 1977a) proposed that this syndrome be termed the "intestinal syndrome", to differentiate it from what he termed the "hepatic syndrome" which develops after massive challenge of partially immune cattle (Van Wyk & Bartsch 1971; Van Wyk *et al.* 1974; Lawrence 1977b).

Despite great differences in the mean dose of cercariae per group, when the time of infection was synchronized, the percentage change in the masses of the individual calves did not differ significantly between the three groups of infected calves at any stage after infection. This was due, largely, to variations between individuals in each group, and to the fact that, with the single exception of calf 18, all the calves underwent a period of acute loss of body mass at much the same time after infection.

Most of the infected animals, except for losing approximately 4 months of production, eventually made complete recoveries and gained mass at a normal rate. It must be remembered that experimental conditions, where calves received only one to two doses of cercariae, are vastly different from field infections. In the latter, cattle are usually exposed repeatedly over a period of months, and can be expected to develop a partial resistance to reinfection (Van Wyk & Bartsch 1971; Van Wyk *et al.*,1974). On the other hand, more deaths may have occurred, had the severely affected experimental animals been exposed to the stresses of field conditions, including gastrointestinal nematode infection and having to graze over large tracts of land (Saad *et al.* 1980).

Unfortunately, very few clinicopathological investigations could be carried out in this study. The striking feature of the disease in calves, is their acute suffering, a much more dramatic reaction than in sheep, which usually slowly waste away and seldom show signs of intense pain (Van Wyk, unpublished observations 1974). The calves were recumbent for only a short time during the terminal stages of the disease

This observation differs from that of McCully & Kruger (1969) who described recumbency of a few days' duration in an ox during the acute stage of non-fatal schistosomosis.

As discussed by McCully & Kruger (1969) and Lawrence & McKenzie (1972), cattle are able to overcome the effect of infection much more effectively than sheep are. It appears that after single infection, calves either die during the acute stage of the disease or recover more or less completely, becoming partially resistant to the effects of reinfection (Van Wyk & Bartsch 1971; Lawrence 1976). This does not occur to the same extent in sheep which, in this laboratory, have died from schistosomosis up to 4 years after reinfection (Van Wyk, unpublished data 1974).

The LD_{50} could not be determined accurately from these results, mainly because of the variation between individual animals in each experimental group. Nevertheless, the percentage death rate differed significantly between the infected groups, and it appears that the LD_{50} is in the region of the highest dose tested (group A, with 248 cercariae/kg^{-1} live mass). This is supported by the results of a subsequent trial in which six of six calves died of their own accord or were *in extremis* between 56 and 59 d after infection with 440 cercariae/kg^{-1} live mass (Van Wyk & Bartsch 1971). This conclusion differs from that of Lawrence (1977a), who, after he had conducted an experiment involving calves of an age similar to that of the calves used in the present study, stated that the "lethal level of [infection] is clearly greater than ...341 cercariae/kg body mass ...". Possible reasons for this apparent discrepancy are differences in cattle breeds and strain of parasite, and the relatively small numbers of calves used in the trials, coupled with large variations in the development of cercariae in individuals, as discussed below.

There were marked variations in the effects of infection between animals in each group. Undoubtedly, this is due mainly to variations in percentage development of cercariae to which the calves were exposed. Van Wyk, Heitmann & Van Rensburg (1975), comparing different methods of infection in sheep, found a variation of 40–76% (mean: 63%) in cercarial development in a group which was infected percutaneously by leg immersion. In the present investigation, the variation in percentage development in the three calves that had died by day 70 after infection, ranged from 47–61%. In an above-mentioned experiment in which six calves received a mean of about 440 cercariae/kg^{-1} live mass, Van Wyk & Bartsch (1971) reported that the worm development varied from 39–77%, with a mean of 63% (Van Wyk, unpublished data 1971).

The subacute clinical syndrome, suspected by Lawrence (1977a) to follow relatively heavy infection of calves on a low plane of nutrition, occurred in about five calves, all of which were on a high plane of nu-

trition. While it is possible that the nutrition of these calves was indeed poor because of anorexia, it should be borne in mind that the observations of Lawrence (1977a) were apparently based on only two calves and some field cases.

The mean dose of 248 cercariae/kg^{-1} live mass administered to calves in group A, is 32,6% more than the dose of 187 cercariae/kg^{-1} in group B and 108,4 % more than the 119 cercariae/kg^{-1} in group C. Therefore, with variations as large as reported previously, an overlap can be expected in the numbers of worms that develop at these dosage levels.

In the case in which the cercariae were observed during infection, they concentrated at the surface of the cercarial suspension within a few minutes after the tail of the animal had been lowered into the suspension. Presumably, the same occurred in the other cases, and spillage of a small volume of suspension at this time would cause a disproportionately large loss of cercariae.

The percentages cercariae which failed to penetrate the calves in the present investigation (0,2–16,8%, with a mean of 4,1%), were relatively high. In subsequent, similar experiments in which cattle were infected by the same method, lower percentages failed to penetrate, even though larger numbers of cercariae were used, e.g. a mean of 2,1% in the trial of Van Wyk & Bartsch (1971), when the dosage rate was 440 cercariae kg^{-1} (Van Wyk, unpublished data 1971). In the latter trial, six of the 12 calves that were challenged after a previous primary infection, were survivors from the 21 used in this trial that is discussed in the present paper. The percentage cercariae that failed to penetrate these six individuals, was a mean of 2,9 % when they were re-infected (Van Wyk, unpublished data 1971). The reason for the difference is unknown, but perhaps factors affecting the physiological state of the intermediate host can be expected to play a role in the viability of cercariae, as the snails survived the infection much better in the latter trial than in the present one. Calves 6 and 7 were excluded from the present trial owing to spillage, but practically no cercariae were spilt when the rest of the calves were infected, so this could not have played a role.

Worms and granulomata have apparently not previously been described in the omental blood vessels. The omental veins drain into the hepatic portal system, hence it is to be expected that some worms would find their way into this site. Possibly this migration occurs only with relatively heavy worm burdens, or else lesions did occur, but were either not observed, or not reported.

The worm distribution in calf 15, which was killed *in extremis* after 378 d, was similar to that of the three which died > 300 d previously (Table 2).

The worms' sex ratio was reversed from the usual ± 60/40% preponderance of males described in previous reports, where *S. mattheei* was used for infecting sheep (Van Wyk *et al.* 1975, 1976) or cattle (Lawrence 1977c). Similarly, in a group of seven sheep infected with cercariae obtained from the same batch of snails as used in the present investigation, more female than male worms developed (Van Wyk *et al.* 1975) and therefore the difference does not appear to be due to the species of host animal. Lawrence's (1977c, 1978) surmise that female *S. mattheei* are eliminated from the host more rapidly than the males are, does not seem to be supported by the limited data from this trial, as the proportion of females recovered from the ox killed *in extremis* after 378 d, was similar to that from two of the three cattle necropsied after 58–70 d (Table 2). Van Rensburg & Van Wyk (1981) also found that when sheep were infected with male and female cercariae that had previously been sexed, their rates of development were similar. Therefore it appears more likely that male:female proportions are determined by disparate sex ratios in cercariae obtained from different batches of snails, than by different rates of elimination of male and female worms, or by differences in the infectivity of cercariae of the two sexes.

Perfusion in cattle is much more difficult and time-consuming than in sheep, and requires careful preparatory dissection to prevent loss of worms. Other than in sheep, the pancreas in calves completely overlies the vena porta and is traversed by numerous blood vessels that ooze blood and obscure the view if damaged. Furthermore, particularly in adult cattle, the common vena porta is very short and is large in diameter. This makes it extremely difficult to sever the vein so that the stumps that remain attached to both the "intestines" and the liver, are long enough to secure the perfusion tubes sufficiently well to prevent loss of worms during perfusion. Despite these difficulties, the method of perfusion is efficient, as it was shown (apparently for the first time) by painstaking dissection of the intestinal and mesenteric veins of one animal, that probably > 98% of the worms had been washed out. It is likely that accuracy will depend on the size and condition (particularly the amount of fat present) of the animal, and the speed at which the perfusion is done. In the first trial conducted at Onderstepoort, in which 29 sheep were necropsied consecutively (Van Wyk *et al.* 1975), a mean of 9,3% (3,3–22,9%) of the *S. mattheei* were not removed. The perfusion of these sheep was rushed because of the knowledge that the helminths could be counted relatively easily *in situ* when the blood had been washed from the intestinal and mesenteric veins (Van Wyk, unpublished observations). In a subsequent trial involving 35 sheep (Van Wyk *et al.* 1976), more time was spent perfusing the sheep, and a mean of only 3,7% (1,2–8,5%) worms remained *in situ* (Van Wyk, unpublished observa-

tions). In the present experiment, a maximum of one animal was perfused per day and this was done with great care, because in cattle it is difficult, if not impossible, to count accurately the helminths that are not removed by perfusion, unless the blood vessels are opened.

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