

STUDIES ON SCHISTOSOMIASIS. 8. THE INFLUENCE OF AGE ON THE SUSCEPTIBILITY OF SHEEP TO INFESTATION WITH *SCHISTOSOMA MATTHEEI*

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ABSTRACT

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Twenty-eight Dorper wethers, allocated according to age into 4 groups of 7 animals each, and 1 group of 7 Merino wethers, were compared for susceptibility to *Schistosoma mattheei* infestation. The group mean ages of the Dorper sheep varied from 5-61 months and their live mass from 25-66 kg, while the Merinos were 8 months old and had a mean mass of 19 kg.

Despite the marked differences in the age and live mass of the Dorper sheep and the inclusion of 2 breeds in the experiment, no statistically significant differences were found in cercariae which failed to penetrate the sheep, the mean percentage development of cercariae to adult worms, worm distribution in the mesenteric and gastric radicles of the portal vein and the pulmonary arterial system, and worms not removed by perfusion.

Significant differences between groups (5% significance level) were found, however, in the number of worms recovered from the hepatic portal system, and in the worm sex ratio.

On 3 occasions the total number of eggs excreted per female schistosome in the mesentery per 24 hours differed significantly between groups, but each time a different group or groups of sheep were responsible for the variation which was probably due, therefore, not to the age or breed of the sheep, but to daily variations in individuals.

Highly significant differences occurred in the infectivity of the 6 cercarial pools used for infestation in spite of standardized collection and handling of the cercariae. Possible reasons for this are discussed and a solution suggested.

Frequent egg counts (5 per sheep per week) were done during the first 25 days of patency, until the sheep were slaughtered. Schistosome ova were detected in the faeces of only 1/18 sheep examined on Day +43 after infestation, and 3/17 on Day +44, whereafter this increased rapidly to 15/34 on Day +45, 25/33 on Day +46, etc.

A highly significant correlation was found between the total worm egg excretion in the faeces of the sheep per day and the numbers of female schistosomes in the mesentery, especially shortly after the onset of egg production.

Résumé

ETUDES SUR LA SCHISTOSOMIASE. 8. INFLUENCE DE L'ÂGE SUR LA RÉCEPTIVITÉ DU MOUTON AU *SCHISTOSOMA MATTHEEI*

La réceptivité au *Schistosoma mattheei* a été comparée chez vingt-huit moutons de race Dorper, classés en 4 lots de 7 animaux suivant leur âge et un seul lot de 7 moutons de race Mérino. Les âges moyens des moutons Dorper ont été de 5 à 61 mois et leur poids de 25 à 66 kg, tandis que les moutons Mérino ont eu 8 mois et ont pesé 19 kg.

Malgré les différences considérables en âge et poids et l'utilisation de deux races différentes, les auteurs n'ont pas pu démontrer des différences statistiquement significatives à l'égard des paramètres suivants: les cercaires qui ont failli à pénétrer les moutons, le pourcentage moyen des cercaires qui ont développé au stade adulte, la distribution des vers dans les branches mésentériques et gastriques de la veine porte et le système artériel pulmonaire et les vers résistants au déplacement à la perfusion.

Des différences significatives entre les différents lots ont cependant été constatées dans le nombre de vers cueillis du système port hépatique et dans le rapport sexuel des vers.

A 3 reprises on a constaté des différences significatives entre les lots dans le nombre total des oeufs produits par la schistosome femelle en 24 heures dans le mésentère, mais chaque fois un lot différent a été responsable de la variation, que l'on a pu donc mettre en rapport avec les variations journalières de chaque animal et non pas avec l'âge ou la race.

Les auteurs ont pu constater des différences hautement significatives dans l'infectiosité des 6 rassemblements de cercaires destinés à la infestation des animaux, malgré des méthodes de collection et de traitement standardisées. On discute sur les causes possibles de ce phénomène et on propose une solution.

Des comptes fréquents (5 par mouton par semaine) des oeufs ont été effectués pendant les 25 premiers jours de la période de patence jusqu'à l'abattage des moutons. Les oeufs ont pu être décelés dans les fèces prélevées, d'un seul mouton sur 18 au jour +43 après infestation, de 3 sur 17 au jour +44 et puis en augmentant rapidement à 15 sur 34 au jour +45, 25 sur 33 au jour +46, etc.

Les auteurs ont pu constater un rapport direct et de grande importance entre le total des oeufs dans les fèces d'un mouton par jour et le nombre de schistosomes femelles dans le mésentère, surtout aussitôt après le début de la production des oeufs.

INTRODUCTION

Susceptibility to infestation with schistosomes varies markedly from one species of animal to another and even between strains of the same species (Stirewalt, Kuntz & Evans, 1951; Stirewalt, Shepperson & Lincicome, 1965; Colley, 1972; Van Wyk, Heitmann & Van Rensburg, 1975). Consequently, it is often necessary to use the target species in experiments to test the development of cercariae under various experimental conditions, even though this species (e.g. sheep or cattle) may be more expensive than readily available, small laboratory animals.

Furthermore there is great individual variation in the numbers of worms which develop following equal or similar cercarial exposure even within a given strain of host (Stirewalt *et al.*, 1951; McCully & Kruger, 1969; Van Wyk *et al.*, 1975), and hence it is essential to use many animals per experiment to make statistical analysis valid (Van Wyk & Groeneveld, 1973).

Purnell (1966), Pellegrino & Katz (1969) and Ghandour & Webbe (1973) reported large differences in worm development in baby and adult mice, but from about 30 days of age, the susceptibility of young mice was the same as that of adults. No similar investigations have yet been carried out in large animals.

It is often difficult to obtain sufficient sheep and cattle of uniform age, mass and breed for a satisfactory statistical analysis. The experiment reported in this paper was planned to test whether sheep varying greatly in age and mass and of different breeds may be used in a single experiment without an excessive resultant variation in worm development. In addition, the percentage worm development, the percentage cercariae which failed to penetrate, the worm-sex ratio and worms remaining *in situ* in the blood vessels after perfusion are compared with the results reported in a previous publication (Van Wyk *et al.*, 1975).

MATERIALS AND METHODS

The sheep

Twenty-eight of the 35 sheep used in the experiment were Dorper wethers, and the remaining 7, Merino, none of which had been exposed to schistosomes.

The sheep were divided into 5 groups (A, B, C, D, E) of 7 sheep each according to age and breed.

The parasite and intermediate host

The origin of the strain of *Schistosoma mattheei* used and its maintenance in the laboratory have already been described (Van Wyk, 1973).

Two groups of *Bulinus* (*Physopsis*) were available for infestation, one raised in bore-hole water with high electrical conductivity (1 000–2 000 mho), and the other in river water with a lower conductivity (450 mhos). After infestation both groups were maintained in river water.

Collection of cercariae and preparation of doses

Care was taken to ensure that all utensils used for collecting and holding cercariae before and during infestation were free of contamination by detergents and that all were thoroughly washed immediately before use.

Cercariae were obtained from at least 275 infested snails at a time. On the 1st day of infestation, only those cercariae which had emerged from the snails raised in river water were used; on the 2nd day, cercariae from both sources.

The snails were washed thoroughly with running water on a coarse sieve to remove cercariae adhering prior to exposure to neon light for cercarial collection. Cercariae were collected in 2 batches from each group of snails on each day of infestation. The 1st batch on each day was collected over $\frac{1}{2}$ h and divided into 2 pools of cercariae (Pools I and II and IV and V, Table 1). Thereafter, a 2nd batch of cercariae was collected from the same snails and these constituted Pools III and VI, respectively.

Cercarial doses were prepared and the numbers of cercariae per dose and the percentage variation of the doses calculated, as described by Van Wyk & Groeneveld (1973). Sufficient aliquots of the cercarial suspensions were counted to ensure a maximum percentage error of 8.8% (5% significance level) in the doses estimated. A maximum of 7 Infestation Series and 3 Estimation Series of aliquots was drawn from any one pool of cercariae (Van Wyk & Groeneveld, 1973).

Infestation

The allocation of cercarial doses to groups of sheep as well as to individuals within groups and the sequence of infestation of the sheep were determined by using tables of random numbers.

After the skin had been thoroughly washed with water, the sheep were infested by having one of their fore-legs submerged for 30 minutes (Van Wyk *et al.*, 1975). A battery of 7 infestation cradles was used for immobilizing the sheep for this purpose (Van Wyk, 1975). Seventeen of the sheep were infested on 1 day, and the rest 2 days later.

No cercariae were older than $4\frac{1}{2}$ hours by the time exposure was completed. To arrive at an estimate of the number of cercariae which had failed to penetrate the sheep, the cercariae remaining in the measuring cylinders after infestation (intact cercariae and separated heads) were counted.

Viability of the cercariae from the 6 pools used for infestation

Approximately $2\frac{1}{2}$ hours after infestation, cercariae from the pools used for infestation were examined to determine the percentage of live cercariae (Van Wyk, 1973).

The numbers of cercariae in the various cercarial pools which developed to adult worms are listed in Table 2.

Worm eggs in the faeces

The sheep were kept under conditions which precluded re-exposure to schistosomes. Total faecal collections were made from each sheep from immediately before patency of the infestation until slaughter. During the first week, the faeces excreted during the previous 24 h were collected daily, mass measured, mixed thoroughly and a 6 g aliquot preserved with formalin. After the 1st week, the total faecal output was collected daily from Monday to Friday, but that collected from Friday morning until Monday morning was pooled, and a single aliquot collected from each sheep. One-tenth of each aliquot (hence a total of 0.6 g of faeces) was examined for eggs after being stained with acid fuchsin by a modification of the method described by Pitchford, Visser, Du Toit, Pienaar & Young (1973). The eggs were concentrated on a series of sieves and the 1/10th aliquots for examination taken after mixing the egg suspension with methyl cellulose (Lawrence, 1970). The aliquots were examined with the aid of a stereoscopic microscope. The number of eggs per gramme of faeces (e.p.g.) and the total number of eggs excreted per day by each female schistosome in the mesentery were calculated from the number of eggs in the aliquot and the faecal mass of each sheep. Group mean egg counts per female per day were made each day and the results used to plot a graph to demonstrate the increase in number over the first 20 days of egg production (Fig. 1). In addition, 3-point moving averages of the group mean e.p.g. were calculated and plotted on a graph (Fig. 2). Also, 3-point moving averages of the daily mean e.p.g. for all the sheep were calculated and compared graphically with similar means for total eggs excreted each day and eggs per female per day in the faeces (Fig. 3).

Coefficients of correlation were calculated for the following:

(a) The total number of eggs excreted per day in the faeces as compared with the number of female schistosomes in the mesentery of each sheep (Table 6);

(b) E.p.g. as compared with the number of female schistosomes in the mesentery of each sheep (Table 7).

For both (a) and (b), separate correlations were calculated for the Dorper sheep only.

Autopsy and worm recovery

The sheep were autopsied a mean of 68,4 days after infestation by the method described by Van Wyk *et al.* (1975) but with the following modification. During perfusion of the mesenteric and gastric radicles of the portal vein (mesentery), the entire intestine was examined and as many worms as possible remaining *in situ* in the blood vessels of the intestine after hard pummeling, were massaged individually into the larger blood vessels. After perfusion, the worms remaining in the mesentery, including the small branches on the intestines, were counted *in situ*. Up to 12 sheep were autopsied and perfused on the same day.

The worms recovered were counted *in toto* and the following values calculated: The ratio of male to female worms; the percentage development of cercariae to adult worms; the percentage cercariae which failed to penetrate; the worm distribution in the mesentery, liver and lungs; the percentage of worms not removed by perfusion.

Statistical evaluation

The Kruskal-Wallis One-Way Analysis of Variance by Ranks Test (Siegel, 1956) and the Kruskal-Wallis Multiple Comparisons Test (Miller, 1966) were used to test for significant differences at the 5% significance level between the groups of sheep. The percentage of cercariae which failed to penetrate was tested for correlation with worm development, and the percentage development in the Dorper sheep was correlated with the age of each sheep. The cercariae of Day 1 and Day 2 of infestation were tested for significant differences in infectivity by means of the Mann-Whitney U Test (Siegel, 1956).

Because of marked variation in the infectivity of the various pools of cercariae used (Table 2), an analysis of variance with groups of sheep and pools of cercariae as factors was made for the purpose of correcting group means for differences in infectivity of the individual pools of cercariae. Since the numbers of animals per group infested with each pool of cercariae differed (Table 2), the least squares estimates of group means were calculated and tested for differences with the aid of an F-test (Steel & Torrie, 1960).

RESULTS

Survival of the snails

The snails raised in bore-hole water started dying in large numbers a few days before the emergence of the first cercariae, while the snails raised in river water survived very well until cercarial emergence, after which they also started dying fairly rapidly, though not at the same rate as the others.

Cercariae were first found on Monday, 12/3/73, and probably started emerging from 9/3/73 onwards.

TABLE 2 Viability (percentage development) of the 6 pools of cercariae used for infestation

Pool	No. of cercariae	Infestation day	Development to adult worms (%)		Cercariae failed to penetrate (%)	
			Mean ± S.D.	Range	Mean ± S.D.	Range
I.....	1 599	1st	63,6 ± 4,2	57,2-69,9	2,9 ± 2,2	0,3- 5,7
II.....	1 561	1st	65,9 ± 8,6	58,4-82,3	1,7 ± 0,9	1,0- 3,3
III.....	1 834	1st	66,9 ± 10,0	58,8-79,9	3,0 ± 2,3	0,7- 6,8
IV.....	1 658	2nd	42,5 ± 11,8	27,1-57,4	8,4 ± 8,6	0,4-24,9
V.....	1 761	2nd	59,5 ± 11,3	49,3-78,1	3,1 ± 2,7	0,7- 7,8
VI.....	1 904	2nd	48,8 ± 7,3	40,2-59,7	5,4 ± 4,9	0,6-13,9

By 14/3/73, when the first sheep were infested, the snails from the bore-hole water appeared so lethargic and were dying at such a fast rate compared with the others which were in a better condition, that the cercariae collected from the former were not used for infestation lest their viability should have been affected by the physiological condition of their snail hosts (Stirewalt & Fregeau, 1968).

On 16/3/73, however, when the remaining sheep were infested, cercariae from all the snails were pooled accidentally, and 45 moribund snails and 275 others were used for cercarial collection.

After 16/3/73, mortality was very rapid, very few snails remaining alive a week later.

Cercarial viability

The percentage live cercariae in each pool used for infestation is listed in Table 1. The mean percentage for all groups was 99,2%.

TABLE 1 Percentage live cercariae in the cercarial pools used for infestation

Cercarial Pool	Number of cercariae examined	Live cercariae (%)
Day 1*		
I+II.....	1 106	99,1
III.....	940	98,9
"moribund" snails ..	1 733	99,6
Day 2**		
IV.....	1 028	98,7
V.....	658	98,9
VI.....	900	99,3
Mean (all pools)....	—	99,2

* Day 1 of infestation (14/3/73). Day 2 was 16/3/73

** Cercariae from "moribund snails" included with Pools IV-VI (see text)

The viability (percentage development to adult worms) of the 3 pools of cercariae used on the first day of infestation (Table 2) was highly significantly higher than that of the 3 pools of the second day (U=24,5; P<0,001).

A highly significant negative correlation (r=-0,6105; P<0,001) existed between the percentage cercariae which failed to penetrate and the percentage worm development. If Sheep 16, 29 and 30, which had the highest percentage non-penetrant cercariae, were excluded from the calculation, however, the negative correlation, r=-0,3434, was slightly lower than the value of r=-0,3494 which denotes significance at the 5% level.

Infestation and worm recovery data are summarized in Table 3.

TABLE 3 Infestation of animals, development, distribution, and sex ratio of worms recovered

Group	Age of sheep (days)*	Live mass (kg)	No. of cercariae	Cercariae failed to penetrate (%)	Development to adult worms (%)	Worm distribution (%)			Worms <i>in situ</i> after perfusion (%)	Worm sex ratio	
						Mesentery	Liver	Lungs		♂ (%)	♀ (%)
A. Dorper:											
1.....	1732	59.8	1904	0.6	40.2	85.8	14.1	0.1	7.8	63.5	36.5
2.....	1947	70.0	1761	1.9	49.3	94.9	5.0	0.1	5.3	56.2	43.8
3.....	1834	62.8	1834	2.7	60.3	92.1	7.8	0.1	1.5	58.6	41.4
4.....	1702	66.1	1599	0.3	63.5	95.3	4.7	0.0	1.6	54.9	45.1
5.....	1908	66.8	1561	1.8	61.2	90.0	10.0	0.0	1.9	57.1	42.9
6.....	1869	41.4	1658	2.6	47.5	90.2	9.8	0.0	1.3	61.0	39.0
7.....	1692	94.8	1834	1.6	75.4	90.7	9.3	0.0	3.1	59.9	40.1
Mean ± S.D.**	61 m	66.0 ± 15.8	—	1.6 ± 0.9	56.8 ± 11.8	91.3 ± 3.2	8.7 ± 3.2	0.04 ± 0.05	3.2 ± 2.5	58.7 ± 3.0	41.3 ± 3.0
C.V.***(%)	—†	—	—	—	20.8	3.6	37.3	125.0	—	5.1	13.8
B. Dorper:											
8.....	1043	67.3	1561	1.4	58.4	85.8	12.9	1.3	1.5	60.4	39.6
9.....	890	46.1	1599	1.1	61.8	91.6	8.4	0.0	2.6	57.0	43.0
10.....	1478	80.0	1599	2.3	57.2	89.9	10.1	0.0	4.6	57.8	42.2
11.....	917	45.9	1761	2.4	58.5	89.4	9.9	0.7	2.5	60.5	39.5
12.....	1051	57.3	1904	6.7	53.4	89.2	10.8	0.0	1.6	62.1	37.9
13.....	1435	88.2	1658	0.4	56.6	87.7	12.1	0.2	2.8	61.3	38.7
14.....	1497	55.5	1658	4.4	31.9	89.4	10.0	0.6	1.9	59.8	40.2
Mean ± S.D.	40 m	62.9 ± 16.4	—	2.7 ± 2.2	54.0 ± 10.1	89.0 ± 1.8	10.6 ± 1.5	0.4 ± 0.5	2.5 ± 1.1	59.8 ± 1.8	40.2 ± 1.8
C.V. (%)	—	—	—	—	18.6	2.0	14.2	123.3	—	3.1	4.6
C. Dorper:											
15.....	510	36.8	1658	3.9	39.8	90.5	9.4	0.1	4.9	59.8	40.2
16.....	510	30.5	1658	24.9	27.1	85.1	14.9	0.0	5.3	62.0	38.0
17.....	517	46.1	1761	0.7	78.1	90.8	9.0	0.2	1.8	58.9	41.1
18.....	510	44.6	1599	5.4	63.0	88.3	11.6	0.1	8.1	59.7	40.3
19.....	503	42.3	1904	2.5	51.3	87.1	12.6	0.3	2.7	61.2	38.8
20.....	512	39.1	1834	0.7	79.9	89.2	10.7	0.1	6.3	60.5	39.5
21.....	512	33.6	1561	3.3	64.2	89.3	8.9	1.8	1.2	58.3	41.7
Mean ± S.D.	17 m	39.0 ± 5.8	—	5.9 ± 8.5	57.6 ± 19.5	88.6 ± 2.0	11.0 ± 2.2	0.4 ± 0.6	4.3 ± 2.5	60.1 ± 1.3	39.9 ± 1.3
C.V. (%)	—	—	—	—	33.8	2.3	20.0	162.2	—	2.1	3.2

* Mean age in months

** Standard deviation

*** Coefficient of variation

† Not calculated

TABLE 3 (continued)

Group	Age of sheep (days)*	Live mass (kg)	No. of cercariae	Cercariae failed to penetrate (%)	Development to adult worms (%)	Worm distribution (%)			Worms <i>in situ</i> after perfusion (%)	Worm sex ratio	
						Mesentery	Liver	Lungs		♂ (%)	♀ (%)
D. Dorper:											
22.....	137	28.2	1904	6.8	45.8	90.0	9.9	0.1	6.2	60.4	39.6
23.....	160	25.9	1561	1.8	67.7	89.1	4.5	6.4	8.5	57.5	42.5
24.....	132	20.7	1834	3.3	60.0	96.8	1.7	1.5	5.1	59.6	40.4
25.....	144	24.6	1761	7.8	51.8	91.7	8.2	0.1	4.8	60.4	39.6
26.....	127	23.2	1561	1.0	82.3	92.2	7.8	0.0	4.2	57.0	43.0
27.....	139	25.0	1599	2.3	69.9	98.0	1.9	0.1	5.0	56.1	43.9
28.....	142	25.9	1761	2.9	60.0	89.0	11.0	0.0	2.1	60.4	39.6
Mean ± S.D.**	5 m	24.8 ± 2.4	—	3.7 ± 2.6	62.5 ± 12.1	92.4 ± 3.6	6.4 ± 3.8	1.2 ± 2.4	5.1 ± 1.9	58.8 ± 1.9	41.2 ± 1.9
C.V. (%)***	—†	—	—	—	19.4	3.9	58.4	202.4	—	3.2	4.5
E. Merino:											
29.....	256	19.6	1658	14.8	37.3	86.3	13.6	0.1	2.3	62.4	37.6
30.....	251	18.9	1904	13.9	42.5	87.6	12.4	0.0	2.2	63.7	36.3
31.....	254	17.3	1658	7.8	57.4	87.7	11.8	0.5	4.4	64.0	36.0
32.....	238	20.0	1561	1.0	61.5	86.1	13.9	0.0	5.2	61.6	38.4
33.....	242	23.2	1904	1.9	59.7	87.2	10.9	1.9	3.5	64.1	35.9
34.....	247	17.7	1599	5.7	66.0	93.1	6.9	0.0	3.2	57.9	42.1
35.....	254	18.9	1834	6.8	58.8	90.3	9.6	0.1	2.6	60.8	39.2
Mean ± S.D.	8 m	19.4 ± 1.9	—	7.4 ± 5.4	54.7 ± 10.6	88.3 ± 2.5	11.3 ± 2.5	0.4 ± 0.7	3.3 ± 1.1	62.1 ± 2.2	37.9 ± 2.2
C.V. (%)	—	—	—	—	19.4	2.9	21.7	188.4	—	3.6	5.9
All groups:											
Mean ± S.D.	—	—	—	4.3 ± 2.4	57.2 ± 3.4	89.9 ± 1.8	9.6 ± 2.1	0.5 ± 0.4	3.7 ± 1.0	59.9 ± 1.4	40.1 ± 1.4
C.V. (%)	—	—	—	55.4	5.9	2.0	21.3	89.0	27.9	2.3	3.4

* Mean age in months

** Standard deviation

*** Coefficient of variation

† Not calculated

TABLE 4 Number of *S. mattheei* ova excreted in the faeces per female schistosome in the mesentery per 24 h

Groups and sheep	Days after infestation																				
	43	44	45	46	47	48	49	**50/52	**51/53	54	55	56	**57/59	**58/60	61	62	63	**64/66	**65/67	68	69
A.....	0	0	11	11	0	22	30	44	83	111	192	305	200	155	233	153	144	392	336	293	93
1.....	0	6	31	31	42	27	105	88	93	203	226	263	209	194	310	418	159	285	203	342	211
2.....	—	—	7	8	0	8	29	—	45	60	66	99	144	140	75	161	116	159	109	278	141
3*	—	—	0	4	10	13	11	51	55	153	93	114	363	182	182	182	181	107	146	—	—
4*	—	—	0	5	18	17	47	65	54	147	216	139	327	258	267	265	265	107	191	234	—
5*	0	0	6	6	14	13	4	63	174	94	95	100	223	248	380	281	388	179	191	179	—
6*	—	—	0	0	17	4	21	27	—	242	166	221	219	347	259	268	387	281	281	215	104
7.....	0,0	2,0	6,6	9,8	14,4	15,2	40,5	56,3	84,0	144,3	150,6	177,3	246,4	206,6	243,7	243,8	249,7	233,8	213,5	256,8	—
S.D.....	—	3,5	5,7	11,0	14,2	8,6	33,8	20,8	47,8	63,0	65,2	84,8	85,1	83,5	96,7	101,2	142,3	104,6	87,1	58,9	—
B.....	—	—	8	0	6	17	—	92	83	105	164	251	241	195	275	176	413	—	171	367	452
8*	—	—	5	4	5	17	—	79	104	105	198	227	170	242	242	176	219	244	253	246	—
9*	—	—	12	30	4	25	—	41	96	65	185	193	198	245	305	335	207	200	222	441	—
10*	0	0	0	0	15	13	18	39	96	70	128	130	205	258	154	149	194	284	256	278	—
11.....	0	5	0	6	6	3	2	19	43	69	90	82	99	166	128	200	126	266	365	403	346
12.....	0	0	0	6	33	—	68	88	187	215	254	163	214	515	369	188	309	332	383	294	—
13.....	0	0	0	8	17	7	27	39	78	77	129	87	99	407	191	174	159	83	153	177	—
14.....	0,0	1,3	4,9	7,7	12,3	13,7	28,8	56,7	98,5	100,2	164,0	161,9	175,1	289,7	237,0	203,7	232,4	234,8	229,0	315,1	—
Mean.....	—	2,5	5,0	10,3	10,5	7,9	28,1	28,9	48,2	58,1	54,3	66,0	56,1	125,2	94,2	66,5	97,9	86,2	88,5	93,0	—
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C.....	0	7	0	0	—	46	30	41	93	237	73	108	202	270	490	279	280	567	954	567	—
15.....	0	0	0	0	0	39	14	38	79	121	94	89	112	208	316	299	200	400	400	713	—
16.....	0	0	0	0	24	—	—	38	87	175	146	166	317	329	212	178	122	547	464	95	—
17.....	0	0	0	13	0	—	—	104	157	107	206	181	119	325	339	193	193	231	319	197	—
18*	0	0	0	19	11	23	—	104	147	195	220	189	201	325	339	153	260	133	366	422	—
19.....	0	0	0	5	17	48	38	152	128	204	182	204	171	404	428	360	282	180	391	366	—
20*	—	—	0	5	24	13	41	92	106	230	197	347	399	380	392	351	282	109	224	294	—
21.....	0,0	1,8	3,1	11,4	8,7	33,8	30,8	86,4	113,9	181,3	159,7	183,4	217,3	307,7	341,6	270,0	222,8	309,6	510,8	284,7	343
Mean.....	—	3,5	6,4	9,3	10,3	15,2	12,1	48,8	30,5	50,6	57,3	83,8	105,2	72,4	104,7	86,9	62,8	193,7	273,1	150,8	—
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
D.....	0	0	6	6	9	25	18	59	88	82	225	108	231	368	191	168	280	188	452	317	259
22.....	0	0	6	19	0	6	—	133	147	230	259	314	244	498	498	788	429	407	580	601	—
23*	—	—	0	5	8	0	31	48	32	120	182	240	235	207	268	368	224	185	269	528	—
24*	0	0	0	5	5	11	—	36	36	94	109	76	84	172	138	148	133	393	294	301	—
25.....	0	0	0	2	10	20	33	64	69	79	153	128	266	266	278	242	294	198	310	420	264
26*	—	—	0	2	10	8	34	—	109	100	139	198	304	217	296	512	313	249	361	182	—
27*	—	—	0	0	10	—	76	—	384	205	273	273	362	439	329	358	299	732	237	400	—
28.....	0,0	0,0	1,6	8,1	9,0	11,7	37,3	68,0	138,2	130,0	191,0	191,0	246,6	286,9	285,4	369,1	282,0	336,0	357,6	392,7	—
Mean.....	—	—	2,7	8,0	6,4	9,3	19,8	37,9	126,4	61,7	61,9	89,8	85,5	97,8	114,2	224,2	98,6	198,5	120,5	142,0	—
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
E. Merino.....	0	0	0	0	—	17	66	65	108	69	58	86	28	49	79	73	108	233	126	138	—
30.....	0	0	0	19	—	43	18	132	152	275	233	176	136	185	313	149	77	245	398	179	—
31.....	0	0	0	8	8	18	29	59	67	139	105	149	235	215	96	37	126	75	187	260	289
32*	—	—	10	15	11	48	76	63	85	310	340	339	290	309	408	493	351	91	213	67	—
33.....	5	0	—	—	—	—	—	34	128	113	—	126	100	68	53	67	109	56	268	207	—
34*	—	—	5	4	24	31	—	58	105	164	230	231	419	343	334	418	301	238	271	219	—
35*	—	—	0	0	0	21	—	39	46	83	125	87	153	220	140	195	92	139	210	172	87
Mean.....	1,3	0,0	2,5	7,2	22,0	29,7	47,3	64,3	98,7	164,7	181,8	170,6	194,4	198,4	203,3	204,6	166,3	153,9	233,6	182,9	—
S.D.....	2,5	—	4,2	8,0	19,1	13,3	28,1	32,2	36,0	93,5	104,3	90,0	131,0	110,6	144,1	180,9	111,1	83,3	89,1	63,3	—
All groups.....	0,3	1,1	3,5	8,7	13,2	20,3	37,3	66,6	106,6	145,4	169,1	176,8	216,0	257,9	262,9	260,0	229,3	254,8	311,7	287,3	—
Mean.....	1,2	0,3	5,0	8,9	12,7	13,7	24,5	34,8	63,9	69,0	66,9	79,0	94,5	104,6	116,4	154,0	107,4	151,9	173,8	125,0	—
S.D.....	39,3	21,6	142,0	102,0	96,6	67,3	65,6	52,2	60,0	47,4	39,6	44,7	43,7	40,5	44,3	59,2	46,8	59,6	55,7	43,5	—

** The first figure refers to sheep marked with asterisks

TABLE 5 Number of *S. mattheei* ova excreted per g of faeces (e.p.g.)††

Groups and sheep	Days after infestation																		
	43	44	45	46	47	48	49	**50/52	**51/53	54	55	56	**57/59	**58/60	61	62	63	**64/66	
A.....	0.0	0.0	1.7	3.3	0.0	10.0	11.7	15.0	21.7	35.1	45.1	61.8	48.4	41.8	66.8	43.4	35.1	80.2	
1.....	0.0	3.3	6.7	13.4	16.7	23.4	33.4	41.8	35.1	100.2	80.2	93.5	93.5	88.5	105.2	203.7	73.5	93.5	
2.....	0.0	0.0	0.0	5.0	0.0	23.4	10.0	48.4	45.1	43.4	65.1	105.2	120.2	90.2	55.1	143.6	108.6	147.0	
3*	—	—	0.0	1.7	5.0	3.3	10.0	15.0	11.7	126.9	65.1	140.3	325.7	182.0	218.8	320.6	267.2	—	
4*	—	—	0.0	1.7	5.0	3.3	10.0	15.0	11.7	41.8	61.8	50.1	76.8	73.5	93.5	—	—	33.4	
5*	—	—	0.0	1.7	5.0	3.3	10.0	15.0	11.7	28.4	28.4	30.1	81.8	101.9	125.3	143.6	153.6	68.5	
6.....	0.0	0.0	1.7	0.0	3.3	1.7	8.4	18.4	51.8	85.2	58.5	101.9	76.8	91.9	110.2	126.9	165.3	152.0	
7.....	0.0	1.1	2.2	4.2	5.6	8.9	16.2	27.3	33.4	65.9	55.6	83.3	121.9	95.7	110.7	163.6	125.3	95.8	
Mean.....	0.0	1.9	2.3	4.8	5.6	7.7	10.1	14.4	14.8	38.1	16.4	37.8	92.8	42.8	53.6	92.6	77.8	46.2	
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B.....	—	—	1.7	0.0	1.7	5.0	—	20.0	16.7	28.4	56.8	100.2	60.1	38.4	71.8	43.4	91.9	80.2	
8*	—	—	1.7	0.0	1.7	6.7	—	23.4	28.4	81.8	53.4	70.1	40.1	55.1	55.1	40.1	58.5	155.3	
9*	—	—	1.7	1.7	1.7	18.4	—	43.4	40.1	197.1	197.1	128.6	128.6	157.0	160.3	257.2	142.0	80.2	
10*	—	—	3.3	8.4	5.0	5.0	—	16.7	40.1	36.7	40.1	40.1	70.1	68.5	45.1	66.8	71.8	83.5	
11.....	0.0	0.0	0.0	0.0	5.0	10.0	5.0	10.0	13.4	20.0	40.1	25.1	48.4	48.4	48.4	31.7	70.1	80.2	
12.....	0.0	1.7	0.0	1.7	8.4	10.0	16.7	25.1	46.8	60.1	63.5	33.4	48.4	116.9	70.1	46.8	78.4	61.8	
13.....	0.0	0.0	1.7	1.7	8.4	1.7	6.7	25.1	28.4	28.4	28.4	21.7	25.1	116.9	36.7	60.1	55.1	45.1	
14.....	0.0	0.0	0.0	1.7	3.3	7.8	8.8	11.2	13.1	42.6	65.1	59.9	25.1	78.3	71.8	84.3	81.1	84.4	
Mean.....	0.0	0.9	1.2	2.2	2.6	5.9	5.3	11.2	13.1	23.6	60.5	41.3	34.0	43.6	45.5	85.6	29.5	37.7	
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
C.....	0.0	1.7	0.0	1.7	—	11.7	8.4	10.0	21.7	50.1	18.4	31.7	63.5	71.8	93.5	105.2	118.6	83.5	
15.....	0.0	0.0	0.0	0.0	0.0	5.0	—	5.0	8.4	13.4	18.4	11.7	18.4	25.1	33.4	38.4	25.1	38.4	
16.....	0.0	0.0	0.0	5.0	10.0	—	1.7	15.0	31.7	61.8	41.8	56.8	103.5	91.9	53.4	58.5	63.5	93.5	
17.....	0.0	0.0	0.0	5.0	0.0	—	—	25.1	26.7	18.4	33.4	36.7	60.1	58.5	53.4	—	23.4	53.4	
18*	—	—	0.0	3.3	3.3	8.4	—	28.4	30.1	45.1	51.8	53.4	60.1	86.8	86.8	40.1	40.1	28.4	
19.....	0.0	0.0	0.0	6.7	3.3	—	—	35.1	33.4	50.1	50.1	65.1	48.4	98.5	126.9	118.6	75.2	58.5	
20*	—	—	0.0	1.7	5.0	16.7	10.0	35.1	33.4	68.5	50.1	91.9	105.2	118.6	131.9	157.0	157.0	213.8	
21*	—	—	0.0	8.4	0.0	5.0	8.0	28.4	27.2	43.9	37.0	49.6	60.6	78.7	82.8	86.3	60.7	83.0	
Mean.....	0.0	0.4	1.2	3.0	3.1	9.4	4.4	11.3	9.8	20.8	17.2	25.9	34.2	30.4	38.0	48.2	35.3	62.2	
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
D.....	0.0	0.0	0.0	1.7	1.7	6.7	3.3	10.0	16.7	20.0	38.4	15.0	40.1	66.8	33.4	28.4	16.7	46.8	
22.....	0.0	0.0	0.0	5.0	0.0	1.7	—	35.1	35.1	53.4	51.8	70.1	51.8	68.5	103.5	158.7	80.2	83.5	
23*	—	—	0.0	1.7	3.3	0.0	8.4	11.7	10.0	28.4	45.1	61.8	60.1	41.8	61.8	80.2	50.1	51.8	
24*	—	—	0.0	1.7	1.7	3.3	—	28.4	10.0	33.4	38.4	21.7	61.8	61.8	53.4	53.4	25.1	53.4	
25.....	0.0	0.0	0.0	1.7	6.7	11.7	15.0	48.4	46.8	48.4	106.9	113.6	135.3	145.3	167.0	133.6	135.3	140.3	
26*	—	—	0.0	1.7	6.7	3.3	—	—	31.7	33.4	43.4	65.1	80.2	56.8	85.2	172.0	93.5	91.9	
27*	—	—	0.0	0.0	3.3	3.3	23.4	—	131.9	81.8	95.2	106.9	115.2	140.3	106.9	113.6	93.5	230.5	
28.....	0.0	0.0	0.0	10.0	6.7	4.4	11.7	23.4	45.4	42.7	60.4	64.9	87.3	83.0	87.3	105.7	85.2	108.3	
Mean.....	0.0	0.0	0.5	3.1	3.6	4.2	6.9	17.4	14.4	20.7	28.5	37.7	39.6	41.8	44.2	53.9	30.4	63.0	
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
E. Merino	0.0	0.0	0.0	0.0	1.7	3.3	3.3	13.4	20.0	13.4	11.7	15.0	5.0	8.4	13.4	13.4	16.7	41.8	
29.....	0.0	0.0	0.0	6.7	15.0	10.0	16.7	36.7	43.4	70.1	56.8	45.1	36.7	45.1	76.8	38.4	25.1	70.1	
30.....	0.0	0.0	0.0	10.0	3.3	11.7	13.4	21.7	28.4	55.1	36.7	61.8	61.8	90.2	38.4	25.1	51.8	53.4	
31.....	0.0	0.0	3.3	13.4	16.7	11.7	16.7	11.7	18.4	41.8	65.1	83.5	66.8	71.8	98.5	131.9	95.2	21.7	
32*	—	—	0.0	3.3	16.7	11.7	—	11.7	40.1	41.8	36.7	41.8	21.7	25.1	21.7	34.4	40.1	23.4	
33.....	1.7	0.0	3.3	3.3	16.7	—	—	35.1	53.4	78.5	98.5	88.5	177.0	220.4	147.0	183.7	153.6	120.2	
34*	—	—	0.0	1.7	11.7	26.7	—	15.0	15.0	35.1	58.5	70.1	58.5	70.1	50.1	63.5	31.7	48.4	
35*	—	—	0.0	0.0	0.0	10.0	12.2	15.0	15.0	35.1	51.1	45.1	54.6	68.0	75.9	68.5	59.2	54.1	
Mean.....	0.4	0.0	1.1	2.5	8.1	12.2	12.5	21.0	31.0	51.1	54.6	54.2	54.9	69.8	63.5	64.8	48.9	48.4	
S.D.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
All groups	0.1	0.4	1.2	3.1	4.5	8.5	11.8	22.7	32.8	49.4	54.5	62.4	76.5	82.3	83.6	100.8	82.9	84.8	
Mean.....	—	—	—	3.3	4.7	6.4	7.3	12.2	22.0	26.1	33.8	34.3	57.4	45.2	46.1	73.6	51.9	50.9	
S.D.....	—	—	—	105.1	105.5	75.3	61.6	53.8	67.0	52.8	61.9	55.0	75.0	54.9	55.2	73.0	62.6	60.0	
C.V. (e.p.g.)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

** The first figure refers to sheep marked with asterisks
 †† E.p.g. data for Day +65/67, +68 and +69 are not included as the animals were starved and this causes an increase in the e.p.g.

The overall mean percentage worm development was $57,2\% \pm 3,4\%$. This figure is derived from means of $56,8\%$ in Group A (Dorpers aged 61 m), $54,0\%$ in Group B (Dorpers aged 40 m), $57,6\%$ in Group C (Dorpers aged 17 m), $62,5\%$ in Group D (Dorpers aged 5 m) and $54,7\%$ in Group E (Merinos aged 8 m). These percentages did not differ significantly ($P > 0,5$) between the various groups of sheep. In addition, when the group means were corrected for differences in infectivity of the individual pools of cercariae, the differences were smaller ($F = 0,23$).

When the Merino sheep were excluded and the 4 groups of Dorper sheep were compared with one another, the P-value was smaller, but the differences between groups were not significant even at 10% significance level ($P > 0,1$).

Because of the smaller difference in the ages of the 2 oldest groups of Dorper sheep (A and B) than that of the other groups, the ages of the Dorper sheep were examined for correlation with the percentage worm development in each sheep, in case the grouping might have affected the above results. There was, however, no significant correlation ($r = -0,207$; $P > 0,1$).

The overall mean values (with the respective P-values for group differences in parentheses) were: $4,3\%$ cercariae failed to penetrate ($0,2 > P > 0,1$); $89,9\%$ ($0,1 > P > 0,05$) of the worms were recovered from the mesentery, $9,6\%$ ($0,02 < P < 0,05$) from the hepatic portal system (liver) and $0,5\%$ ($0,5 > P > 0,3$) from the pulmonary arterial system (lungs); the worm sex ratio was $59,9\% \pm 1,4\%$ ($0,02 < P < 0,05$) males to $40,1\% \pm 1,4\%$ ($0,02 < P < 0,05$) females and $3,7\%$ ($0,2 > P > 0,1$) of the worms were not removed by perfusion and were counted *in situ*.

Significant differences occurred between groups in worms recovered from the livers ($0,02 < P < 0,05$, Kruskal-Wallis Test statistic, $H = 9,8$) and in the worm sex ratio. The mean worm sex ratio of the Merino sheep was $62,1\%$ males: $37,9\%$ females, whereas that of the 28 Dorpers was $59,4\%$ males: $40,7\%$ females, a difference of $+2,7\%$ males to $-2,7\%$ females in favour of the former. The differences were small and it was not possible to determine by means of the Kruskal-Wallis Multiple Comparisons Test which group(s) were responsible for these differences.

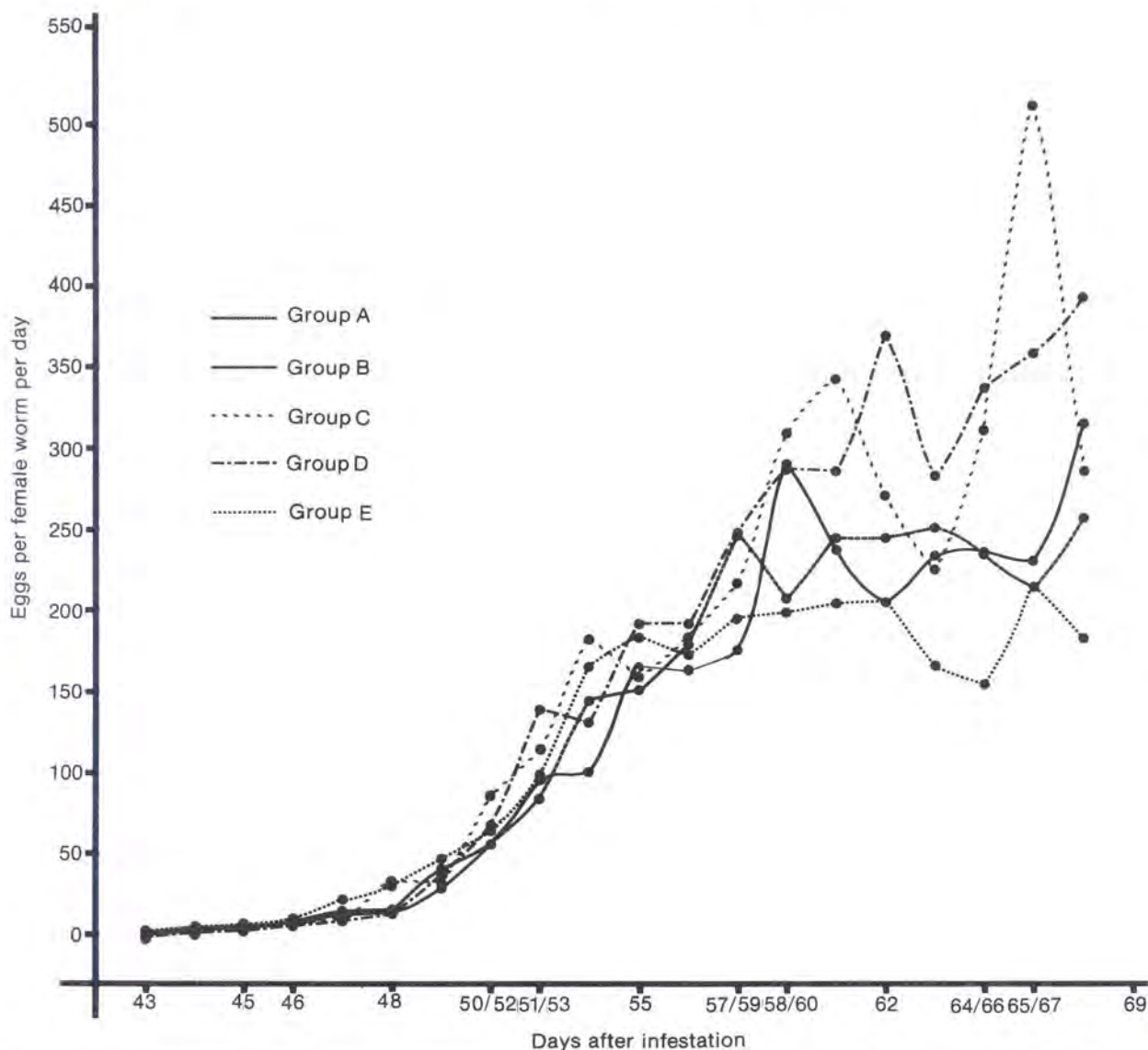


FIG. 1 *S. mattheei* ova recovered in the faeces, expressed as the mean number per female worm in the mesentery of each group per day

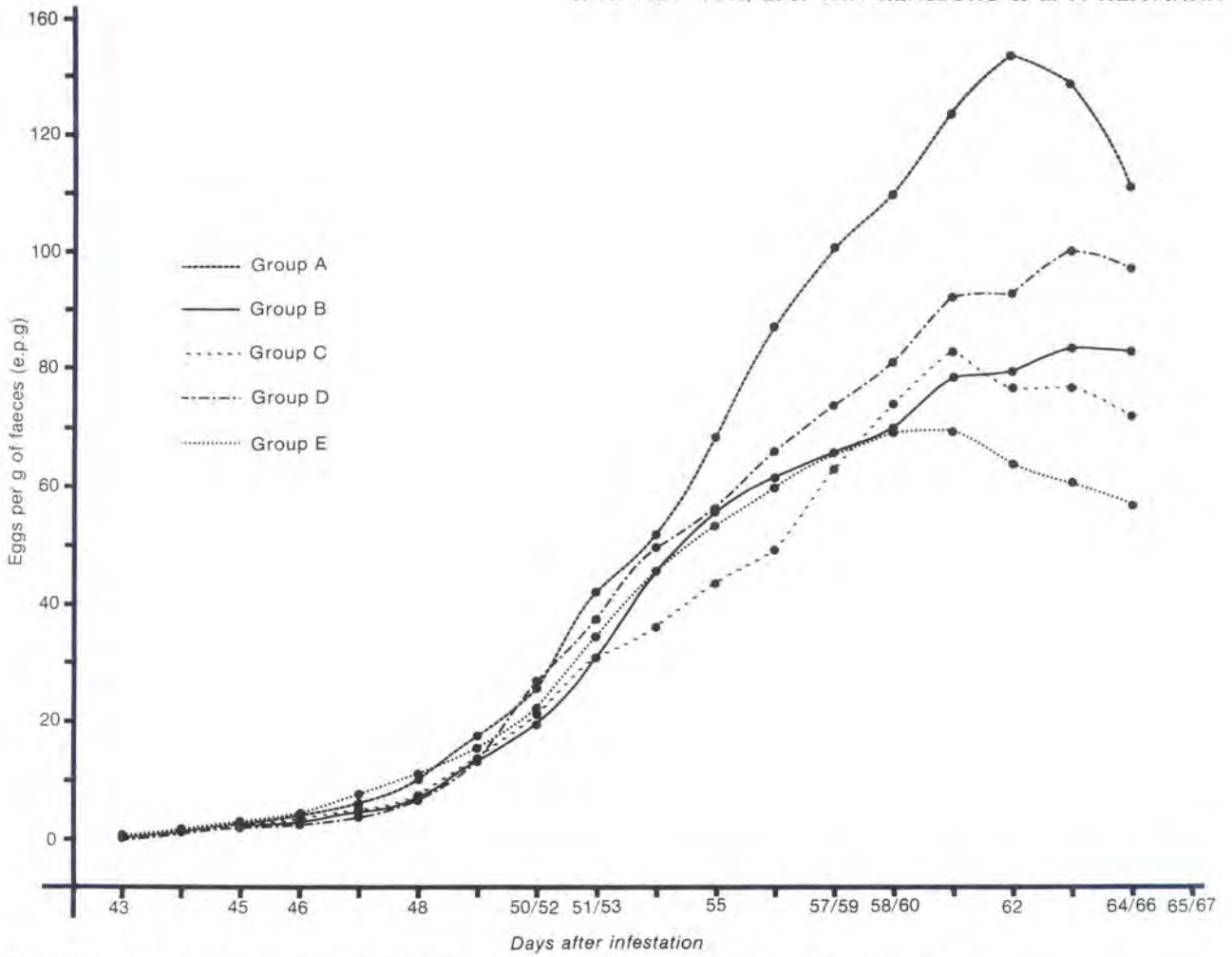


FIG. 2 *S. mattheei* ova excreted per g of faeces (E.p.g.) [3-point moving averages of the means per group]

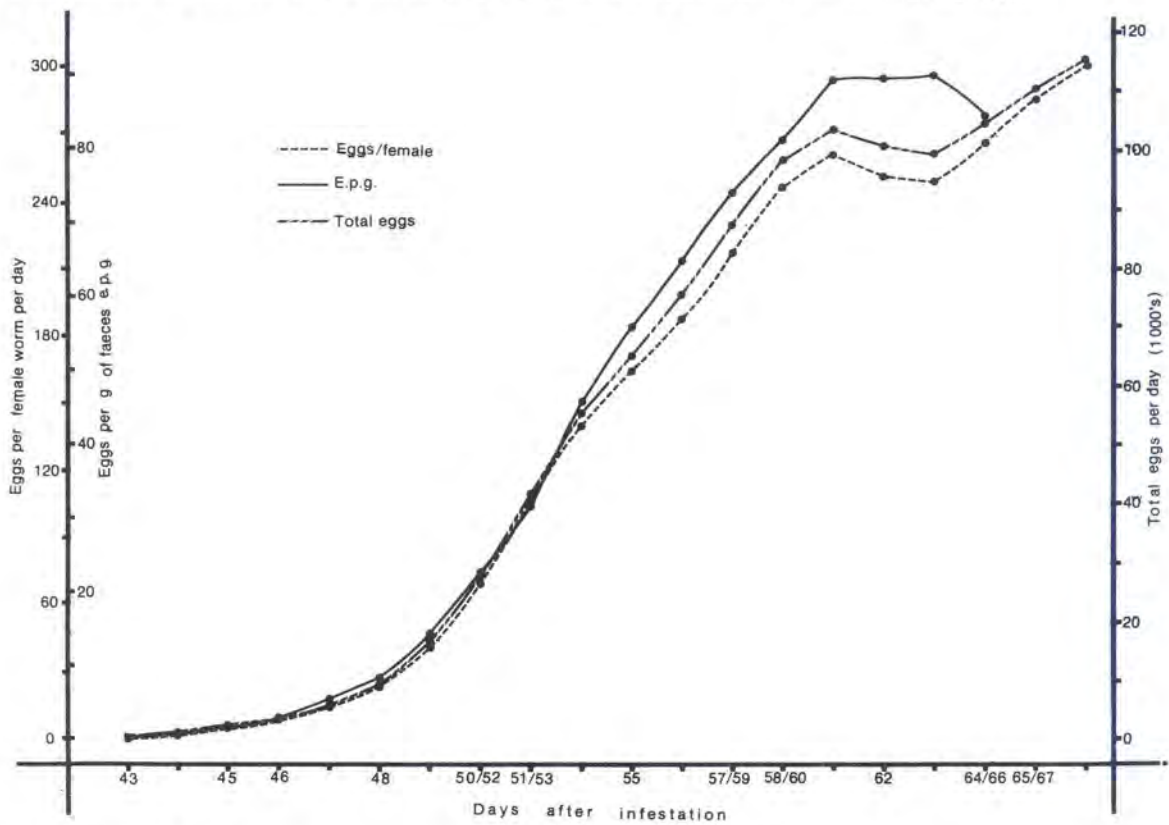


FIG. 3 A comparison of the increase of the total ova excreted per day; ova excreted per female worm in the mesentery per 24 hours; and the ova per g of faeces (3 point moving averages of the mean daily values)

Egg excretion (Fig. 1, 2 and 3; Tables 4 and 5)

Schistosome ova were detected in the faeces of only 1/18 sheep examined on Day +43 and 3/17 on Day +44, after which these increased rapidly to 15/34 on Day +45, 25/33 on Day +46, etc. By Day +48 ova had been detected in the faeces of all 35 sheep.

The mean number of eggs passed in the faeces per day per female worm in the mesentery increased from 0.3 at Day +43 to 13 at Day +47, 37 at Day +49, 145 at Day +54, 177 at Day +56, 263 at Day +61 and 287 at Day +68 (Table 4). Hence the egg count rose very rapidly from Day +48 and more or less Day +61, and thereafter appeared to increase more gradually (Fig. 1 and 2). The relative rates of increase of the eggs excreted per female per day and the e.p.g. were very similar to one another and also to the mean total number of eggs excreted per sheep per day in the faeces (Fig. 3).

The inter-group differences in egg excretion per female schistosome per 24 hours (Table 4) were significant on 3 occasions only, P being <0.05 at Day +48, +65/67 and +68. On each of the 3 occasions different groups of sheep accounted for these differences.

The coefficients of correlation, presented in Tables 6 and 7, respectively, represent:

(a) the total number of eggs excreted per day in the faeces compared with the number of female schistosomes in the mesentery of each sheep, and

(b) the e.p.g. compared with the number of female schistosomes in the mesentery of each sheep.

On all the days for which calculations were made, a significant or highly significant correlation was found between the total egg excretion in the faeces and the numbers of female worms in the mesentery. In the case of the Dorpers alone, the correlation was less highly significant than when data from all 5 groups were used for the calculations.

The correlation between the e.p.g. counts and numbers of female worms was less significant than for total egg excretion in the faeces and the number of female worms. Once again the Dorpers alone gave less significant results.

Furthermore, the correlation was more significant soon after the onset of egg production by the worms than towards the end of the period for which egg determinations were done.

TABLE 6 Coefficients of correlation: Total eggs excreted per day in faeces vs numbers of female schistosomes in the mesentery of each sheep

Category	Days after infestation	P-value	r=	No. of observations
All the sheep (Groups A-E)	50/52	< 0.01**	0.4908	32
	56	< 0.001***	0.6024	35
	61	< 0.001***	0.5538	34
	63	< 0.001***	0.6352	33
	64/66	< 0.02*	0.4192	33
	68	< 0.01**	0.4725	34
Dorpers only (Groups A-D)	50/52	< 0.01**	0.5338	25
	56	< 0.001***	0.6146	28
	61	< 0.01**	0.5575	27
	64/66	< 0.05*	0.4002	26
	68	< 0.05*	0.4256	27

* Significant
** Significant—highly significant
*** Highly significant

TABLE 7 Coefficients of correlation: E.p.g. of faeces compared with the number of female schistosomes in the mesentery of each sheep

Category	Days after infestation	P-value	r=	No. of observations
All the sheep (Groups A-E)	50/52	< 0.02*	0.4293	32
	56	< 0.001***	0.5557	35
	61	< 0.01**	0.4451	34
	63	< 0.05*	0.3825	33
	64/66	< 0.05*	0.3460	33
	65/67	< 0.05*	0.3618	33
	68	n.s.—	0.2910	34
Dorpers only (Groups A-D)	50/52	< 0.02*	0.4788	25
	56	< 0.01**	0.5316	28
	61	< 0.05*	0.4058	27
	63	n.s.—	0.3070	26
	64/66	n.s.—	0.3099	26
	68	n.s.—	0.2233	27

* Significant
** Significant—highly significant
*** Highly significant
— Not significant

DISCUSSION

In addition to the LD₅₀, the usual parameters for measuring the susceptibility of the primary host to schistosomes are the percentage and extent of worm development, distribution of worms in the body and worm egg production (Stirewalt *et al.*, 1951; Bradley, 1967). We investigated 2 of these. Unfortunately, only the faeces and not the organs of the sheep were examined for eggs as the organs were damaged during storage.

Despite the marked variations in ages and masses of the Dorper sheep, and despite inclusion of 2 breeds in the trial, no significant differences (5% significance level) were demonstrated between the 5 groups in respect of the number of cercariae which failed to penetrate, worm development, worm distribution (mesentery and lungs) and worms remaining *in situ* after perfusion.

Despite the care taken to standardize cercarial collection, division into pools and the actual making up of the doses for infestation, highly significant differences were found in the infectivity of the 6 pools of cercariae used. Indeed, these differences had a much greater effect on percentage worm development than either the age or the breed of sheep, as these did not affect development significantly (5% significance level). Similar differences in the infectivity of various batches of cercariae were reported recently by Maddison, Norman, Geiger & Kagan (1970).

Cercariae collected first after exposure of the snails to light appeared to be as infective as those which emerged towards the end of the period of collection (Pools III and VI compared with the rest). However, cercariae collected on the 2nd day of infestation were much less infective than those of the 1st day ($U=24, 5$; $P<0.001$).

The only difference in method on the 2 days of infestation was the inclusion of cercariae from snails in poor condition on the 2nd day, but it seems unlikely that this difference could have affected the results as markedly as appears here. The snails in question comprised only about 14% of the total used for cercarial collection on Day 2 of infestation, and the percentage live cercariae was similar both between the 2 groups of snails on Day 1 of infestation and between the 2 days of infestation (Table 1). Nevertheless, the

possibility should be investigated further in case water in which snails in poor condition are placed for cercarial collection has a deleterious effect on the viability of cercariae from snails in relatively good condition.

It must be remembered, however, that, although the group of snails kept previously in tap water died more quickly than the others, mortality in both infested groups was rapid. Stirewalt & Fregeau (1968) found that the infectivity of cercariae collected at various times after the start of cercarial emergence from groups of snails, varied, and concluded that the physiological condition of the snails may have been responsible. The snails in the present experiment did not survive a comparable time, but their physiological condition must have been deteriorating rapidly and this may have caused the differences in infectivity between the 2 days.

The effect of differences in infectivity between cercarial pools can be minimized by infesting an equal number of animals from each test group with cercariae from every cercarial pool.

Although the negative correlation found between cercariae which apparently failed to penetrate the sheep but remained in the suspension after infestation, and the percentage worm development would appear to indicate that this is a valid method for estimating worm development, this probably holds true only if the species of animal and circumstances of infestation are suitable for maximum worm development. This deduction is based on the following: A mean of 4.3% cercariae apparently failed to penetrate these sheep. In similar trials by Van Wyk *et al.* (1975), however, in which a mean of only 0.88% cercariae apparently failed to penetrate their sheep, the differences in penetration between the 4 groups of sheep they infested percutaneously were not significant, even though significant differences did exist between the percentage worm development of the 4 groups. Furthermore, Van Rensburg (1972) recorded negative or very poor worm development despite good apparent cercarial penetration in susceptible multimammate rats treated with a protective soap before infestation.

The mean percentage worm development was lower in this experiment (57.2% \pm 3.4%) than in a previous trial (63.0% \pm 13.8%) where the same method of infestation and various batches of cercariae were used in Merino wethers (Van Wyk *et al.*, 1975), but in view of the large coefficients of variation these results are comparable.

These findings differ from those of other workers with baby and adult mice, where significant differences were shown in worm development (Purnell, 1966; Pellegrino & Katz, 1969; Ghandour & Webbe, 1973). Similar differences may possibly be found if newborn lambs are compared with adult sheep.

The worms recovered from the liver differed significantly between the 4 groups of sheep. Possibly the youngest sheep, in which the smallest percentage of worms was located in the liver, offered less resistance than the older sheep to the full development and migration of the worms to the target location in the mesentery.

More male and fewer female worms were recovered from the Merinos than the Dorper Sheep, but the difference is small (+2.72% males: -2.72% females) and may be ignored for practical purposes. In another group of Merinos examined by Van Wyk *et al.* (1975), the mean ratio of male: female worms was

also slightly higher than in the Dorpers in the present trial. Nevertheless this variation was small in comparison with that in a further trial in which Van Wyk *et al.* (1975) recovered more female worms than males from Dorper wethers. They deduced that this was probably due to variations in the cercarial sex ratio from different batches of snails rather than differences in development of male and female cercariae in the host animal, a hypothesis supported by this investigation.

As far as can be ascertained, this is the first detailed study on the time of onset of schistosome egg excretion in the faeces of sheep and the rate of increase during the early stages of patency. Lawrence (1974) studied the relative concentration of eggs in the faeces of a sheep (e.p.g. of faeces) at weekly intervals from 6 weeks after infestation. He found the first ova 7 weeks after infestation, but unfortunately did not state the number of sheep which were positive.

The egg excretion varied markedly from sheep to sheep and from day to day in the same sheep. The e.p.g. varied much more than total egg excretion per day. These findings and correlations with worm numbers (below) show that, even though they entail more work, the determination of the total egg excretion per 24 h is more rewarding than the determination of only the relative egg concentration (e.p.g.) in the faeces.

After a relatively slow rate of increase in egg excretion in the faeces per female schistosome per day from Day +43 to Day +48, a period of very rapid increase ensued until about Day +61. Thereafter, it appeared that a plateau had been reached (Fig. 3) and at the end of the investigation the egg excretion was increasing only slowly. The e.p.g. and total egg excretion per day followed a similar trend (Fig. 3). Although it would appear from the graph that egg production was levelling off towards the end of the experiment, it is probable that there would have been a further increase if the experiment had not been terminated. Lawrence (1974) found that the e.p.g. of 3 sheep infested with *S. matthei* almost doubled between 10 and 20 weeks after infestation. Similarly Massoud (1973) reported a threefold increase in the concentration of *S. bovis* eggs in sheep faeces between 9 and 18 weeks after infestation. Nevertheless, the egg excretion in the mesentery per day was only 121 at 18 weeks, compared with a mean of 287 at 10 weeks in the present investigation (Table 4). This should be pursued further to obtain figures for long-standing infestations.

The egg excretion per female in the mesentery per 24 h differed significantly between groups on 3 occasions viz. Day +48, +65/67 and +68. From Table 4 and Fig. 1 it is obvious, however, that there was considerable variation from day to day both in the same sheep and in the mean egg excretion per group of sheep, and this probably accounts for the fact that, on each of the 3 occasions when significant differences occurred between groups, different groups of sheep were responsible. For example, on Day +48, Groups C and E, and on Day +65/67 Groups C and D were much higher, while on Day +68, Group E was much lower than the rest. These variations between groups can probably be ignored since they are not consistent, though they might have been clearly resolved if the study had been continued longer.

Although there was only a relatively small variation in the numbers of worms per animal, the total number of eggs excreted in the faeces per day showed a highly significant correlation with the numbers of female schistosomes in the mesentery (Table 6). Nevertheless, towards the end of the investigation, the correlation was much less reliable. A similar trend was observed in the correlation between the worm numbers and the e.p.g. counts (Table 7), which was less reliable than the total egg excretion for estimating worm development. It would be very interesting to study egg excretion in long-standing infestations which differ markedly in worm burdens to determine whether this trend is continued.

The highly significant correlation at the early stages between egg production and the number of worms could conceivably be used for predicting the worm development in an animal. In investigations in which the worm burdens are crucial, the sheep may be allocated to groups according to their egg excretion.

Massaging worms out of small blood vessels during the perfusion of the mesentery appears to be justified. In this experiment, only 3.7% worms were not removed by perfusion plus massage, whereas 9.3% worms remained in another study where this method was not used (Van Wyk *et al.*, 1975).

Vascular perfusion was more laborious in the larger sheep than in the smaller animals, and larger amounts of perfusion fluid were required. The vigorous pummelling of the intestines in the former sheep, which were fatter than the others, resulted in damage to the peritoneum and, the instruments and organs being unavoidably covered with a layer of slippery fat, complicated worm recovery. The mean percentages of worm development and worms not removed by perfusion, would indicate, however, that worm recovery was as effective in these as in the smaller sheep.

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