Anthelmintic treatment with Bithionol failed to remove the worms resulting from immunization as 23,958 of these worms were recovered *post mortem*. The immunity in this bovine was still complete 553 days after initial infestation as only 21 worms from the challenge infestation were recovered.

(v) *The administration of live, adult paramphistomes per os:* Live, adult paramphistomes were collected from the rumens of experimentally infested sheep, goats and cattle slaughtered over a period of 28 days. The worms were washed and dosed in a solution of 0·85 per cent NaCl to a bovine (B50) on the day that they were collected, until a total of 5,629 worms had been dosed. It was challenged with 250,000 metacercariae 103 days after the last administration of worms and slaughtered 58 days after challenge.

Symptoms of paramphistomiasis developed after challenge and 102,823 worms of the challenge infestation were recovered at autopsy. Of the 5,629 paramphistomes dosed to produce immunity 3,480 were recovered, and were all present in the rumen.

Challenge infestation

(*a*) *Migration:* The worm distributions in the majority of the controls are given in Table 10. The worm distributions of the challenge infestations in some of the immune cattle are compared with those of susceptible cattle with light or heavy worm burdens of approximately the same age in Table 25.

In the immune cattle migration was delayed not only from the posterior intestine to the first three metres for periods exceeding 34 days (B36 and B44) but from the intestine to the rumen for longer than 51 days (B49).

A similar distribution is seen in heavily infested susceptible cattle for periods up to 40 days after infestation (B17, Table 10). In lightly infested susceptible cattle 96·9 per cent of the worms were present in the anterior three metres of intestine within 10 days of infestation and migration to the rumen was nearly complete at 35 days. The low challenge worm burdens in the immune cattle hardly migrated at all when compared with the similar but heavier worm burdens in the lightly infested, susceptible cattle.

In six of the immune cattle, which are not included in Table 25, all the worms of the challenge infestation were present in the first three metres of small intestine (B35, B38, B41, B42, B43 and B46, Table 24). In the animals which were not immune, worm distribution was similar to that in susceptible animals with large worm burdens (B30 to B33 and B50, Table 24).

(*b*) *Worm size:* Worm measurements were made on as many worms as were available up to a maximum of 30, from each of six cattle which were completely immune. These measurements are compared with those from small or large populations in susceptible cattle (see Table 26).

The growth rate of worms originating from the challenge infestations in the immunized cattle is not only considerably slower than that of worms in susceptible cattle with light worm burdens, but also than that in susceptible cattle with heavy worm burdens of the same age. The 18-day old worms in one immune animal (B34) were scarcely larger than four-day old worms from a lightly infested, susceptible bovine (B1). In the other immune cattle, 24 to 34-day old worms were smaller than 14-day old worms in a heavily infested, susceptible animal (B12). It is obvious that the growth rate of worms in immune cattle is retarded for periods varying from 14 to 30 days when compared with that of worms from susceptible cattle and because of this retarded rate of growth, migration is delayed.

Discussion

In cattle initial infestations with 2,500 metacercariae proved to be insufficient and no resistance to reinfestation developed. Infestations with 40,000 normal or X-irradiated metacercariae stimulated a rapid immune response, which was fully effective 28 days after initial infestation to a challenge of 101,000 metacercariae and still effective 279 days after immunization to a challenge of 250,000 metacercariae.

After multiple infestations of 500 metacercariae three times a week, for 31 weeks, immunity was still complete more than a year later even to the enormous challenge of more than 1,500,000 metacercariae.

Michel (1962) found that an immune response could be elicited 11 days after vaccination of cattle with larvae of *D. viviparus*. The present findings confirm the rapid development of this immune reaction.

The effects of immunity on the worms

Similar observations to those in sheep were made in cattle but whereas most of the reactions were mild in sheep they had a very marked effect on the worms in immune cattle.

(1) Excystation of the challenge infestation is not inhibited. This was demonstrated in an immune bovine (B49) in which, after challenge, 85 per cent of metacercariae recovered from the faeces had excysted.

(2) The attachment of the newly excysted worms is prevented and these worms are eliminated. An immune bovine (B45) slaughtered eight days after challenge with 240,000 metacercariae harboured only 911 worms of the challenge infestation indicating that the majority of worms had already been expelled. It would appear that the intestinal environment in immune cattle is so unfavourable that the young worms are unable to attach and are expelled.

It is possible that this elimination takes place immediately after excystation. This occurred in two cattle (B27 and B28, Table 13) which had received regular doses of metacercariae over a prolonged period to within three days of slaughter. No worms were recovered from the small intestine of one of these animals while the other harboured only one.

(3) Because of the rapid expulsion of the immature worms the worm burdens that result from challenge are reduced to such an extent that they are negligible.

The total worm burden of the 16 completely immune cattle in Table 24 is 3,451 compared to a total challenge of 5,005,000 metacercariae, a reduction to less than one thousandth of the number of metacercariae dosed. These figures are even more striking when compared with the total worm burden of the seven control animals which had received 200,000 to 305,000 metacercariae. The total burden of these animals was 753,815 worms whereas the total number of metacercariae dosed was 1,715,000 (Table 24).

(4) The growth of worms in immune cattle is retarded to such an extent that 34 and 51-day old worms do not approach the size of 10 and 21-day old worms in lightly infested susceptible cattle (Table 26). This delay in growth is probably due to a completely hostile intestinal environment which has already caused the expulsion of the majority of worms from the challenge infestation and now also inhibits the growth of the few remaining worms.

Douvres (1962) showed that precipitates formed around the body openings and on the cuticle of larvae of *Oesophagostomum radiatum* Rudolphi, 1803, cultivated in media containing extracts of intestinal tissues of previously infested animals. Similar reactions might occur *in vivo* in immune animals reinfested with *P. microbothrium* and result in expulsion or retarded growth of the challenge infestation.

(5) Many of the few remaining worms of the challenge infestations are attached posterior to the first three metres of small intestine. This may be due to the anterior three metres of intestine no longer being a suitable habitat; or to the worms being so stunted that their normal migration from the posterior intestine to the first three metres is delayed. In susceptible, lightly infested cattle the vast majority of worms has migrated to the first three metres by 10 days and their average acetabular size is 0·40 mm, while in immune cattle the acetabula are only 0·33 mm at 34 days.

(6) Migration from the small intestine to the rumen is delayed. One reason for this could be that the worms are so stunted that at 51 days of age the acetabular measurements have not reached 0·56 mm, which is the minimum average size before migration can take place (*vide supra*); another, that the worms may grow and start migrating only to be expelled before reaching the rumen. This creates the false impression that migration is delayed because the entire residual worm burden is confined to the intestine.

(7) Elimination of the immunizing worm burden appears to take place in some animal after challenge. Two calves each immunized with 40,000 metacercariae had residual worm burdens of this immunizing dose of only four and 12, compared with burdens of 21,246 and 17,908 worms in the two adult controls which received 40,000 metacercariae. This suggests that " self-cure " might have taken place in the calves after challenge. This is not a consistent finding as immune adult cattle of the same age as the controls retained 20,569 and 10,698 worms of their original burdens after challenge (B34 and B35, Table 24) indicating a variable elimination of the immunizing infestation.

(8) The pathogenic effects of the challenge infestation are reduced. This reduction is entirely due to the elimination of the challenge infestation in immune cattle. In those animals which were not immune, symptoms developed after challenge and no reduction in pathogenicity was evident.

Factors governing immunity

The attainment of a state of immunity to *P. microbothrium* is not as delicately balanced in cattle as it is in sheep, nor are the factors that govern it as stringent.

(1) Immunity is dependent upon the number of metacercariae dosed initially and, therefore, on the number of young paramphistomes which excyst, attach and migrate during the process of immunization. For this reason a single infestation of 2,500 metacercariae is insufficient to produce immunity but an infestation of 40,000 or more results in complete immunity.

The wide margin between the pathogenicity of the immunizing infestation, which gives rise to approximately 20,000 worms and the lethal infestation of 160,000 worms, indicate that infestations considerably in excess of 40,000 metacercariae would give rise to immunity and not death. This is confirmed in two bovines (B37 and B49) which received 100,000 and 176,000 metacercariae respectively: the one (B37) showed no symptoms but the other exhibited weight loss and diarrhoea. Both

these animals were completely immune when challenged 214 and 553 days later. This is in direct contrast to sheep in which an initial infestation of 100,000 metacercariae would probably produce death, and 176,000 metacercariae would definitely do so.

(2) Immunity is not dependent upon the number of worms which reach the rumen. Bovines 32 and 33 had ruminal worm burdens of 756 and 1,121 respectively and were not immune. The cattle which were immunized with X-irradiated metacercariae had similar ruminal worm burdens and yet were completely immune.

It would appear that it is the initial stimulus provided by the immature worms that determines the degree of immunity that will develop. If the findings in sheep can be transposed to cattle, it is the continued presence of worms which ensures maintenance of this degree of immunity.

(3) These observations indicate that immunity is dependent upon the immunizing infestation completing the normal life cycle. This is confirmed in one bovine (B50) in which the intestinal phase of the life cycle was bypassed by dosing adult paramphistomes *per os*. Although a large number of these paramphistomes became established in the rumen no immunity to subsequent challenge developed.

If the relative suitability of sheep and cattle as the normal hosts of *P. microbothrium* are now compared, taking both the life cycle and immune responses into consideration, it is obvious that cattle are the normal hosts.

In cattle it is only calves that exhibit symptoms after experimental infestations with moderate numbers (40,000) of metacercariae. This confirms field observations where mortalities in calves were common while older cattle survived (Boray, 1959; Butler & Yeoman, 1962). Thus both the young host and the parasite perish. In older cattle, moderate single or multiple infestations lead to the survival of the host with little or no pathogenic effect and the parasite remains established for years. Massive challenge of these hosts causes no symptoms and the challenge infestation is eliminated with little or no reduction in the initial adult worm burden. The host-parasite relationship is to the mutual benefit of both members of the association.

This relationship in sheep only reaches the delicate balance to the mutual benefit of host and parasite under strictly limited experimental conditions. Usually the host dies breaking both links of the association. In the completely immune sheep the host survives but the parasite is eliminated both in its patent and immature stages. The limited studies in goats point to a similar situation to that in sheep.

Included in this immunological study are some of the serological responses of the hosts and these are described below.

3. *Serology*

The serological responses of the host to infestation with *P. microbothrium* may play a role in the development of immunity and as such form part of the host-parasite relationship. A detailed serological investigation was not carried out but the responses which were determined will be enumerated here with descriptions of the methods employed.

A. *The intradermal allergic test*

This test has been used as an aid in the diagnosis of schistosomiasis (for review see Kagan & Pellegrino, 1961) and fascioliasis (Soulsby, 1954; Wikerhauser & Bartulić, 1961; Patnaik & Das, 1961).

The same test using three different antigens prepared from *P. microbothrium* was carried out.

Materials and methods

Preparation of antigens

(i) *Saline extracted antigen:* One gram of fresh, clean, filter paper dried immature paramphistomes was macerated and extracted overnight at 4°C in 9 ml of a 0·85 per cent NaCl solution. The suspension was centrifuged at 1,500 revolutions per minute for 15 minutes and the supernatant fluid collected and stored at −20°C.

(ii) *Metacercarial antigen:* Metacercariae were collected, dried, weighed and one gram macerated and extracted overnight at 4°C in 9 ml of a 0·85 per cent NaCl solution. The suspension was centrifuged and the supernatant fluid stored at −20°C.

(iii) *Boiled alcohol precipitated antigen:* This antigen was prepared by combining the methods of Stewart (1948) and Ershov (1959).

Fresh paramphistomes were collected from the rumen or small intestine of artificially infested sheep, cleaned, dried on filter paper, weighed and macerated. One gram was suspended in 25 ml of de-ionised water. This suspension was boiled by placing the containing vessel in a bath of boiling 20 per cent NaCl for 10 minutes. After cooling the supernatant fluid was pipetted off, centrifuged lightly and five times its volume of 96 per cent ethyl alcohol added. After standing at 4°C for 20 hours the alcohol was pipetted off and the sediment dried, dissolved in de-ionised water, freeze-dried and stored at −20°C until required.

Experimental animals

Sheep with immature or adult paramphistome, fasciola, schistosome and nematode infestations, and worm-free sheep were used.

Intradermal test

The saline extracted and metacercarial antigens were allowed to thaw before use. The boiled alcohol precipitated antigen was dissolved in veronal buffer so that one gram of the original paramphistome material was dissolved in 19 ml of buffer.

A McLintock pre-set tuberculin syringe was used to inject 0·1 ml of antigen intradermally in the axillary region of sheep where there is no wool and skin reactions can readily be observed.

The colour and size of the papule formed by the injection were noted immediately and carefully watched for three minutes. Thereafter observations were made at 15 minute intervals for a further hour or two.

Interpretation

A positive reaction caused the appearance of a dark red to purple area at the site of injection surrounded by a large oedematous wheal within one to 30 minutes of injection.

A suspicious reaction caused a slight colour change and a small oedematous swelling.

Results

The results obtained with each of the three antigens are summarized in Table 27.

Not one of the antigens was specific although the results favour the saline extracted antigen. The boiled alcohol precipitated antigen gave negative reactions with *P. microbothrium* infestations but was positive for *F. hepatica*.

Discussion

The test without the use of other diagnostic aids would appear to be of limited value in the diagnosis of paramphistomiasis.

Using the intradermal allergic test in cattle infested with *F. hepatica*, Soulsby (1954) was able to make a positive diagnosis in approximately 90 per cent of cases. Wikerhauser & Bartulić (1961), however, found that although 79·5 per cent of cattle with hepatic lesions due to *F. hepatica* gave typically positive reactions when a metabolic antigen was used, these reactions did not necessarily indicate the presence of liver fluke. Patnaik & Das (1961) made similar observations in cattle infested with *Fasciola gigantica* Cobbold, 1885.

B. *The complement fixation test*

Standard complement fixation tests done on the sera of sheep infested with *P. microbothrium* indicated that these sera were markedly anti-complementary. This anti-complementary activity made interpretation of the test impossible.

Methods of eliminating it such as mixing the sera with liquid paraffin and centrifuging, or incubating at 62°C, for 30 minutes were unsuccessful and as a final resort a more complicated test had to be used.

Jarrett, Jennings, McIntyre, Mulligan, Thomas & Urquhart (1959) employed a modification of the complement fixation test to determine the serological response of cattle to *D. viviparus* infestation. They assessed the potency of a serum in terms of its " Index Ratio " as recommended by Rice (1942). This method of assessment takes the anti-complementary activity of a serum into consideration and was adopted with certain modifications to determine the index ratio of sera from sheep infested with *P. microbothrium*.

Materials and methods

Serology

Test sera: Blood was collected from the jugular veins of experimental animals, allowed to clot at room temperature, the sera separated and stored at − 20°C until required.

Complement: Guinea-pig serum was collected, freeze-dried in 0·5 ml ampoules and stored at 4°C. Each batch was titrated and the amount of complement equal to one 50 per cent haemolytic unit calculated (Kabat & Mayer, 1948). For testing, a dilution was prepared so that 0·25 ml contained the desired number of 50 per cent haemolytic units for a single test.

Antigen: Antigen was prepared by a combination of the methods of Stewart (1948) and Ershov (1959) (*vide supra*). Complement was titrated in the presence of various dilutions of antigen to detect any anti-complementary activity of the antigen. It was found that a one in 20 dilution of antigen in veronal buffer was not anti-complementary and this dilution was used in the subsequent tests.

Sheep erythrocytes: A worm-free lamb was bled directly into an equal volume of Alsevers' solution (Kabat & Mayer, 1948). The resultant suspension of red blood cells was gently agitated and transferred into sterilized McCartney bottles which were stored at 4°C. When required red cells were transferred to a centrifuge tube, veronal buffer added and mixed. The tube was centrifuged at approximately 2,000 rpm until all the cells were down. The supernatant was discarded and fresh veronal buffer added, mixed and centrifuged. This was repeated three times to remove all traces of the Alsevers' solution.

Finally the washed red blood cells were standardized at a two per cent dilution in veronal buffer by centrifuging at 2,000 rpm for 10 minutes to obtain the actual volume.

Haemolysin: Haemolysin was prepared by injecting a rabbit intravenously with 2 ml, 3 ml and 4 ml of 50 per cent sheep red cells in veronal buffer at four-day intervals. The rabbit was bled five days later, the serum separated and stored at − 20°C.

Haemolysin was titrated in the presence of veronal buffer and antigen. The highest dilution at which complete haemolysis occurred was regarded as equal to one unit of haemolysin. In the subsequent complement fixation tests four units of haemolysin were used to sensitize the sheep red cells (Kabat & Mayer, 1948).

Control-sera: Sera were collected from new-born, worm-free lambs and adult sheep infested with *H. contortus* or *F. hepatica* and these sera were used as controls.

Setting up the test: (1) Sera were diluted one in five with veronal buffer and inactivated in a water bath at 50°C for 30 minutes.

(2) Haemolysin was diluted with veronal buffer to give four units per volume and inactivated at 50°C for 30 minutes.

(3) An equal volume of haemolysin was added to a two per cent suspension of red blood cells in veronal buffer and incubated at 38°C for 10 minutes.

(4) Complement was diluted with veronal buffer so that 0·25 ml contained either 1, 2, 4, 6 or 8, 50 per cent haemolytic units.

(5) Antigen was diluted one in 20 with veronal buffer.

(6) The tubes containing the reagents were kept until required in a water-bath maintained at 4°C by the addition of ice.

The test was then set up as shown in Table 28. For each dilution of serum containing complement and antigen a similar dilution was set up containing complement and no antigen, thus the anti-complementary activity of the serum at the various used could be read. Identical tests with sera from worm-free lambs and adult sheep infested with *H. contortus* or *F. hepatica* were set up.

The tubes containing the reagents described in Table 28 were agitated and incubated at 38°C for 45 minutes; 0·5 ml of the suspension containing sensitized red blood cells was then added to each tube. The tubes were shaken and re-incubated at 38°C for 45 minutes. After storage overnight at 4°C the test was read without further addition of veronal buffer.

Reading the test: The test was read in an E.E.L. colorimeter using filter 625.

Series A: Tubes 1 and 2 which contained only sensitized red cells and veronal buffer were used to zero the colorimeter for the reading of tubes 3 and 4 which contained sensitized red cells and excess complement and gave the reading for 100 per cent haemolysis.

Series B: Tube 5 was used to zero the colorimeter for the rest of the series.

Group I. Tubes 6 to 9 indicated the haemolysis occurring with a certain dilution of serum, complement and no antigen, tubes 10 to 13 that occurring with the addition of antigen.

Groups II and III were duplicates of Group I but at higher dilutions of serum.

Interpreting the test: The test was interpreted as indicated by Jarrett *et al.*, (1959), with the exception that the calculation was based on tubes in which the percentage haemolysis lay between 15 and 85 per cent and not 20 to 80 per cent as they suggested. The potency of the serum was expressed in terms of its index ratio which equals the amount of complement necessary to produce 50 per cent haemolysis in the presence of immune serum and antigen, divided by the amount of complement necessary to produce 50 per cent haemolysis in the presence of immune serum alone (Rice, 1942). Where possible index ratios for each dilution of serum used were calculated and the average index ratio for the particular serum sample estimated.

Experimental animals: Five sheep (S103, S107, S108, S124 and S125) of which the parasitological results were given in a previous section (Table 17) were used in this experiment. [These sheep were Sheep 9, 10, 11, 7 and 8 respectively (Horak & Clark, 1963)].

Results

The fluctuations in the index ratio of the sera of two sheep (S124 and S125) at various intervals after infestation and challenge, are illustrated in Fig. 12.

After infestation the index ratio of Sheep 124 climbed to 31 at the height of the pathogenic reaction. It then declined after treatment with Lintex and remained low after challenge to show a slight terminal rise at the time the sheep was slaughtered *in extremis*.

After initial infestation the index ratio of Sheep 125 climbed to a peak of 64 at the height of the pathogenic reaction and then declined after treatment with Lintex. After challenge the index ratio climbed to 79. This sheep was partially immune (Table 17).

The index ratio of Sheep 103 was 60 at the time of challenge and rose erratically to 114 after reinfestation; while the index ratio in Sheep 107, which was 148, declined to 90. Both these sheep were partially immune (Table 17).

Sheep 108, which was not immune, had an index ratio of 96 at the time of challenge, this declined to a value of 11 at the time that the sheep died.

The average index ratio obtained from 10 samples of sera from worm-free lambs was 6; that from a *H. contortus* infested sheep 16 and from a *F. hepatica* infested sheep 14.

Discussion

The antigen appears to be specific for the diagnosis of *P. microbothrium* infestation. It exhibited practically no reaction with the sera from worm-free lambs and reacted only slightly with the sera from sheep infested with *F. hepatica* and *H. contortus*.

It appears that a low index ratio indicates a state of complete susceptibility to *P. microbothrium*, while a high ratio indicates a reaction to initial infestation, but not necessarily a state of immunity unless it remains high or is boosted by challenge.

Anthelmintic treatment which removed practically the entire initial worm burden, seemed to interfere with the maintenance of a high index ratio, which declined considerably after treatment (S124 and S125, Fig. 12).

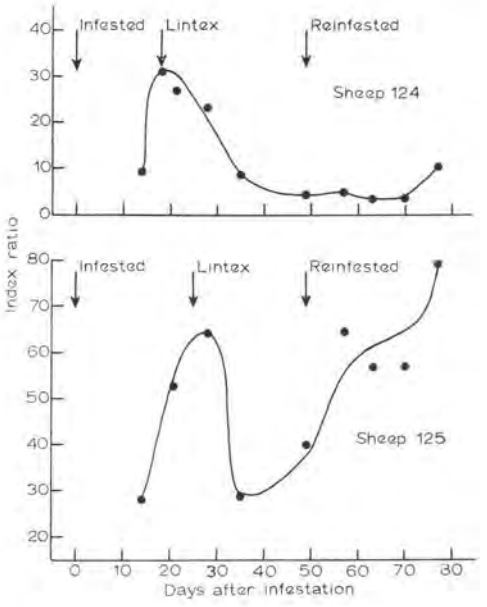

FIG. 12.—The index ratios of two sheep after infestation, treatment and re-infestation

Because of the involved nature of this test and the difficult interpretation of results, it is not a practical method for use as a diagnostic aid, nor does a positive result indicate a state of immunity in the host.

A simpler serological test was used and the results are presented below.

C. *Serum precipitates around live worms*

The formation of precipitates around live nematode larvae incubated in immune serum has been described by Sarles (1937) and Taffs (1961). Wikerhauser (1961a) demonstrated similar precipitates surrounding newly excysted *F. hepatica* incubated in immune serum. These precipitates formed particularly at the body openings but were also noticeable on the cuticle.

Attempts were made to demonstrate similar precipitates around live *P. microbothrium* incubated in immune serum.

Materials and methods

Test sera: These were collected from one infested and three immune sheep; one infested goat; six immune and two infested cattle; and from two paramphistome-free lambs and six cattle, and stored at − 20°C. The term " infested " implies that the animals were infested with *P. microbothrium* but not immune to it.

Paramphistomes: On the day that a test was to be done adult worms were recovered from the rumen or immature worms from the small intestine of artificially infested animals. The worms were thoroughly washed in 0·85 per cent saline and kept until required in saline at 38°C, for a short period never exceeding 30 minutes.

Setting up the test: The sera were allowed to thaw, 0·5 ml was withdrawn from each sample and pipetted into a well of a plastic haemagglutination plate. Duplicate or quadruplicate tests were set up with each serum sample. In addition 0·5 ml of 0·85 per cent saline was pipetted into each of two or four separate wells.

A single, living, adult or immature paramphistome was introduced into each well containing serum or saline and the whole plate was incubated at 38°C.

Tests were set up on five separate occasions using paramphistomes recovered from various hosts. In addition newly excysted paramphistomes were incubated in serum obtained from an immune and from a paramphistome-free bovine.

Reading the test: Observations were made at approximately hourly intervals and the amount and distribution of the precipitate noted. Final readings were made four to 18 hours after commencement of the test.

Results

The most striking results were obtained from the sera of the immune and infested cattle (B18, B19, B34, B35, B37, B41, B42 and B45, Table 24). Within two and a half hours marked precipitates formed around the adult worms, which were particularly noticeable at the excretory pore, genital pore and on the cuticle of the anterior portion of the worm. The precipitate increased in quantity and reached a maximum density approximately four to five hours after the start of incubation (see Plate 5). These precipitates were not as marked around immature worms.

PLATE 5.—Precipitates surrounding a paramphistome incubated in immune cattle serum

Only small quantities of precipitates formed around the worms immersed in serum from paramphistome-free cattle, and in saline the formation of precipitates was delayed for at least 18 hours.

Small amounts of precipitate formed around the newly excysted paramphistomes incubated in immune cattle serum but none formed in the paramphistome-free cattle serum.

Sera from two cattle in which large amounts of precipitate formed when initially tested, were negative when tested two years later, indicating that prolonged storage affects the potency of serum.

In the sera of immune sheep and the infested goat slight precipitates were evident at 18 hours of incubation (S67, Table 13; S115 and S117, Table 17; G7, Table 2) and in only one of four wells containing serum from the infested sheep (S124, Table 17). Around worms in serum from paramphistome-free lambs and in saline, precipitates only started forming after a period of 18 hours.

Discussion

The simplicity and rapidity with which this serological test can be read in cattle are recommendations for its use as a diagnostic aid.

In the experiments on the immunization of sheep, goats and cattle (*vide supra*) it was shown that cattle developed a complete immunity, while the immunity in sheep and goats permitted some of the worms from the challenge infestation to become established. The present findings support these observations, in that a host's ability to develop complete immunity is confirmed by the amount of precipitate which forms around live worms incubated in its serum. What role, if any, these precipitates may play in immunity is not known.

A series of experiments has been described in which the trematode *P. microbothrium* and the domestic ruminants which act as its definitive hosts were discussed separately. It now remains to consider them together in the host-parasite relationship under field conditions.

PART III

Host-parasite Relationships

The preceding studies have shown that cattle are the normal definitive hosts of *P. microbothrium*. Morbidity and mortality is more marked in sheep both under experimental conditions and in the field. Therefore, more emphasis has been placed on experimental investigations in these animals.

Dinnik (1964) has discussed the epizootiology of the acute disease with particular emphasis on the biological aspects. There are, however, additional factors such as prevailing climatic conditions, animal husbandry practices and camp rotation which play an important role in South Africa. Taking these into consideration the author has evolved the following hypothesis on the epizootiology of acute paramphistomiasis.

The epizootiology of acute paramphistomiasis

Most of the outbreaks of acute paramphistomiasis occur during the dry months from autumn to spring, i.e. from March to September in South Africa. This appears anomalous when the aquatic nature of the intermediate snail host *B. (B.) tropicus* is considered. This anomaly can, however, be explained in the light of the following factors.

During the warm summer months, i.e. from October to March, the snails multiply and become infested with *P. microbothrium*. Water is plentiful and the infested snails and the metacercariae are widely dispersed on the herbage surrounding water sources. At the same time grazing is lush and stock graze over large areas only coming to the water source to drink. Consequently if infestation is acquired it is generally light.

In the late summer the grass seeds and from March to September the only green grazing is that surrounding dams, pans, streams and marshes. As little or no rain falls during these months the water surface area recedes, the snail populations become concentrated and the surrounding grazing heavily infested with metacercariae. The livestock concentrate on this green grass and soon become heavily infested.

It is customary to graze cattle prior to or simultaneously with sheep on the same pasture. Cattle being the most suitable hosts contaminate the pasture continuously with large numbers of eggs of *P. microbothrium*. They rarely suffer from pathogenic effects as they can tolerate large worm burdens and rapidly become immune. Sheep, however, are more susceptible and soon acquire heavy infestations and die in large numbers.

A further complication is the system of camp rotation employed by most farmers. Camps with a natural water supply are saved for winter-grazing as the herbage remains green. Animals are introduced into these camps in March and kept there until after the first rains of spring.

Thus the vicious cycle is completed. Ever-receding water supplies during the arid winter and spring with a resultant concentration of metacercariae in a small area, the mixing of cattle and sheep and the enforced winter grazing on infested pastures, give rise to marked mortalities particularly in sheep.

Although wild antelopes are frequently infested with adult paramphistomes outbreaks of acute paramphistomiasis are unknown, unless man interferes with their natural grazing habits and confines them to restricted areas. Numerous mortalities in a herd of Bontebok, *Damaliscus dorcas dorcas* (Pallas, 1766) confined to an area of 800 morgen* were reported by Van der Walt & Ortlepp (1960). These animals suffered mainly from " lungworm and conical flukes ".

It can justly be claimed that acute paramphistomiasis is a man-made disease created by the imposition of farming practices on the natural grazing habits of ruminants.

Control measures must be adopted not necessarily to eliminate the parasite but rather to ensure a degree of equilibrium in the host-parasite relationship.

Control

(i) *Grazing and water sources*

If paddocks with a natural water supply are used for summer grazing only, cattle, calves and sheep can be grazed together. Snails and metacercariae will be widely spread and the infestation that may be acquired could well be immunizing in nature.

However, when a paddock with a natural water supply is continuously grazed, cattle should not graze prior to, or with sheep. Adult and young cattle should also graze separate pastures.

Grazing adjacent to natural water supplies should not be used in winter. These areas should be fenced, the water pumped to a reservoir, treated with a molluscicide and gravitated to drinking troughs, suitably constructed to prevent leakage or soiling. Strydom (1963) was able to control bilharziasis by the application of these simple control measures.

(ii) *Chemo-therapy*

In an outbreak of acute paramphistomiasis before any treatment is considered the whole flock or herd must be removed from the infested paddock. Treatment must be aimed at removing only the pathogenic immature worms and for this purpose Lintex is an excellent anthelmintic in sheep but not in cattle (Horak, 1962b, 1964). The whole flock must be treated because an apparently healthy sheep can die within three days of showing symptoms.

Bithionol is effective against both adult and immature worms in sheep (Horak, 1965b), but must not be used indiscriminately because removal of the ruminal worm burden may interfere with immunity.

If on a routine faecal examination a diagnosis of adult paramphistome infestation is made no treatment should be administered as these worms are not pathogenic. Their removal may also interfere with an immune state in the host.

Because anthelmintics are inefficient in cattle (Horak, 1964, 1965b) other control measures have to be considered.

*One morgen = 10,000 square yards or 2⅛ acre

(iii) *Immunization*

The successful immunization of cattle under experimental conditions has been conclusively demonstrated (*vide supra*). In those areas where paramphistomiasis is enzootic this is a distinct practical possibility. If successful, this may well be an invaluable prophylactic measure in these areas.

Summary and Conclusions

The life cycle of *Paramphistomum microbothrium* Fischoeder, 1901, in experimentally infested sheep, goats and cattle was compared and discussed. It is concluded, that of these domestic ruminants, cattle are the normal definitive hosts of *P. microbothrium*. The worms in cattle grow larger, migrate more rapidly, lay more eggs, live longer and the percentage take after migration is higher than that in either sheep or goats.

Massive infestation of sheep and cattle resulted in retarded growth and migration of the worms.

Metacercariae exposed to X-rays were dosed to sheep and the effects on the life cycle studied.

The pathological anatomy, clinical pathology and symptoms of acute paramphistomiasis are described in detail and their inter-relationship is discussed.

Adult cattle and sheep were successfully immunized by repeated dosage of small numbers of metacercariae (500 to 1,500). Adult cattle were readily immunized either with single doses of 40,000 to 176,000 un-irradiated metacercariae or with single or divided doses of 40,000 irradiated metacercariae. Although adult sheep could be immunized, this was only completely successful with a single or divided dose of 40,000 metacercariae. Immunization of adult goats was successful with a single or divided dose of 40,000 metacercariae.

The effects of immunity on paramphistomes are a marked reduction in numbers, retarded growth and rate of migration.

The intradermal allergic test, a modification of the complement fixation test and the formation of precipitates around live worms incubated in sera from infested hosts were investigated.

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TABLE 1.—*The effect of age on metacercarial viability*

Sheep No.	Age of metacercariae in days	No. of metacercariae dosed	Days between infestation and slaughter	No. of worms recovered	Percentage take
S 11*	47 to 63	210,000	26	70,678	33·7
S 12*	8 to 15	175,000	25	70,139	40·1
S 13	70 and 77	169,000	14	21,797	12·9
S 14	8 to 12	167,000	15	75,579	45·3
S 15	122	45,000	24	30	0·07
S 16	7 to 12	72,000	23	29,729	41·3
S 17	239 and 240	70,000	20	427	0·6
S 18	4 and 5	72,000	22	37,825	52·5
S 19	508 and 539	282,000	7	168	0·06
S 20	14 to 18	170,000	8	71,231	41·9

*Slaughtered *in extremis*

TABLE 2.—*Percentage take and worm distribution in sheep, goats and cattle*

Animal No.	Host	Age of infestation in days	No. of metacercariae dosed	No. of worms recovered	Percentage take	Worm distribution expressed as a percentage							
						Rumen	Omasum	Abomasum	Small intestine				Caecum and Colon
									1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
S 1	Sheep	4	10,000	6,292	62·9	0·00	0·00	4·77	83·39	6·20	3·73	1·91	0·00
G 1	Goat	4	10,000	5,955	60·0	0·00	0·00	0·00	88·51	7·96	3·36	0·17	0·00
B 1	Bovine	4	10,000	5,551	55·5	0·00	0·00	1·08	72·83	21·17	3·84	1·08	0·00
S 2	Sheep	10	10,000	7,411	74·1	0·00	0·00	0·27	98·18	1·28	0·27	0·00	0·00
G 2	Goat	10	10,000	6,324	63·2	0·00	0·00	0·63	98·74	0·57	0·06	0·00	0·00
B 2	Bovine	10	10,000	5,501	55·0	0·00	0·00	2·18	96·86	0·78	0·18	0·00	0·00
S 3	Sheep	20	10,000	7,207	72·1	0·38	0·51	5·26	93·70	0·10	0·03	0·01	0·01
G 3	Goat	20	10,000	5,306	53·1	0·02	0·00	1·34	98·38	0·07	0·04	0·02	0·13
B 3	Bovine	21	10,000	4,914	49·1	0·00	0·18	7·29	92·27	0·18	0·00	0·04	0·04
S 4	Sheep	34	10,000	1,088	10·9	96·60	1·93	0·28	1·19	0·00	0·00	0·00	0·00
G 4	Goat	34	10,000	2,904	29·0	1·03	0·45	18·46	76·72	1·27	0·90	0·96	0·21
B 4	Bovine	35	10,000	3,602	36·0	98·67	0·67	0·30	0·28	0·05	0·00	0·03	
S 5	Sheep	48	5,000	384	7·7	95·83	2·09	0·26	1·30	0·00	0·00	0·00	0·52
G 5	Goat	48	5,000	950	19·0	92·95	5·90	0·21	0·00	0·84	0·00	0·10	0·00
B 5	Bovine	48	5,000	2,381	47·6	100·00	0·00	0·00	0·00	0·00			
S 6	Sheep	97	5,000	77	1·5	97·40	0·00	2·60	0·00	0·00	0·00		
G 6	Goat	97	5,000	237	4·7	99·58	0·42	0·00	0·00	0·00	0·00	†	
B 6	Bovine	97	5,000	2,721	54·4	100·00	0·00	0·00	0·00				
S 7	Sheep	391	5,000	2,157	43·1	100·00	0·00						
G 7 *	Goat	391	5,000	634	12·7	100·00	0·00						
S 8	Sheep	391	10,000	2,568	25·7	100·00	0·00						
G 8 *	Goat	391	10,000	2,338	23·4	100·00	0·00						
S 9	Sheep	487	2,000	55	2·8	100·00	0·00	0·00					
G 9	Goat	487	2,000	8	0·4	100·00	0·00	0·00					
B 9	Bovine	487	2,000	893	44·7	100·00	0·00						

*Bovines in these groups were not killed (see text)

†All blank spaces indicate that these organs were not examined

TABLE 3.—*The size in mm of paramphistomes recovered from sheep, goats and cattle*

Animal No.	Host	Age of infestation in days	Site of attachment	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
	Artificial excystation.....	3 hours		0·22 (0·20–0·27)	0·13 (0·11–0·17)	0·12 (0·10–0·14)	0·07 (0·06–0·08)
S 1.....	Sheep.....	4	1st 3 metres....	0·42 (0·29–0·55)	0·24 (0·19–0·30)	0·20 (0·14–0·25)	0·18 (0·14–0·23)
G 1.....	Goat.....	4	1st 3 metres....	0·61 (0·41–0·77)	0·25 (0·20–0·31)	0·23 (0·18–0·28)	0·20 (0·15–0·25)
B 1.....	Bovine.....	4	1st 3 metres....	0·52 (0·35–0·66)	0·22 (0·18–0·25)	0·20 (0·15–0·23)	0·17 (0·13–0·20)
S 2.....	Sheep.....	10	1st 3 metres....	1·00 (0·74–1·27)	0·47 (0·39–0·58)	0·42 (0·36–0·48)	0·36 (0·31–0·41)
G 2.....	Goat.....	10	1st 3 metres....	1·40 (1·08–1·76)	0·55 (0·41–0·64)	0·52 (0·40–0·58)	0·39 (0·31–0·43)
B 2.....	Bovine.....	10	1st 3 metres....	1·19 (0·87–1·75)	0·55 (0·43–0·66)	0·47 (0·36–0·56)	0·40 (0·33–0·45)
S 3.....	Sheep.....	20	1st 3 metres....	1·56 (1·34–1·86)	0·75 (0·62–1·04)	0·62 (0·48–0·86)	0·56 (0·44–0·64)
G 3.....	Goat.....	20	1st 3 metres....	2·09 (1·24–3·58)	0·65 (0·46–0·78)	0·59 (0·38–0·72)	0·52 (0·40–0·62)
B 3.....	Bovine.....	21	1st 3 metres....	2·32 (1·86–2·92)	1·03 (0·88–1·24)	0·83 (0·72–0·92)	0·66 (0·56–0·72)
S 4.....	Sheep.....	34	Rumen.....	2·74 (2·08–3·48)	0·94 (0·76–1·18)	0·83 (0·64–0·98)	0·70 (0·52–0·82)
G 4.....	Goat.....	34	1st 3 metres....	2·39 (1·40–3·74)	0·84 (0·52–1·24)	0·77 (0·48–1·12)	0·63 (0·40–0·84)
B 4.....	Bovine.....	35	Rumen.....	3·42 (2·16–4·28)	1·09 (0·96–1·30)	1·04 (0·90–1·28)	0·77 (0·64–0·9₁)
S 5.....	Sheep.....	48	Rumen.....	3·6 (2·8–4·8)	1·3 (1·0–1·5)	1·2 (0·9–1·4)	0·9 (0·8–1·0)
G 5.....	Goat.....	48	Rumen.....	4·0 (3·0–5·2)	1·3 (1·0–1·5)	1·2 (1·0–1·5)	1·0 (0·7–1·2)
B 5*.....	Bovine.....	48	Rumen.....	4·8 (3·8–5·7)	1·4 (1·2–1·6)	1·4 (1·2–1·5)	1·0 (0·9–1·2)
S 6.....	Sheep.....	97	Rumen.....	4·4 (3·5–5·8)	2·0 (1·7–2·4)	1·8 (1·5–2·3)	1·4 (1·2–1·7)
G 6.....	Goat.....	97	Rumen.....	4·8 (4·0–5·8)	2·2 (1·8–2·5)	2·0 (1·6–2·3)	1·5 (1·0–1·8)
B 6.....	Bovine.....	97	Rumen.....	6·6 (4·7–8·1)	2·4 (2·0–2·8)	2·2 (1·8–2·5)	1·5 (1·3–1·8)
S 7.....	Sheep.....	391	Rumen.....	4·6 (3·6–5·7)	1·8 (1·3–2·1)	1·7 (1·1–1·9)	1·1 (0·9–1·3)
G 7.....	Goat.....	391	Rumen.....	6·1 (4·2–8·5)	2·8 (2·3–3·3)	2·5 (2·0–3·0)	1·6 (1·3–2·0)
S 8.....	Sheep.....	391	Rumen.....	4·2 (3·3–5·6)	1·6 (1·3–1·8)	1·5 (1·2–1·7)	1·0 (0·8–1·2)
G 8.....	Goat.....	391	Rumen.....	5·6 (4·3–7·3)	2·2 (1·9–2·6)	2·0 (1·7–2·3)	1·4 (1·0–1·6)
S 9.....	Sheep.....	487	Rumen.....	8·4 (4·8–10·7)	2·8 (2·1–3·3)	2·7 (1·2–3·3)	1·8 (1·3–2·3)
G 9†.....	Goat.....	487	Rumen.....	6·5 (5·5–7·4)	2·5 (2·2–2·8)	2·4 (2·0–2·7)	1·6 (1·3–1·8)
B 9.....	Bovine.....	487	Rumen.....	8·4 (6·7–10·6)	3·8 (3·1–4·3)	3·5 (2·8–4·0)	2·2 (1·8–2·5)

*Only 19 worms measured as others were accidentally killed with heat

†Only eight worms recovered and measured

TABLE 4.—*Worm distribution in the first three metres of small intestine*

Animal No.	Host	Age of infestation in days	No. of worms recovered from 1st 3 metres	Worm distribution expressed as a percentage								
				1st 33 cm	2nd 33 cm	3rd 33 cm	4th 33 cm	5th 33 cm	6th 33 cm	7th 33 cm	8th 33 cm	9th 33 cm
S 10.....	Sheep.....	29	76,930	11·70	8·67	29·62	20·05	18·87	8·65	1·76	0·68	
G 10*.....	Goat.....	23	83,694	11·89	3·56	23·57	14·11	15·95	15·87	8·57	5·06	1·42
B 10*.....	Bovine.....	33	77,867	20·36	7·38	11·31	14·14	9·88	10·27	7·30	10·28	9·08

*Died or slaughtered *in extremis*

TABLE 5.—*Fluctuations in faecal worm egg counts*

Time of faecal collection	Eggs per gm of faeces			
	Sheep	Goat	Cattle	
	S 7	G 7	B 51	B 52
6– 8 a.m.....		42		
8–10 a.m.....	61	124	586	173
10–12 noon.....	94	173	705	168
12– 2 p.m.....	104	304	724	429
2– 4 p.m.....	78	240	638	233
4– 6 p.m.....	74	218		
6– 8 p.m.....	73	240		

TABLE 6.—*The estimated average daily egg production of a single fertile paramphistome*

Day of faecal collection	E.p.g. 1st 1 gm faecal sample	E.p.g. 2nd 1 gm faecal sample	Average E.p.g.	Daily faecal output in gm	Total daily egg output	Paramphistomes recovered from the rumen	
1	311	310	310·5	267	82,904	Anterior pillar (Dorsal).....	375
2	327	300	313·5	205	64,267	Posterior pillar (Dorsal).....	84
3	354	309	331·5	94	31,161	Posterior pillar (Ventral).....	378
4	381	366	373·5	75	28,013		
5	286	309	297·5	241	71,697	Total.....	837
6	267	271	269·0	162	43,578	Percentage containing eggs.....	85·3
Average	321·0	310·8	315·9	174	53,603	Eggs/fertile worm/day.....	75·1

TABLE 7.—*Percentage take and worm distribution in sheep with moderate and heavy infestations*

Sheep No.	Age of infestation in days	No. of metacercariae dosed	No. of worms recovered	Percentage take	Worm distribution expressed as a percentage								
					Rumen	Omasum	Abomasum	Gall-bladder	Small intestine				Caecum and Colon
									1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
S 21	3	200,000	115,267	57·6	0·000	0·000	0·087	0·000	47·498	42·857	4·685	4·873	0·000
S 22	5	203,000	95,560	47·1	—	0·000	0·811	—	69·150	17·246	4·317	8·476	0·000
S 23	10	107,000	78,170	73·1	0·032	0·000	0·026	0·000	94·614	3·966	0·863	0·397	0·102
S 24	14	68,000	24,930	36·7	0·177	0·080	4·412	0·024	93·329	1·480	0·185	0·152	0·161
S 25	14	72,000	55,251	76·7	0·014	0·000	0·652	0·000	98·568	0·489	0·224	0·022	0·031
S 26	20	69,000	25,816	37·4	0·287	0·054	11·051	0·004	87·155	0·465	0·225	0·166	0·593
S 27*	19	201,000	95,163	47·8	0·052	0·019	5·623	0·004	89·320	4·027	0·380	0·203	0·372
S 28	21	10,000	4,944	49·4	0·04	0·49	8·86	0·00	87·34	0·83	0·28	2·16	
S 29	21	88,000	73,197	83·2	0·007	0·059	11·995	0·004	72·862	12·227	1·824	0·306	0·716
S 30	22	20,000	15,950	79·8	0·74	0·81	1·38	0·00	96·38	0·55	0·06	0·02	0·06
S 31*	22	201,000	107,641	53·6	0·006	0·002	0·632	0·008	98·673	0·519	0·024	0·019	0·117
S 32	23	20,000	5,821	29·1	12·01	3·14	12·66	0·00	71·17	0·45	0·19	0·12	0·26
S 33	23	72,000	56,091	77·9	0·151	0·011	6·953	0·005	90·728	1·849	0·141	0·116	0·046
S 34*	28	170,000	46,897	27·6	2·280	0·343	6·685	0·011	89·217	1·083	0·168	0·056	0·147
S 35*	27	167,000	87,768	52·6	0·153	0·539	7·730	0·010	89·292	1·191	0·254	0·183	0·648
S 36*	30	170,000	40,039	23·6	0·914	0·050	12·663	0·027	85·067	0·495	0·162	0·187	0·435
S 10	29	202,000	78,225	38·7	0·015	0·064	0·707	0·004	98·345	0·350	0·222	0·207	0·086
S 37	36	73,000	4,121	5·6	96·82	0·95	0·39	0·00	1·82	0·00	0·02	0·00	0·00
S 38	36	71,000	36,936	52·0	3·734	1·183	10·099	0·119	83·658	0·401	0·219	0·360	0·227
S 39	40	70,000	15,809	22·6	70·49	4·96	3·23	0·00	21·09	0·14	0·00	0·01	0·08
S 40	39	80,000	31,212	39·0	12·287	1·839	19·118	0·003	66·449	0·150	0·010	0·026	0·118
S 41	42	77,000	8,533	11·1	99·92	0·01	0·00	0·00	0·07	0·00	0·00	0·00	0·00
S 42	43	78,000	53,699	68·8	2·441	0·259	20·038	0·019	76·612	0·182	0·136	0·149	0·164
S 43	48	72,000	6,144	8·5	98·76	0·70	0·10	0·00	0·39	0·02	0·03	0·00	0·00
S 44	50	78,000	20,891	26·8	72·80	4·08	11·36	0·00	11·69	0·03	0·01	0·03	0·00
S 45	63	74,000	14,685	19·8	99·64	0·01	0·10	0·00	0·16	0·00	0·00	0·01	0·08
S 46	79	30,000	2,176	7·3	99·68	0·27	0·00	0·00	0·05	†			
S 47	86	30,000	1,049	3·5	99·71	0·29	0·00	0·00					
S 48	106	40,000	10,246	25·6	98·73	1·10	0·17						
S 49	190	5,000	2,580	51·6	100·00								
S 50	211	43,000	6,418	14·9	99·97	0·03							
S 51	548	20,000	837	4·2	100·00	0·00							
S 52	984	10,000	1,610	16·1	100·00	0·00	0·00	—	0·00				

*Died or slaughtered *in extremis*

†Blank spaces indicate that these organs were not examined

TABLE 8.—*The size in mm of paramphistomes recovered from sheep with moderate and heavy infestations*

Sheep No.	Age of infestation in days	*Total No. of worms recovered	Site from which worms were measured	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
	3 hours		Artificial excystation	0·22 (0·20–0·27)	0·13 (0·11–0·17)	0·12 (0·10–0·14)	0·07 (0·06–0·08)
S 53	8	25,705	1st 3 metres	1·16 (0·73–1·38)	0·42 (0·27–0·50)	0·40 (0·27–0·48)	0·33 (0·23–0·39)
S 23	10	78,170	1st 3 metres	0·98 (0·71–1·14)	0·39 (0·31–0·45)	0·36 (0·28–0·44)	0·29 (0·22–0·32)
S 24	14	24,930	1st 3 metres	1·29 (1·05–1·62)	0·55 (0·43–0·67)	0·51 (0·40–0·61)	0·40 (0·33–0·46)
S 25	14	55,251	1st 3 metres	1·23 (0·91–1·63)	0·50 (0·40–0·60)	0·48 (0·39–0·58)	0·37 (0·30–0·43)
S 54	20	6,734	1st 3 metres	1·57 (1·04–2·32)	0·74 (0·44–0·90)	0·62 (0·38–0·80)	0·55 (0·36–0·64)
S 27	19	96,163	1st 3 metres	1·01 (0·74–1·59)	0·47 (0·34–0·64)	0·40 (0·30–0·60)	0·37 (0·27–0·51)
S 16	23	29,729	1st 3 metres	1·83 (1·08–2·58)	0·79 (0·52–1·04)	0·70 (0·48–0·92)	0·58 (0·42–0·72)
S 33	23	56,091	1st 3 metres	1·82 (1·08–2·96)	0·72 (0·46–1·04)	0·67 (0·36–0·98)	0·52 (0·34–0·74)
S 12	25	70,139	1st 3 metres	0·90 (0·59–1·72)	0·44 (0·29–0·74)	0·40 (0·27–0·66)	0·33 (0·23–0·59)
S 11	26	70,678	1st 3 metres	1·09 (0·77–1·58)	0·54 (0·39–0·73)	0·46 (0·34–0·61)	0·41 (0·30–0·57)
S 37	36	4,121	Rumen	2·13 (1·68–2·82)	0·99 (0·80–1·32)	0·88 (0·72–1·08)	0·61 (0·48–0·74)
S 38	36	36,936	Rumen	1·73 (1·24–2·32)	0·86 (0·54–1·08)	0·72 (0·44–0·96)	0·52 (0·36–0·68)
S 39	40	15,809	Rumen	2·30 (1·44–3·12)	0·85 (0·68–1·08)	0·73 (0·56–1·08)	0·61 (0·44–0·76)
S 55	40	17,498	Rumen	2·35 (1·60–3·32)	0·98 (0·76–1·26)	0·88 (0·68–1·12)	0·66 (0·50–0·80)
S 41	42	8,533	Rumen	2·98 (1·96–3·84)	1·02 (0·82–1·24)	0·93 (0·70–1·12)	0·70 (0·50–0·80)
S 56	42	17,384	Rumen	2·41 (1·44–3·20)	0·90 (0·66–1·18)	0·82 (0·60–1·08)	0·60 (0·44–0·70)
S 57	46	20,754	Rumen	2·08 (1·26–2·88)	0·98 (0·68–1·32)	0·86 (0·56–1·16)	0·62 (0·38–0·8[illegible]
S 43	48	6,144	Rumen	2·7 (1·9–3·7)	1·1 (0·9–1·3)	1·0 (0·7–1·2)	0·7 (0·5–0·[illegible]
S 58	57	†18,032	Rumen	2·6 (1·8–3·4)	0·9 (0·7–1·2)	0·9 (0·7–1·0)	0·6 (0·5–0·8)
S 45	63	14,685	Rumen	2·7 (1·6–3·7)	1·1 (0·7–1·5)	1·0 (0·7–1·3)	0·7 (0·4–0·9)
S 46	79	2,176	Rumen	2·4 (2·1–3·3)	1·2 (1·0–1·4)	1·0 (0·9–1·2)	0·8 (0·7–0·9)
S 47	86	1,049	Rumen	4·0 (3·0–5·7)	2·0 (1·8–2·3)	1·8 (1·5–2·0)	1·2 (1·1–1·4)
S 49	190	2,580	Rumen	3·9 (2·5–4·8)	1·7 (1·2–2·0)	1·5 (1·2–1·8)	1·1 (0·8–1·3)
S 50	211	6,418	Rumen	5·8 (5·1–6·8)	1·5 (1·3–1·9)	1·3 (1·0–1·6)	1·0 (0·8–1·2)
S 51	548	837	Rumen	7·9 (6·0–10·8)	2·7 (2·2–3·3)	2·6 (2·2–3·0)	‡

*Column 3 gives the total number of worms recovered from all organs and not only those from the organ in which measurements were made

†18,016 of these worms were recovered from the rumen

‡Not measured

TABLE 9.—*The size in mm of paramphistomes recovered from sheep, in relation to their site of attachment*

Sheep No.	Age of infestation in days	No. of worms in each organ	Organ	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
S 59	24	1,370	Rumen	1·85 (1·50–2·14)	0·89 (0·78–1·06)	0·77 (0·64–0·90)	0·62 (0·52–0·72)
		1,093	Omasum	1·91 (1·66–2·06)	0·99 (0·90–1·10)	0·84 (0·76–0·92)	0·67 (0·58–0·78)
		2,316	Abomasum	2·03 (1·60–2·34)	1·04 (0·78–1·22)	0·86 (0·66–0·96)	0·70 (0·58–0·80)
		10,005	1st 3 metres	2·34 (1·70–2·76)	1·05 (0·74–1·20)	0·93 (0·68–1·04)	0·72 (0·54–0·82)
S 40	39	3,835	Rumen	2·40 (1·68–3·20)	0·85 (0·64–1·04)	0·76 (0·56–0·96)	0·61 (0·46–0·76)
		5,967	Abomasum	2·14 (1·52–3·72)	0·72 (0·48–1·16)	0·65 (0·46–1·04)	0·52 (0·36–0·70)
		20,740	1st 3 metres	2·22 (1·10–3·54)	0·69 (0·52–1·16)	0·62 (0·46–1·06)	0·53 (0·36–0·84)
S 60	40	1,369	Rumen	1·59 (0·99–2·25)	0·75 (0·53–1·08)	0·63 (0·42–0·91)	0·56 (0·40–0·74)
		2,773	Abomasum	1·61 (1·25–2·57)	0·76 (0·53–0·99)	0·63 (0·46–0·84)	0·56 (0·42–0·72)
		18,888	1st 3 metres	1·55 (1·01–2·33)	0·80 (0·58–1·11)	0·63 (0·45–0·94)	0·56 (0·41–0·72)
S 42	43	1,311	Rumen	1·86 (1·20–2·60)	0·71 (0·50–0·96)	0·63 (0·48–0·84)	0·48 (0·40–0·60)
		10,760	Abomasum	2·02 (1·60–2·80)	0·69 (0·54–0·92)	0·58 (0·44–0·80)	0·49 (0·40–0·62)
		41,140	1st 3 metres	1·50 (0·90–2·60)	0·67 (0·44–1·20)	0·57 (0·40–1·02)	0·45 (0·30–0·70)
S 57	46	7,264	Rumen	2·08 (1·26–2·88)	0·98 (0·68–1·32)	0·86 (0·56–1·16)	0·62 (0·38–0·80)
		11,840	1st 3 metres	1·51 (1·00–2·00)	0·78 (0·52–1·08)	0·63 (0·40–0·94)	0·51 (0·36–0·70)
S 44	50	15,209	Rumen	2·28 (1·72–2·96)	1·03 (0·76–1·52)	0·92 (0·68–1·24)	0·60 (0·44–0·84)
		853	Omasum	1·93 (1·48–3·24)	0·98 (0·76–1·28)	0·82 (0·60–1·16)	0·60 (0·48–0·84)
		2,372	Abomasum	1·94 (1·40–2·56)	0·91 (0·60–1·16)	0·78 (0·48–1·04)	0·53 (0·40–0·68)
		2,441	1st 3 metres	2·05 (1·52–2·56)	1·03 (0·80–1·32)	0·86 (0·60–1·08)	0·62 (0·48–0·72)
S 48	106	2,149	Ant. Pillar	2·09 (1·68–2·72)	0·89 (0·72–1·26)	0·79 (0·68–1·08)	0·62 (0·54–0·70)
		4,150	Rumen Wall	2·24 (1·62–2·98)	0·93 (0·66–1·26)	0·83 (0·56–1·12)	0·64 (0·50–0·82)
		1,907	Post. Pillar	2·23 (1·50–2·96)	0·96 (0·68–1·24)	0·86 (0·64–1·06)	0·66 (0·50–0·86)

Ant. Pillar and Post. Pillar = The anterior and posterior ruminal pillars

TABLE 10.—*Percentage take and worm distribution in cattle*

Bovine No.	Age of infestation in days	No. of metacercariae dosed	No. of worms recovered	Percentage take	Worm distribution expressed as a percentage								
					Rumen	Omasum	Abomasum	Gall-bladder	Small intestine				Caecum and Colon
									1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
B 11	8	100,000	55,265	55·3	0·000	—	0·290	—	72·523	25·170	1·800	0·217	
B 12	14	113,000	57,084	50·5	0·009	—	7·795	0·000	69·844	21·104	1·186	0·023	0·039
B 13	20	110,000	55,616	50·6	0·014	0·611	2·499	0·009	96·424	0·146	0·085	0·198	0·014
B 14	23	250,000	108,223	43·3	0·000	0·000	1·367	0·000	73·700	23·735	0·585	0·613	
B 15	28	50,000	16,714	33·4	63·06	16·87	3·09	0·00	16·84	0·11	0·01	0·01	0·01
B 16	28	250,000	102,103	40·8	0·255	0·078	2·774	0·000	37·739	28·794	20·881	9·479	
B 10*	33	256,000	172,696	67·5	0·045	0·064	0·689	0·020	45·089	45·606	5·705	2·270	0·512
B 17*	40	305,000	161,654	53·0	0·016	0·036	0·458	0·000	34·857	33·702	29·149	1·303	0·479
B 18	46	40,000	21,246	53·1	90·54	8·68	0·50	—	0·28	@			
B 19	53	40,000	17,908	44·8	79·20	13·31	1·18	—	6·21	0·02	0·08		
B 20	52	204,000	44,196	21·7	97·989	1·674	0·174	0·000	0·149	0·002	0·005	0·002	0·005
B 21	52	200,000	72,252	36·1	19·681	1·785	3·460	0·010	74·185	0·797	0·024	0·015	0·043
B 22	62	19,000	10,060	52·9	100·00	0·00	0·00						
B 23	205	150,000	13,855	9·2	99·96	0·00	0·00	—	0·04				
B 24	737	2,500	903	36·1	100·0								

@Blank spaces indicate that these organs were not examined

*Died, paramphistomiasis

TABLE 11.—*The size in mm of paramphistomes recovered from cattle*

Bovine No.	Age of infestation in days	*Total No. of worms recovered	Site from which worms were measured	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
	3 hours		Artificial excystation	0·22 (0·20–0·27)	0·13 (0·11–0·17)	0·12 (0·10–0·14)	0·07 (0·06–0·08)
B 25.....	7	49,163	1st 3 metres.....	0·83 (0·54–1·11)	0·30 (0·25–0·37)	0·29 (0·24–0·37)	0·23 (0·17–0·27)
B 12.....	14	57,084	1st 3 metres.....	1·38 (0·79–1·67)	0·57 (0·40–0·68)	0·51 (0·34–0·63)	0·43 (0·26–0·49)
B 13.....	20	55,616	1st 3 metres.....	1·52 (1·20–1·88)	0·71 (0·52–0·82)	0·61 (0·44–0·70)	0·52 (0·40–0·60)
B 15.....	28	16,714	1st 3 metres.....	2·43 (1·96–3·14)	0·91 (0·80–1·12)	0·85 (0·76–1·00)	0·64 (0·56–0·74)
B 26.....	27	66,659	1st 3 metres.....	1·79 (1·38–2·20)	0·79 (0·64–0·94)	0·68 (0·48–0·82)	0·60 (0·50–0·68)
B 18.....	46	21,246	Rumen.....	2·4 (2·1–2·8)	1·1 (0·9–1·3)	1·0 (0·8–1·1)	0·7 (0·6–0·8)
B 19.....	53	17,908	Rumen.....	2·9 (2·1–4·2)	1·1 (0·8–1·6)	1·1 (0·8–1·5)	0·7 (0·6–1·0)
B 20.....	52	44,196	Rumen.....	3·7 (2·6–4·9)	1·4 (1·0–1·7)	1·3 (1·0–1·7)	0·9 (0·7–1·2)
B 21.....	52	72,252	Rumen.....	3·1 (2·1–4·7)	1·1 (0·8–1·5)	1·1 (0·8–1·4)	0·7 (0·5–1·0)
B 22.....	62	10,060	Rumen.....	4·5 (3·3–6·5)	1·9 (1·6–2·3)	1·7 (1·5–2·1)	1·1 (0·9–1·3)
B 23.....	205	13,855	Rumen.....	4·0 (2·2–5·5)	1·7 (1·1–2·4)	1·5 (1·0–2·0)	1·0 (0·7–1·3)
B 24.....	737	903	Rumen.....	6·7 (5·8–8·2)	3·1 (2·7–3·5)	2·8 (2·5–3·2)	1·7 (1·4–2·0)

* Note.—Column 3 gives the total number of worms recovered from all organs and not only those from the organ in which measurements were made

TABLE 12.—*The size in mm of paramphistomes recovered from cattle, in relation to their site of attachment*

Bovine No.	Age of infestation in days	No. of worms in each organ	Organ	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
B 15.....	28	10,541	Rumen.....	2·43 (1·96–3·14)	0·91 (0·80–1·12)	0·85 (0·76–1·00)	0·64 (0·56–0·74)
		2,820	Omasum.....	2·20 (1·80–4·48)	0·92 (0·58–1·22)	0·82 (0·54–1·22)	0·64 (0·46–0·92)
		516	Abomasum.....	2·34 (1·76–4·00)	0·95 (0·84–1·18)	0·85 (0·74–1·04)	0·65 (0·56–0·78)
		2,815	1st 3 metres.....	2·35 (1·68–2·90)	1·05 (0·66–1·28)	0·93 (0·62–1·16)	0·70 (0·48–0·80)
B 17.....	40	56,347	1st 3 metres.....	1·41 (0·71–1·93)	0·71 (0·35–0·96)	0·60 (0·31–0·79)	0·52 (0·29–0·65)
		54,480	2nd 3 metres....	1·50 (0·94–1·87)	0·78 (0·45–0·99)	0·66 (0·38–0·83)	0·55 (0·32–0·67)
		47,120	3rd 3 metres.....	1·52 (0·90–1·90)	0·80 (0·57–0·98)	0·66 (0·45–0·81)	0·56 (0·37–0·68)
B 9.....	487	828	Ant. Pillar.....	8·43 (6·67–10·58)	3·76 (3·08–4·33)	3·46 (2·75–4·00)	2·18 (1·83–2·50)
		65	Post. Pillar.....	7·98 (6·25–9·50)	3·69 (2·75–4·58)	3·36 (2·42–3·92)	2·07 (1·75–2·33)

Ant. Pillar and Post. Pillar = The anterior and posterior ruminal pillars

TABLE 13.—*Multiple infestation of sheep and cattle*

No.	Host	Method of infestation (No. of doses and metacercariae)	Infestation period in days	Total No. of meta-cercariae dosed	Days between last dose and slaughter	No. of worms recovered	Percentage take	Worm distribution expressed as a percentage.		
								Anterior to pylorus	1st 3 metres of intestine	Remainder of intestine
(i) Random infestation of sheep over a short period										
S 61	Sheep	6 Doses of 2,000– 9,000 each	7	31,000	7	9,550	30·8	6·89	40·98	52·13
S 62	Sheep	6 Doses of 4,000–12,000 each	7	41,000	16	20,615	50·3	1·69	97·94	0·37
S 63	Sheep	4 Doses of 8,000–21,000 each	6	55,000	16	22,997	41·8	0·99	98·62	0·39
S 64	Sheep	9 Doses of 10,000–27,000 each	10	172,000	16	74,820	43·5	2·18	93·15	4·67
S 65	Sheep	13 Doses of 1,000–23,000 each	22	102,000	13	38,058	37·3	16·17	81·42	2·41
(ii) Regular prolonged infestation of sheep										
S 66	Sheep	2,000 (6 x week).....	35	60,000	1	27,259	45·4	11·49	74·85	13·66
S 67	Sheep	4,000 (6 x week).....	458	1,560,000	29	22,017	1·4	52·40	47·19	0·41
S 68	Sheep	6,000 (6 x week).....	69	360,000	2*	71,442	19·8	2·59	48·23	49·18
S 69	Sheep	6,000 (6 x week).....	62	324,000	1*	59,950	18·5	2·94	75·49	21·57
S 70	Sheep	8,000 daily.....	52	400,000	0*	113,541	28·4	12·90	67·40	19·70
S 71	Sheep	16,000 daily.....	26	416,000	1*	150,699	36·2	2·45	87·90	9·65
(iii) Regular prolonged infestation of sheep and cattle										
S 72	Sheep	500 (3 x week).....	184	46,000	1	14,039	30·5	76·99	22·60	0·41
S 73	Sheep	1,000 (3 x week).....	184	92,000	2	13,736	14·9	70·86	28·76	0·38
B 27	Bovine	1,000 (3 x week).....	190	94,000	1	5,627	6·0	100·00	0·00	0·00
S 74	Sheep	1,500 (3 x week).....	155	100,500	4	18,124	18·0	77·93	21·79	0·28
B 28	Bovine	1,500 (3 x week).....	184	139,500	3	3,244	2·3	99·97	0·03	0·00

*Died or slaughtered *in extremis*

TABLE 14.—*X-Irradiation of metacercariae*

Sheep No.	Age of infestation in days	No. of metacercariae dosed	X-Irradiation	No. of worms recovered	Percentage take	Worm distribution expressed as a percentage		
						Anterior to pylorus	1st 3 metres of intestine	Remainder of intestine
(i) X-Irradiation of metacercariae								
S 54	20	10,000	Control	6,734	67·3	3·8	96·1	0·1
S 75	21	10,000	Control	4,946	49·5	6·8	90·4	2·8
S 28	21	10,000	Control	4,944	49·4	9·4	87·3	3·3
S 76	21	10,000	1 kr	7,265	72·7	2·8	96·6	0·6
S 77	21	10,000	2 kr	4,182	41·8	1·2	97·3	1·5
S 78	21	10,000	4 kr	218	2·2	18·8	78·9	2·3
S 79	21	10,000	8 kr	4	0·04	0·0	75·0	25·0
S 49	190	5,000	Control	2,580	51·6	100·0	@	
S 80	189	5,000	1 kr	1,569	31·4	100·0		
S 81	189	5,000	2 kr	94	1·9	100·0		
S 82	189	5,000	4 kr	0	0·0	0·0		
S 83	189	5,000	8 kr	0	0·0	0·0		
(ii) First generation after X-irradiation								
S 84	20	10,000	1st generation	4,704	47·0	2·5	96·7	0·8
S 85	189	5,000	Control	1,655	33·1	100·0		
S 86	189	5,000	1st generation	1,238	24·8	100·0		
S 87	189	5,000	1st generation	2,380	47·6	100·0		

@ Blank spaces indicate that these organs were not examined

TABLE 15.—*The effect of X-irradiation on the size (in mm) of paramphistomes*

Sheep No.	Age of infestation in days	X-Irradiation	Total No. of worms recovered	Site from which worms were measured	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
(i) X-Irradiation of metacercariae								
S 49.....	190	Control	2,580	Rumen	3·9 (2·5–4·8)	1·7 (1·2–2·0)	1·5 (1·2–1·8)	1·1 (0·8–1·3)
S 80.....	189	1 kr	1,569	Rumen	4·2 (3·1–5·5)	1·9 (1·4–2·2)	1·7 (1·4–2·0)	1·2 (1·0–1·4)
S 81.....	189	2 kr	94	Rumen	4·0 (3·0–5·3)	2·0 (1·5–2·5)	1·8 (1·5–2·3)	1·2 (1·0–1·7)
(ii) First generation after X-irradiation.								
S 85.....	189	Control	1,655	Rumen	4·3 (3·6–5·5)	2·1 (1·8–2·4)	1·9 (1·6–2·3)	1·3 (1·1–1·7)
S 86.....	189	1st generation	1,238	Rumen	3·8 (3·1–4·6)	1·8 (1·5–2·3)	1·6 (1·3–2·0)	1·2 (1·0–1·4)
S 87.....	189	1st generation	2,380	Rumen	4·3 (3·0–5·4)	2·1 (1·6–2·4)	1·9 (1·5–2·2)	1·4 (1·1–1·6)

TABLE 16.—*The effect of X-irradiation on the faecal worm egg count*

Sheep No.	X-Irradiation	No. of worms recovered	Average No. of eggs per gram of faeces days after infestation				
			0–80	81–110	111–140	141–170	171–189
(i) X-Irradiation of metacercariae							
S 80	1 kr	1,569	0·0	14·8	8·3	19·3	3·0
S 81	2 kr	94	0·0	0·5	0·3	1·0	2·7
S 82	4 kr	0	0·0	0·0	0·7	0·0	0·0
S 83	8 kr	0	0·0	0·0	0·3	0·3	0·0
(ii) First generation after X-irradiation							
S 85	Control	1,655	0·0	139·5	828·4	1114·5	1284·5
S 86	1st generation	1,238	0·0	42·3	186·8	226·5	233·5
S 87	1st generation	2,380	0·0	253·0	712·0	1234·0	300·0

TABLE 17.—*The immunization of sheep*

Sheep No.	Immunization Procedure		Days between end of immunization and challenge	Challenge			
	No. of metacercariae dosed	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	Percentage take
	Controls of challenge infestation						
S 88	Control			169,000	22*	72,145	42·7
S 12	Control			175,000	25*	70,139	40·1
S 35	Control			167,000	27*	87,768	52·6
S 34	Control			170,000	28*	46,897	27·6
S 36	Control			170,000	30*	40,039	23·6
S 89	Control			170,000	36*	66,556	39·2
S 21	Control			200,000	3	115,267	57·6
S 90	Control			197,000	19	105,974	53·8
S 27	Control			201,000	19*	96,163	47·8
S 31	Control			201,000	22*	107,641	53·6
S 11	Control			210,000	26*	70,678	33·7
S 10	Control			202,000	29	78,225	38·7
	Controls of immunizing infestation						
S 48	40,000 Slaughtered 106 days later.....	10,246		Control			
S 91	20,000 + 20,000, 33 days later and slaughtered 73 days later	9,462		Control			
	Immunized sheep						
	Suckling lambs						
S 92	20,000 + 20,000, 32 days later.....	1,191	43	170,000	31*	70,697	41·6
S 93	20,000 + 20,000, 32 days later.....	1,926	43	169,000	33*	40,160	23·8

TABLE 17.—*The immunization of sheep* (continued)

Sheep No.	Immunization Procedure		Days between end of immunization and challenge	Challenge			
	No. of metacercariae dosed	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	Percentage take
	Weaned lambs under one year of age						
S 94	20,000 + 20,000, 32 days later	4,928	48	202,000	29	38,104	18·9
S 95	20,000 (2 kr) + 20,000 (2 kr), 32 days later	5,041	48	201,000	29	89,345	44·5
S 96	40,000	5,404	80	207,000	29	65,114	31·5
S 97	40,000 (2 kr)	1,909	80	200,000	29	74,625	37·3
	Adult sheep (i) *Single or double immunizing infestations*						
S 98	500	123	264	200,000	22*	84,378	42·2
S 99	1,000	Not Counted	264	200,000	25*	71,525	35·8
S 100	2,000	924	264	202,000	26*	76,067	37·7
S 101	20,000	4,989	376	191,000	28	9,521	5·0
S 102	20,000	1,061	1,075	205,000	48	38,329	18·7
S 103	25,000	5,752	63	200,000	48	13,673	6·8
S 104	20,000 + 20,000, 32 days later	2,353	37	200,000	35	126	0·06
S 105	40,000	815	69	200,000	35	1,073	0·5
S 106	40,000	2,942	1,075	203,000	48	85	0·04
S 107	50,000	9,445	63	202,000	55	18,341	9·1
S 108	75,000	7,388	63	202,000	22*	75,273	37·3
	(ii) *X-Irradiation of immunizing infestatings*						
S 109	17,000 (1 kr)	5,136	74	210,000	24	92,480	44·0
S 110	17,000 (2 kr)	2,533	74	200,000	24	44,214	22·1
S 111	19,000 (4 kr)	21	74	200,000	24	81,353	40·7
S 112	32,000 (8 kr)	0	74	203,000	24	125,967	62·1

TABLE 17.—*The immunization of sheep* (continued)

Sheep No.	Immunization Procedure		Days between end of immunization and challenge	Challenge			
	No. of metacercariae dosed	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	Percentage take
	(iii) *Multiple immunizing infestations*						
	(*a*) *Challenge immediately after immunization*						
S 113	415,000 (1,500, 3 x week).....	17,342	0	183,000	4	82,517	45·1
S 114	320,000 (1,000, 6 x week).....	11,784	0	196,000	14	2,523	1·3
S 115	46,500 (500, 3 x week).....	6,517	3	202,000	27	3,665	1·8
S 116	93,000 (1,000, 3 x week).....	7,586	3	202,000	25	2,214	1·1
S 117	141,000 (1,500, 3 x week).....	16	1	202,000	24	739	0·4
	(b) *Pregnancy and delayed massive challenge*						
S 118†	46,500 (500, 3 x week).....	1,303	202	200,000 + 206,000 30 days later	17*	58,475	14·4
S 119‡	93,000 (1,000, 3 x week).....	4,109	198	408,000	30*	118,614	29·1
	(iv) *Immunization followed by anthelmintic treatment*						
S 120	50,000 + 100mg/kg Bithionol 21 days later...	0	42§	170,000	28	31,014	18·2
S 121	50,000 + 100mg/kg Bithionol 28 days later...	0	42	170,000	28	66,737	39·3
S 122	50,000 + 100mg/kg Bithionol 35 days later...	0	41	171,000	29	43,025	25·2
S 123	40,000 + 330mg/kg Freon 698 days later.....	50	54	205,000	22	119,466	58·3
S 124¶	169,000 + 50mg/kg Lintex 18 days later.....	2	31	200,000	28*	53,476	26·7
S 125¶	174,000 + 50mg/kg Lintex 25 days later.....	370	24	204,000	32	18,162	8·9
	(v) *Other attempts at immunization*						
S 126	277 Adult paramphistomes *per os*.....	0	66	171,000	20	70,995	41·5
S 127	1,988 Adult paramphistomes *per os*.....	51	154	208,000	29	41,281	19·8
S 128	4,703 Adult paramphistomes *per os*.....	2,870	56	205,000	29	48,727	23·8
S 129¶	1·5 G macerated paramphistomes I.P.....		20	179,000	13*	87,208	48·7
S 130¶	20ml blood from immune sheep I.V. 3 x week for three weeks		-18	146,000	20	107,410	73·6

*Died or slaughtered *in extremis*

†Lambed 26 days after first challenge (see text)

‡Lambed 19 days after challenge (see text)

§Period in days between anthelmintic treatment and challenge in all treated sheep (see text)

¶Weaned lambs under one year old

TABLE 18.—*Worm distribution in immunized sheep*

Sheep No.	Age of challenge infestation in days	No. of worms recovered	Worm distribution expressed as a percentage				
			Anterior to abomasum	Abomasum	1st 3 metres of intestine	Remainder of small intestine	Caecum and Colon
S 92	31*	70,697	0·04	1·61	83·45	14·76	0·14
S 97	29	74,625	0·20	7·65	67·15	24·89	0·11
S 98	22*	34,378	0·14	3·51	67·27	24·93	4·15
S 109	24	92,480	0·02	0·03	75·52	24·41	0·02
S 114	14	2,523	0·00	0·00	61·83	35.79	2·38
S 119	30*	118,614	0·01	0·49	50·73	47·36	1·41
S 124	28*	53,476	0·06	3·39	45·52	35·40	15·63
S 123	22	119,466	0·00	0·27	79·60	20·06	0·07
S 126	20	70,995	0·04	2·09	80·78	17·08	0·01
S 130	20	107,410	0·00	1·90	86·39	11·71	0·00

*Died or slaughtered *in extremis*

TABLE 19.—*Comparative migratory rates in susceptible and immunized sheep*

Sheep No.	Age of infestation in days	No. of worms recovered	Worm distribution expressed as a percentage							
			Rumen	Omasum	Abomasum	Small intestine				Caecum and Colon
						1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
Susceptible sheep										
S 4	34	1,088	96·60	1·93	0·28	1·19	0·00	0·00	0·00	0·00
S 37	36	4,121	96·82	0·95	0·39	1·82	0·00	0·02	0·00	0·00
S 5	48	384	95·83	2·09	0·26	1·30	0·00	0·00	0·00	0·52
S 43	48	6,144	98·76	0·70	0·10	0·39	0·02	0·03	0·00	0·00
S 44	50	20,891	72·80	4·08	11·36	11·69	0·03	0·01	0·03	0·00
Challenge infestation in immunized sheep										
S 104	35	126	83·3	0·8	0·0	15·9	0·0	0·0	0·0	0·0
S 105	35	1,073	7·83	5·13	4·75	81·73	0·37	0·00	0·19	0·00
S 106	48	85	90·6	0·0	0·0	9·4	0·0	0·0	0·0	0·0
S 102	48	38,329	6·53	0·21	5·69	86·10	1·07	0·10	0·10	0·20
S 103	48	13,673	16·19	0·84	3·00	77·62	1·81	0·16	0·23	0·15
S 107	55	18,341	25·35	0·59	2·71	70·94	0·21	0·02	0·08	0·10

TABLE 20.—*The size in mm of paramphistomes recovered from the small intestine of susceptible or immunized sheep after challenge*

Sheep No.	Age of infestation in days	No. of worms recovered	Susceptible or immunized	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
S 54	20	6,734	Susceptible	1·57 (1·04–2·32)	0·74 (0·44–0·90)	0·62 (0·38–0·80)	0·55 (0·36–0·64)
S 101	28	9,521	Immunized	1·41 (0·96–2·20)	0·54 (0·36–0·86)	0·47 (0·32–0·70)	0·39 (0·28–0·56)
S 16	23	29,729	Susceptible	1·83 (1·08–2·58)	0·79 (0·52–1·04)	0·70 (0·48–0·92)	0·58 (0·42–0·72)
S 120	28	31,014	Immunized	1·54 (0·78–2·22)	0·59 (0·36–0·94)	0·54 (0·32–0·76)	0·42 (0·22–0·56)
S 33	23	56,091	Susceptible	1·82 (1·08–2·96)	0·72 (0·46–1·04)	0·67 (0·36–0·98)	0·52 (0·34–0·74)
S 94	29	38,104	Immunized	1·51 (1·00–1·96)	0·57 (0·42–0·78)	0·54 (0·40–0·80)	0·42 (0·32–0·54)
S 59	24	14,856	Susceptible	2·34 (1·70–2·76)	1·05 (0·74–1·20)	0·93 (0·68–1·04)	0·72 (0·54–0·82)
S 125	32	18,162	Immunized	1·46 (0·78–1·96)	0·71 (0·28–0·99)	0·60 (0·24–0·80)	0·50 (0·22–0·64)
S 38	36	36,936	Susceptible	1·57 (0·88–2·38)	0·70 (0·44–1·08)	0·62 (0·40–0·96)	0·48 (0·32–0·64)
S 105	35	1,073	Immunized	1·60 (0·84–2·40)	0·56 (0·38–0·74)	0·49 (0·32–0·70)	0·43 (0·28–0·52)
S 42	43	53,699	Susceptible	1·50 (0·90–2·60)	0·67 (0·44–1·20)	0·57 (0·40–1·02)	0·45 (0.30–0·70)
S 102	48	38,329	Immunized	1·30 (0·93–2·12)	0·59 (0·36–0·98)	0·50 (0·31–0·90)	0·43 (0·26–0·64)

TABLE 21.—*The immunization of goats*

Goat No.	Immunization Procedure		Days between end of immunization and challenge	Challenge			
	No. of metacercariae dosed	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	Percentage take
	Controls of the challenge infestation						
G 11	Control			168,000	27*	47,307	28·2
G 10	Control			200,000	23*	86,135	43·1
G 12	Control			200,000	29*	71,579	35·8
	Immunized goats						
	Suckling kids						
G 13	20,000 + 20,000, 32 days later	3,956	37	173,000	24*	45,046	26·0
G 14	20,000 + 20,000, 32 days later	654	37	169,000	46	3,372	2·0
	Adult goats						
G 15	20,000 + 20,000, 34 days later	1,598	48	199,000	42	84	0·04
G 16	40,000	1,966	82	198,000	42	2,392	1·2

*Died or slaughtered *in extremis*

TABLE 22.—*Comparative migratory rates in susceptible and immunized goats*

Goat No.	Age of infestation in days	No. of worms recovered	Worm distribution expressed as a percentage							
			Rumen	Omasum	Abomasum	Small intestine				Caecum and Colon
						1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
Susceptible goats										
G 4	34	2,904	1·03	0·45	18·46	76·72	1·27	0·90	0·96	0·21
G 5	48	950	92·95	5·90	0·21	0·00	0·84	0·00	0·10	0·00
Challenge infestation in immunized goats										
G 15	42	84	22·0	13·0	12·0	42·0	0·0	0·0	0·0	11·0
G 16	42	2,392	11·96	2·68	11·04	71·24	1·21	0·25	0·79	0·83
G 14	46	3,372	0·80	2·17	0·74	94·51	0·98	0·68	0·00	0·12

TABLE 23.—*The size in mm of worms recovered from the small intestine of susceptible and immunized goats after challenge*

Goat No.	Age of infestation in days	No. of worms recovered	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
Susceptible goats						
G 3	20	5,306	2·09 (1·24–3·58)	0·65 (0·46–0·78)	0·59 (0·38–0·72)	0·52 (0·40–0·62)
G 4	34	2,904	2·39 (1·40–3·74)	0·84 (0·52–1·24)	0·77 (0·48–1·12)	0·63 (0·40–0·84)
Immunized goats						
G 15*	42	84	1·35 (1·14–1·56)	0·62 (0·54–0·70)	0·58 (0·48–0·68)	0·48 (0·44–0·52)
G 16	42	2,392	1·60 (0·66–2·58)	0·60 (0·30–0·88)	0·56 (0·28–0·82)	0·45 (0·24–0·66)

*Only three worms were measured

TABLE 24.—*The immunization of cattle*

Bovine No.	Immunization Procedure		Days between immunization and challenge	Challenge			
	No. of metacercariae dosed	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	Percentage take
	Controls of the challenge infestation						
	Calves						
B 20	Control			204,000	52	44,196	21·7
B 21	Control			200,000	52	72,252	36·1
	Adult cattle						
B 14	Control			250,000	23	108,223	43·3
B 16	Control			250,000	28	102,103	40·8
B 29	Control			250,000	29	92,691	37·1
B 10	Control			256,000	33*	172,696	67·5
B 17	Control			305,000	40*	161,654	53·0
	Controls of the immunizing infestation						
B 18	40,000 Slaughtered 46 days later	21,246		Control			
B 19	40,000 Slaughtered 53 days later	17,908		Control			
	Immunized cattle						
	Fourteen day old calves						
B 30	20,000 + 20,000 28 days later	4	35	204,000	42	48,392	23·7
B 31	40,000	12	63	201,000	44	33,759	16·8
	Adult cattle						
	(i) *Single immunizing infestations*						
B 32	2,500	756	853	251,000	30	52,519	20·9
B 33	2,500	1,121	924	261,000	43	81,071	31·1
B 34	40,000	20,569	28	101,000	18	29	0·03
B 35	40,000	10,698	35	101,000	17	0	0·0
B 36	40,000	15,089	279	250,000	34	8	0·003
B 37	100,000	17,889	214	209,000	24	1,312	0·6

*Died, paramphistomiasis

†Days between treatment and challenge (see text)

TABLE 24.—*The immunization of cattle* (continued)

Bovine No.	Immunization Procedure: No. of metacercariae dosed	Immunization Procedure: No. of worms recovered	Days between immunization and challenge	Challenge: No. of metacercariae dosed	Challenge: Days between challenge and slaughter	Challenge: No. of worms recovered	Challenge: Percentage take
	(ii) *X-Irradiation of immunizing infestations*						
B 38	20,000 (2 kr) + 20,000 (2 kr), 32 days later	766	44	250,000	28	21	0·008
B 39	40,000 (2 kr)	1,122	42	250,000	29	107	0·04
B 40	40,000 (2 kr)	1,490	56	250,000	22	33	0·01
B 41	40,000 (2 kr)	1,204	71	250,000	28	3	0·001
B 42	40,000 (2 kr)	1,007	71	250,000	28	10	0·004
B 43	40,000 (2 kr)	1,002	76	250,000	28	7	0·003
B 44	40,000 (2 kr)	856	279	250,000	34	622	0·25
	(iii) *Multiple immunizing infestations* (a) *Normal challenge*						
B 45	631,500 (1,500, 3 x week)	19,452	108	240,000	8	911	0·4
B 46	46,500 (500, 3 x week)	10,314	10	211,000	33	45	0·02
B 47	93,000 (1,000, 3 x week)	205	161	298,000	30	130	0·04
	(b) *Massive challenge*						
B 48	46,500 (500, 3 x week)	1,066	376	792,000 over 18 days + 781,000, 38 days later	22	182	0·01
	(iv) *Immunization followed by anthelmintic treatment*						
B 49	176,000 + Bithionol at 40 mg/kg 294 days later	23,958	259†	272,000	51	31	0·01
	(v) *Adult paramphistomes* per os						
B 50	5,629 Adult paramphistomes *per os* over 28 days	3,480	103	250,000	58	102,823	41·1

*Died, paramphistomiasis

†Days between treatment and challenge (see text)

TABLE 25.—*Comparative worm distribution in susceptible and immune cattle*

Bovine No.	Age of infestation in days	No. of worms recovered	Worm distribution expressed as a percentage							
			Rumen	Omasum	Abomasum	Small intestine				Caecum and Colon
						1st 3 metres	2nd 3 metres	3rd 3 metres	Rest	
Susceptible cattle with small worm burdens										
B 1	4	5,551	0·00	0·00	1·08	72·83	21·17	3·84	1·08	0·00
B 2	10	5,501	0·00	0·00	2·18	96·86	0·78	0·18	0·00	0·00
B 3	21	4,914	0·00	0·18	7·29	92·27	0·18	0·00	0·04	0·04
B 4	35	3,602	98·67	0·67	0·30	0·28	0·05	0·00	0·03	
B 5	48	2,381	100·00	0·00	0·00	0·00	0·00	*		
Susceptible cattle with large worm burdens										
B 11	8	55,265	0·000	—	0·290	72·523	25·170	1·800	0·217	
B 14	23	108,223	0·000	0·000	1·367	73·700	23·735	0·585	0·613	
B 16	28	102,103	0·255	0·078	2·774	37·739	28·794	20·881	9·479	
B 10	33	172,696	0·045	0·064	0·689	45·109	45·606	5·705	2·270	0·512
B 21	52	72,252	19·681	1·785	3·460	74·195	0·797	0·024	0·015	0·043
Challenge infestation in immune cattle										
B 45	8	911	0·0	0·0	5·5	63·8	16·2	1·3	13·2	0·0
B 34	18	29	0·0	0·0	0·0	0·0	7·0	0·0	93·0	
B 40	22	33	9·0	0·0	0·0	76·0	0·0	0·0	9·0	6·0
B 37	24	1,312	0·0	0·0	0·0	73·0	27·0	0·0	—	
B 39	29	107	0·0	0·0	12·2	79·5	3·7	0·0	0·9	3·7
B 47	30	130	0·0	0·0	5·0	83·0	10·0	0·0	2·0	
B 36	34	8	0·0	—	0·0	0·0	37·0	63·0	0·0	
B 44	34	622	0·0	—	0·0	22·0	19·0	47·7	11·3	
B 49	51	31	0·0	10·0	0·0	87·0	3·0	0·0	0·0	

*All blank spaces indicate that these organs were not examined

TABLE 26.—*The size in mm of worms recovered from the small intestine of susceptible and immune cattle*

Bovine No.	Age of infestation in days	No. of worms recovered	Length Average (Range)	Breadth Average (Range)	Depth Average (Range)	Acetabulum Average (Range)
Susceptible cattle with small worm burdens						
B 1	4	5,551	0·52 (0·35–0·66)	0·22 (0·18–0·25)	0·20 (0·15–0·23)	0·17 (0·13–0·20)
B 2	10	5,501	1·19 (0·87–1·75)	0·55 (0·43–0·66)	0·47 (0·36–0·56)	0·40 (0·33–0·45)
B 3	21	4,914	2·32 (1·86–2·92)	1·03 (0·88–1·24)	0·83 (0·72–0·92)	0·66 (0·56–0·72)
Susceptible cattle with large worm burdens						
B 25	7	49,163	0·83 (0·54–1·11)	0·30 (0·25–0·37)	0·29 (0·24–0·37)	0·23 (0·17–0·27)
B 12	14	57,084	1·38 (0·79–1·67)	0·57 (0·40–0·68)	0·51 (0·34–0·63)	0·43 (0·26–0·49)
B 13	20	55,616	1·52 (1·20–1·88)	0·71 (0·52–0·82)	0·61 (0·44–0·70)	0·52 (0·40–0·60)
B 26	27	66,659	1·79 (1·38–2·20)	0·79 (0·64–0·94)	0·68 (0·48–0·82)	0·60 (0·50–0·68)
Challenge infestation in immune cattle						
B 34*	18	29	0·51 (0·42–0·58)	0·23 (0·21–0·26)	0·21 (0·19–0·23)	0·17 (0·15–0·18)
B 37	24	1,312	1·15 (0·86–1·51)	0·36 (0·31–0·46)	0·36 (0·31–0·43)	0·29 (0·25–0·34)
B 47	30	130	1·46 (0·90–2·28)	0·42 (0·28–0·88)	0·41 (0·28–0·74)	0·33 (0·24–0·58)
B 36†	34	8	1·03 (0·99–1·06)	0·35 (0·33–0·38)	0·33 (0·30–0·37)	0·25 (0·24–0·28)
B 44	34	622	1·34 (0·79–2·53)	0·48 (0·35–0·79)	0·46 (0·33–0·84)	0·33 (0·23–0·55)
B 49‡	51	31	1·92 (0·82–2·34)	0·75 (0·39–0·99)	0·69 (0·34–0·89)	0·51 (0·30–0·64)

*8 worms measured †8 worms measured ‡28 worms measured

TABLE 27.—*The intradermal allergic test*

Infestation	Total No. of sheep	No. positive	No. suspicious	No. negative
Saline extracted antigen				
Paramphistome	27	16	4	7
Liver fluke	2	1	1	0
Schistosome	1	1	0	0
Nematode	16	4	4	8
Worm-free controls	13	0	2	11
Metacercarial antigen				
Paramphistome	3	3	0	0
Nematode	5	1	2	2
Worm-free controls	1	0	1	0
Boiled alcohol precipitated antigen				
Paramphistome	9	0	7	2
Liver fluke	2	2	0	0
Nematode	2	0	0	2

TABLE 28.—*The complement fixation test*

		Tube No.	Serum (ml)	Serum dilution	No. of 50 per cent units of complement/ 0·25 ml	Antigen (ml)	Veronal buffer (ml)
Series A	0 Per Cent Haemolysis	1	0		0	0	1·25
		2	0		0	0	1·25
	100 Per Cent Haemolysis	3	0		8	0	1·00
		4	0		8	0	1·00
Series B	Zero set	5	0·25	1/10	0	0·75	0·25
	Group I	6	0·25	1/10	2	0·0	0·75
		7	0·25	1/10	4	0·0	0·75
		8	0·25	1/10	6	0·0	0·75
		9	0·25	1/10	8	0·0	0·75
		10	0·25	1/10	2	0·75	0
		11	0·25	1/10	4	0·75	0
		12	0·25	1/10	6	0·75	0
		13	0·25	1/10	8	0·75	0
	Group II	14	0·25	1/20	2	0	0·75
		15	0·25	1/20	4	0	0·75
		16	0·25	1/20	6	0	0·75
		17	0·25	1/20	2	0·75	0
		18	0·25	1/20	4	0·75	0
		19	0·25	1/20	6	0·75	0
		20	0·25	1/20	8	0·75	0
	Group III	21	0·25	1/40	1	0	0·75
		22	0·25	1/40	2	0	0·75
		23	0·25	1/40	4	0	0·75
		24	0·25	1/40	2	0·75	0
		25	0·25	1/40	4	0·75	0
		26	0·25	1/40	6	0·75	0

Incubate at 38° C for 45 minutes
Add 0·5 ml of sensitized red blood cells to each tube
Incubate at 38° C for a further 45 minutes