STUDIES ON HAEMONCHUS CONTORTUS INFECTIONS IN MERINO SHEEP

A Thesis

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by

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CHAPTER 1

GENERAL INTRODUCTION

It is well established that sheep and goats have an important role to play in meeting the rapidly expanding worldwide demand for meat and other animal products. This increasing demand is being met by the utilisation of marginal lands, the continued improvement of pastures and the application of intensified methods of husbandry. Under almost all of these systems of management the problem of parasitic disease is probably the greatest obstacle to the achievement of this objective. Indeed since intensification of livestock invariably facilitates the transmission of parasitic diseases these tend to be of increasing economic importance both by their depressant effect on mutton and wool production and in constituting a constant threat to sudden losses through acute disease.

Of all parasitic diseases the range of species associated with gastro-intestinal nematodiases are the most significant both in distribution and economic significance and the majority of sheep and goats are hosts to a variety of these throughout their lives. Of the many pathogenic nematode species Haemonchus contortus is generally recognised as the most important in sheep and goats in the vast tropical and sub-tropical areas. The main features of the life-cycle of this species, which is outlined in Appendix A, have been established by the work of various authors (Veglia, 1915; Fourie, 1931; Andrews, 1942; Stoll, 1943) and the epidemiology and practical significance of haemonchosis from work in Australia by Gordon (1948, 1950) and Swan (1970).

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It would appear that the invidious reputation enjoyed by this ubiquitous parasite is probably based on four particular aspects of its host-parasite relationship.

The first of these is the great biotic potential of H. contortus. Whereas each female of the other common species of gastro-intestinal nematodes of sheep and goats normally produces about 100 eggs daily at the peak of egg production, the female H. contortus normally produces in excess of 10,000 daily (Soulsby, 1965). Thus, given favourable climatic conditions for the development of H. contortus eggs to infective larvae it is apparent that pastures will quickly become heavily contaminated with larvae.

The speed with which development from the egg to the infective stage can occur is the second characteristic of H. contortus infection. While most nematode species require around 6 or 7 days for their eggs to develop to infective larvae, under optimal circumstances those of H. contortus will become infective larvae in as little as four days (Soulsby, 1965). ?

The two other important aspects of the host-parasite relationship concern first its pathogenic effect in the host and secondly the reaction of the host to reinfection. It is well established that the primary pathogenic effect of H. contortus infections is the anaemia caused by the haematophagic habits of the developing and adult stages (Fourie, 1931; Andrews, 1942; Baker, Cook, Douglas and Cornelius, 1959; Clark, Kiesel and Goby, 1962; Baker and Douglas, 1966;

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Whitlock and Georgi, 1968). Since each adult has been shown to ingest up to 0.05 ml of blood daily it is apparent that an infestation of several thousand worms, a not uncommon natural occurrence, will quickly produce gross anaemia and frequently death.

The final aspect, i.e., the reaction of the host to the parasite, is rather unusual in that there is little evidence of the development of a useful degree of acquired immunity against H. contortus in sheep and goats under normal grazing management, and in tropical and sub-tropical areas sheep and goats become infected at an early age and are often continually and successfully infected throughout life (Gordon, 1948, 1950; Lopez and Urquhart, 1967).

The survival of sheep which are set-stocked on permanent pasture and not subject to regular and frequent anthelmintic treatment is customarily attributed to the periodic expulsion of the entire adult worm burden (Gordon, 1948). This reaction, termed the self-cure phenomenon, has been shown under experimental conditions to have an immunological basis dependent on a hypersensitivity reaction of the abomasal mucosa (Stewart, 1953). In the field this is only intermittently exhibited and apparently confers no protection against immediate reinfection (Gordon, 1948; Lopez and Urquhart, 1967).

The work described in this thesis was initially directed towards the elucidation of the self-cure phenomenon as a flock phenomenon under natural grazing conditions. As a consequence of these studies it became apparent that several important aspects of the epidemiology and of the clinical

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syndromes associated with haemonchosis required further investigation. The thesis is therefore essentially a description of haemonchosis of sheep in an endemic area supported, where indicated, by observations obtained by experimental infections. Each of the separate aspects studied is prefaced by a review of the relevant literature and the details of many individual observations which are not included in the text may be found in the various appendices. Some results, for example the individual faecal egg counts and worm burdens at autopsy, were too voluminous to be included and these are available at The Veterinary School of the University of Glasgow.

CHAPTER 2.

A STUDY OF THE EPIDEMIOLOGY OF HAEMONCHOSIS IN A MERINO FLOCK IN EAST AFRICA

INTRODUCTION

Despite the importance of H. contortus in the economy of sheep and goat production in tropical and sub-tropical regions the epidemiology of haemonchosis has remained relatively unstudied in almost all of these areas. Most of the information available is dependent on the observations of Gordon and other workers (Gordon, 1948, 1949, 1950, 1958; Hughes, 1960; Swan, 1970) in Australia. These authors followed the seasonal fluctuations of H. contortus eggs in the faeces of infected sheep and showed that during certain periods i.e., when rainfall was in excess of two inches (50 mm) per month and the mean maximum temperature was above 18°C (65°F), faecal egg counts increased markedly, presumably due to increased numbers of infective larvae becoming available on the pasture. They also found that this increase was quickly followed by a dramatic fall, often to zero, in the faecal egg counts of the entire flock. This they described as the "self-cure phenomenon", a term first used by Stoll (1929) (see Chapter 6). They also showed that clinical haemonchosis occurred in all sheep irrespective of age and previous experience of infection.

These Australian observations have formed the epidemiological basis for the control of haemonchosis wherever the disease is prevalent, despite gross differences in patterns of temperature and rainfall and in the methods of husbandry and breeds of sheep in many of these areas. One such area is the semi-arid region of East Africa which supports large numbers of wool sheep and in which haemonchosis, although recognised as a serious disease of frequent occurrence (Anon, 1948; Round, 1962) remains largely undescribed. For example, the seasonal incidence and precise significance of the disease is unrecorded and control measures are frequently only initiated when the disease is clinically apparent. The experiments described in this chapter were designed to study haemonchosis in a specific area of known endemicity with the object of extending knowledge of the epidemiology and significance of this disease in conditions characteristic of commercial sheep enterprise in semi-arid tropical regions of Africa. Such investigations would, it was thought, provide a greater understanding of the aetiology of its occurrence and thereby contribute towards improved methods of its control.

These observations extended over a period of two years during which a number of parameters were studied. These were the weekly fluctuations in the faecal egg counts of H. contortus, the monthly variations in the abomasal worm burdens, the course of the haematological indices and the development of clinical signs in both ewes and lambs. In addition tracer sheep were used to estimate the seasonal variation in levels of infective larvae on the pasture.

MATERIALS AND METHODS

Location of the Experiment

Observations were made from September, 1969 to July, 1971 at the National Animal Husbandry Research Station, Naivasha, Kenya, which is situated 50 miles west of Nairobi on the floor of the Rift Valley at $0^{o}40'$S. and $36^{o}26'$E. at an altitude of approximately 2,000 metres. It is a region

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of semi-arid climate with an available moisture water index of 30 to 40 (Pratt, Greenway and Gwynne, 1966). Strong desiccating winds during the dry season are a feature of the area. The annual rainfall averages about 600-700 mm but there is considerable variation between years. The monthly rainfall figures indicate that although some rain usually falls in every month there are normally only two rainy seasons with sufficient rainfall to allow grass growth to occur. These two seasons usually extend from March to June (the "long rains") and September to November (the "short rains"). The mean monthly maximum temperature normally varies from 22° to 30°C (72 to 86°F) and the mean monthly minimum temperature from 6° to 18°C (43 to 64°F). Frost occurs rarely and the drier months have the highest variation in temperature. Principal natural grazing consists of Naivasha Star Grass (Cynodon plectostachyum), the less palatable Common Star Grass (Cynodon dactylon), Kikuyu Grass (Pennisetum clandestinum) and the grass Harpachne shimperi also occurs in certain areas. The smooth rolling pastures are supported on a loamy fine sand soil with no profile development other than a more humic silt loam surface which was derived relatively recently from unconsolidated volcanic ash (Gethin-Jones and Scott, 1970).

Experimental Animals

The sheep were part of the commercial flock of pure-bred Merinos born and reared on the National Animal Husbandry Research Station, Naivasha and were set-stocked at a density of about 1 per acre on permanent pastures known to be endemic

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for haemonchosis. This permanent flock consisted of 80 Merino ewes, aged 2 to 5 years with their pure-bred Merino lambs which were born in July each year and which were grazed during these studies with the ewes until they were about 10 months old. Following treatment of all the ewes with thiabendazole* at 100 mg/kg, in July 1969 i.e., at the start of the period of observation, no further anthelmintics were administered throughout the course of the study. Mineral lick was offered ad libitum as the only form of supplementary feeding.

Each month 4 ewes and 2 lambs were withdrawn at random from the permanent flock and removed indoors. After being maintained for 2 weeks in parasite-free conditions to allow development of recently ingested larvae to a stage where they would be readily recovered and, incidentally, distinguished from larvae inhibited in their development (Armour and Bruce, 1974), the ewes and lambs were autopsied and the number of adult and immature nematodes in the gastro-intestinal system was estimated by the methods described later. However, this procedure had to be temporarily abandoned in the lambs between March and July 1970 since the numbers had been excessively depleted by deaths from haemonchosis.

In order to maintain a relatively consistent stocking rate, groups of sheep were withdrawn at intervals from the original farm flock in order to replace those which had been removed by the regular sample autopsies.

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* "Thibenzole" Merck Sharp & Dohme, Nairobi, Kenya.

"Tracer" sheep aged 6 to 12 months were used to assess the relative availability of infective larvae on the pasture during successive months. These were born and reared under parasite-free conditions indoors. Their parasite-free status was confirmed by repeated faecal examinations and by sample autopsies at regular intervals. Pairs of tracer sheep were put out to graze with the field flock for successive 2 or 4 week periods throughout the course of the studies. Following the period of grazing on endemic pasture, the tracer sheep were brought indoors and after being maintained for a further 2 weeks, under conditions which precluded reinfection, they were autopsied and the abomasal helminths recovered by the methods described later. This procedure provided an indirect estimate of the relative numbers of infective larvae available on the pastures during successive 2 or 4 week periods throughout the course of the study.

A clinical examination of all the lambs and ewes was made at least twice weekly and the course of clinical developments was followed more frequently during outbreaks of acute disease.

Parasitological Techniques

Faecal samples were collected from the rectum of each animal at least weekly and the faecal nematode egg count was performed by a modified McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948). The fluctuations in mean faecal egg counts were recorded separately for ewes and lambs. Soft or fluid faeces were infrequently encountered

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and no allowance was therefore made for variations in faecal consistency.

Infective larvae were recovered from faecal cultures periodically by methods similar to those described by Roberts and O'Sullivan (1950). The larval stages were identified using the descriptions of Whitlock (1960). At no time during the course of these studies did H. contortus larvae comprise less than 95% of the total, infective larvae of T. axei, Oesophagostomum spp., Strongyloides spp. and Bunostomum spp. being occasionally observed.

Examination of the morphological types of vulval flap morphology of the adult H. contortus females indicated a distribution of 80%-90% linguiform, 5-10% knobbed and 5-10% smooth which lies within the definition of H. contortus contortus as defined by Das and Whitlock (1960).

Haematological Techniques

Blood samples were collected from the jugular vein using ethylenediaminetetracetate as anticoagulant. This was done at weekly intervals alternately from 20 of the ewes and 20 of the lambs. Packed cell volumes (PCV) were determined by the microhaematocrit method (Fisher, 1962) and haemoglobin concentration (Hb) was estimated by the oxyhaemoglobin method (Dacie and Lewis, 1966). Red blood cell counts (RBC) were made in an electronic counter* by the method of Weide, Trapp, Weaver and Lagace (1962).

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* Coulter Electronics Ltd., London, England.

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The mean corpuscular volume (MCV) was calculated from the packed cell volume and the red cell count from the following formula.

$$\frac{\text{PCV (\%)} \times 10}{\text{r.b.c.} \times 10^{-6}} = \text{MCV } (\mu m^3)$$

The mean corpuscular haemoglobin content (MCHC) was calculated from the haemoglobin concentration and the packed cell volume from the following formula.

$$\frac{\text{Hb (g/100 ml)} \times 100}{\text{PCV}} = \text{MCHC (\%)}$$

Autopsy Procedure

At autopsy the abdomen was opened and following ligation of the pylorus the abomasum and omasum were immediately removed. After separation of the omasum and omental fat the abomasum was placed on a surgical tray and incised along its greatest curvature. A sample of abomasal contents was taken into a universal bottle for pH determination with a glass electrode**. A gross examination of the abomasal mucosa was made and any pathological changes were recorded and photographed.

After removal of the abomasum for parasitological examination, (*vide infra*) the remaining abdominal organs were examined superficially. The liver, adrenals and intestines were removed. If ascitic fluid was present the volume was measured after carefully draining the abdomen. The sternum was divided longitudinally and an examination of the thoracic organs was made. After inspection for pleural

** Beckman Expandomatic, Beckman Ltd.

effusions the pericardium was incised and the heart removed. The lungs were examined superficially and then the trachea and the major bronchi were opened and the presence of any exudate noted.

The carcase was dressed, weighed and an assessment of its general condition was made. The left femur was dissected out and the superficial muscles removed. It was later divided longitudinally with a tenon saw for examination of the bone marrow. The distribution of red marrow was recorded photographically for later correlation with parasitological data.

Parasitological Techniques

The duodenum, small intestine and large intestine were washed out separately in warm water and the contents passed through a sieve (size 200 mesh) to recover any nematodes present. These were floated off the sieve into a bowl of water, then picked out and placed in 10% formol-saline for later identification.

Meteorological Observations

The daily rainfall was obtained from rainfall gauges placed on the pasture and relative humidity and maximum and minimum temperatures were made available by the Naivasha Meteorological Station situated about one mile distant.

Pasture Analysis

Samples of pasture were collected monthly for chemical analysis from multiple random samples taken at regular intervals from a zig-zag route so that representative samples of pasture from each area of the paddock were obtained.

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RESULTS

Faecal Egg Counts

The fluctuations in the mean weekly strongyle faecal egg counts, shown by coproculture to be almost entirely *H. contortus* throughout, were calculated for the 80 ewes and 40 lambs and the results are illustrated in Fig. 1. The mean faecal egg counts of the ewes, treated with thiabendazole in 1969, began to rise in November 1969 and after several fluctuations, particularly a drop to 700 epg in April 1970, were subsequently stabilised around 4,000 eggs per gram (epg) until May 1971, when there was a sudden dramatic fall to a mean of about 200 epg. However, this was quickly followed by a rise and within 1 to 2 months the faecal egg count had returned to former levels. In the lambs the mean faecal egg counts were generally similar and in November 1969, and in July 1970, the new born lambs quickly attained the same level as the ewes. The fall in faecal egg counts in May 1971 was equally marked but the return to the previous high levels occurred more rapidly and was complete within 2 to 4 weeks. Evidence of a peri-parturient egg rise in the ewes was not seen in July 1970.

Worm Burdens

The mean monthly adult *H. contortus* burdens recovered at the serial autopsies of the ewes and lambs which had been withdrawn randomly from the permanent flock are shown in Fig. 1. (the detailed data are recorded in Appendix B, Table 1). A marked seasonal variation was observed in both the

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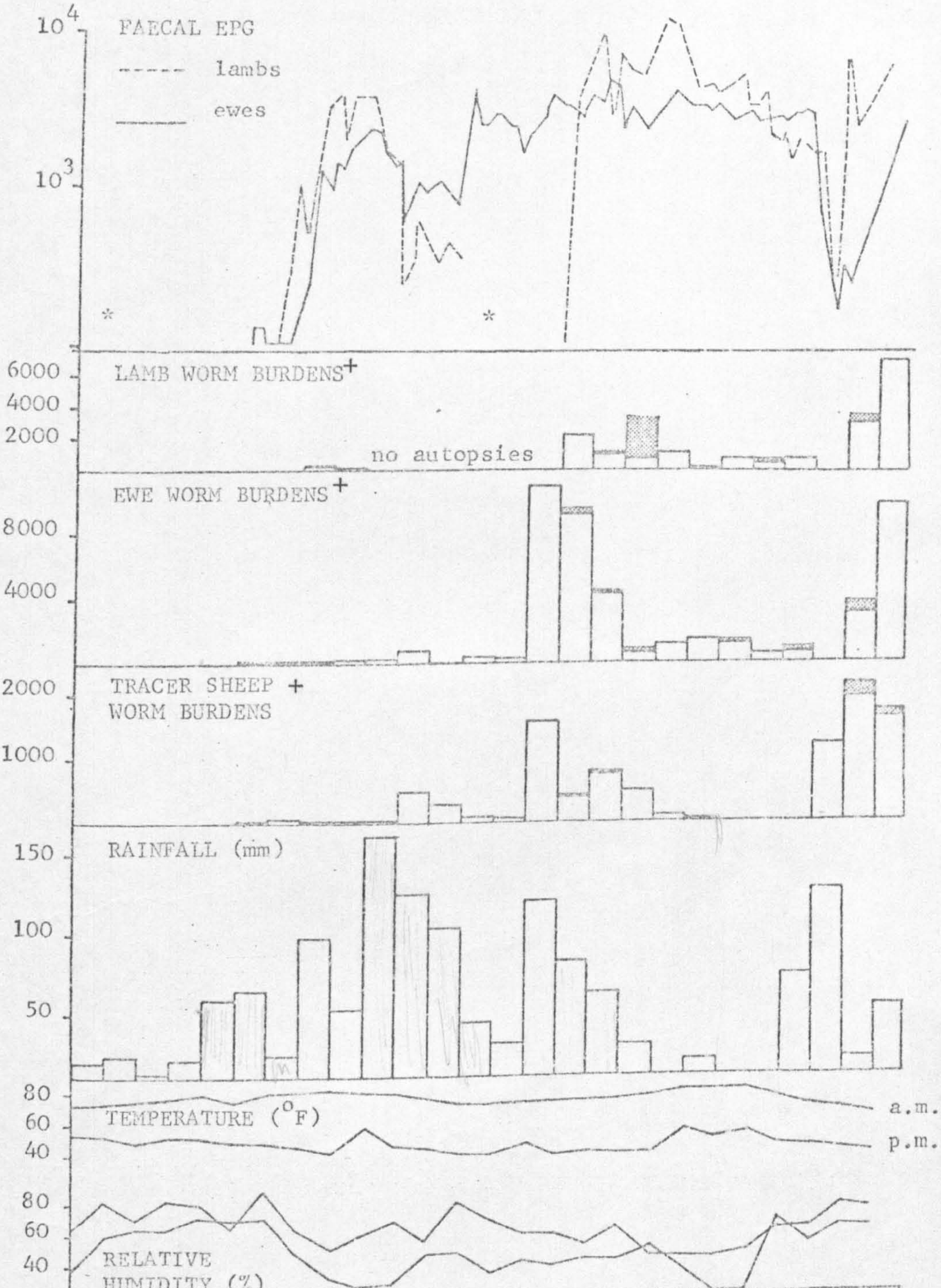

Fig. 1. The mean weekly faecal egg counts of the flock of Merino ewes and lambs, naturally infected with H. contortus, and their mean monthly worm burdens as determined by sample autopsies in relationship to the mean monthly larval challenge, as established in tracer sheep, and the mean monthly rainfall, maximum and minimum temperatures and relative humidity.

* Times of lambing.

+ Shaded areas indicate proportion of inhibited larvae.

ewes and the lambs and although the pattern was similar the worm burdens were lower in the lambs.

In the ewes the highest mean worm burdens ranging between 3,500 and 11,000 occurred between August and October 1970 and in June and July of the following year. During the remainder of the 21 month period of observation the mean worm burdens were much lower, ranging between 30 and 1500. In the lambs the comparative figures were between 2000 and 7000 and between 0 and 1500. Since both ewes and lambs were, as described previously, held indoors for 2 weeks before autopsy, only adult and inhibited larvae were recovered. In fact, the latter were only present in significant numbers on one occasion in the lambs, in November 1970 when a mean of 1500 of 2200 worms were inhibited.

Tracer Sheep

The mean number of adult *H. contortus* recovered at autopsy of tracer sheep grazed serially for 2 or 4 weeks periods with the permanent flock are shown in Appendix B, Table 2 and the mean monthly results are illustrated in Fig. 1. There was a marked seasonal variation of the numbers of *H. contortus* which established during each grazing period. This ranged from 0 in March 1971, to 2136 in June 1971, with a maximal establishment of 3093 *H. contortus* in the first two weeks of June 1971. The number of larvae in which development remained inhibited in the tracer sheep after 2 weeks in parasite-free conditions indoors did not reach a significant proportion of the total worm burden at any period of the year except on a single occasion as described above (see Appendix B, Table 2).

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Haematology

The mean fortnightly PCV and Hb values of 20 ewes and 20 lambs (Tables 3 and 4 of Appendix B) in relation to the mean faecal egg counts, adult worm burdens and larval challenge are shown in Fig. 2. From these it is apparent that an *H. contortus* infection of moderate proportions was established over the period December 1969 to August 1970 (i.e., rising from 48 to 11,000 *H. contortus* in the sheep selected for autopsy). The PCV and Hb values dropped progressively to around 25% and 7 respectively. A large proportion of this heavy infection persisted over the ensuing two months during which the PCV and Hb values continued to fall to 20% and 5g respectively. Following this, there occurred a period during which the sheep carried light infections of around 1000 adult *H. contortus*. Despite this the PCV and Hb values remained unchanged until the sudden loss of these infections eight months later in April 1971, when both returned to within approximately normal limits. During the entire period the anaemia in the lambs was characterised by a steadily increasing MCV; this was not studied in the ewes. The MCHC in both ewes and lambs remained within approximately normal limits throughout the periods of observation.

It is important to note that the haematological values recorded above are the mean values of the sample of 20 ewes and 20 lambs. Since these all suffered from clinical haemonchosis in varying degrees the mean haematological results do not indicate the degree of individual variation. Further information on this aspect is given in Chapter 5.

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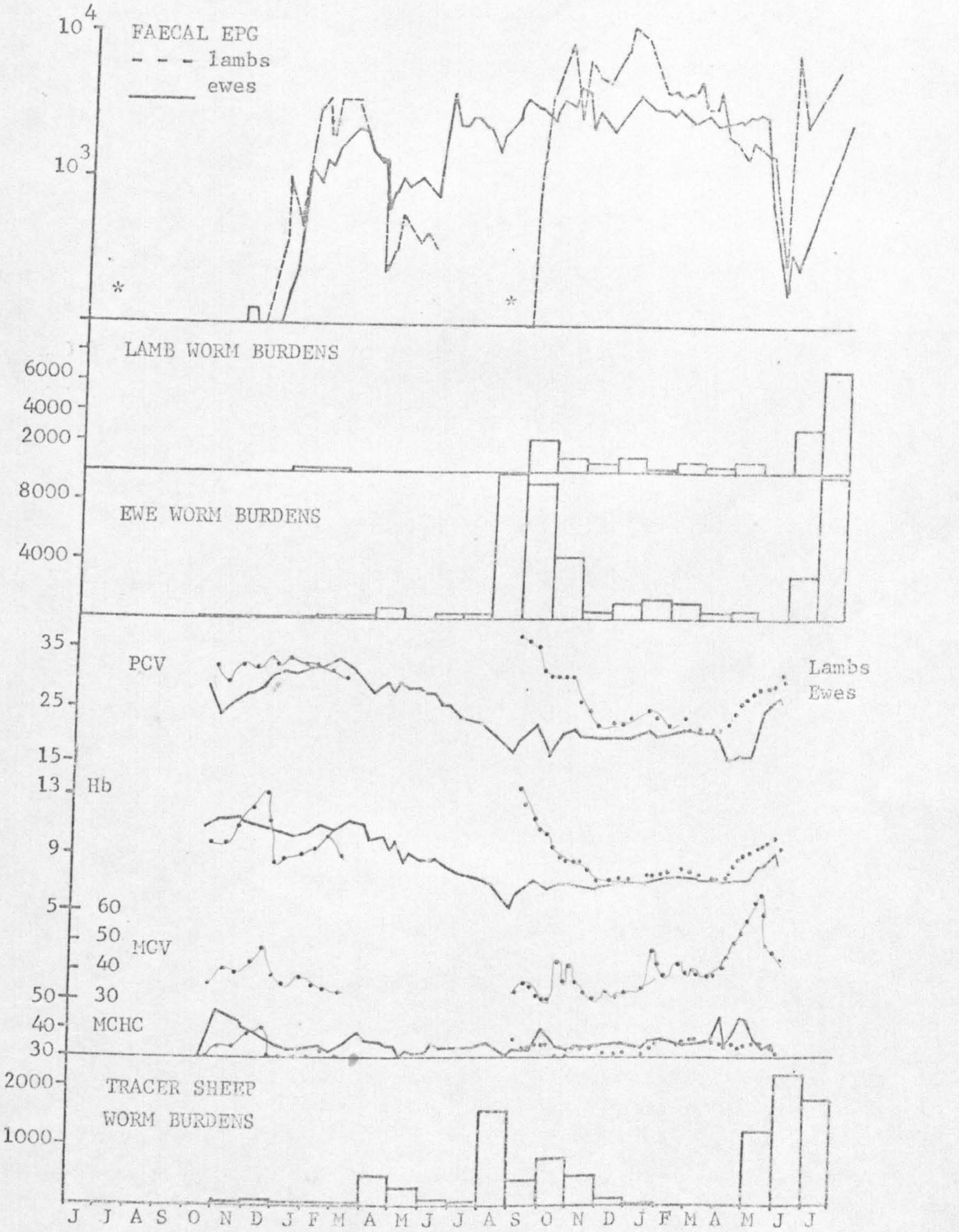

Fig. 2. The mean two-weekly haematological parameters of a flock of Merino ewes and lambs naturally infected with H. contortus in relationship to weekly faecal egg counts, mean monthly adult worm burdens and larval challenge as monitored by the establishment of infection in tracer sheep.

* Times of lambing

Mean Weights of H. contortus

The average mean wet weight of H. contortus adults found at post-mortem in the abomasum of the ewes varied considerably throughout the period of study. It was found that in general the mean wet weight of the adult parasites showed a negative correlation with the total number present (Fig. 3; see also Table 5 of Appendix B). Of particular interest were the two periods August and September 1970, and December 1970 to April 1971. During the first of these periods when the population was composed of a large number of around 10,000 young adult H. contortus, the mean wet weight was low, i.e., 43 mg per adult. Over the second period of 5 months during which fresh infections were not being acquired, there was a small established population of around 1,000 worms whose mean wet weight was 92 mg. Despite this the faecal egg counts of H. contortus eggs were continuously maintained at the level attained by the previous heavy infections of young adults whose population had a similar sex ratio (i.e., male:female about 1:1.2).

Climatic Data

The monthly rainfall figures over the experimental period are shown in Fig. 1. These, in general, followed the typical pattern of "long-rains" in March and April and "short-rains" later in the year. During these periods rainfall figures range from about 60 to 160 mm per month and during the remainder of the period of rainfall figures ranged between 0 and 50 mm per month.

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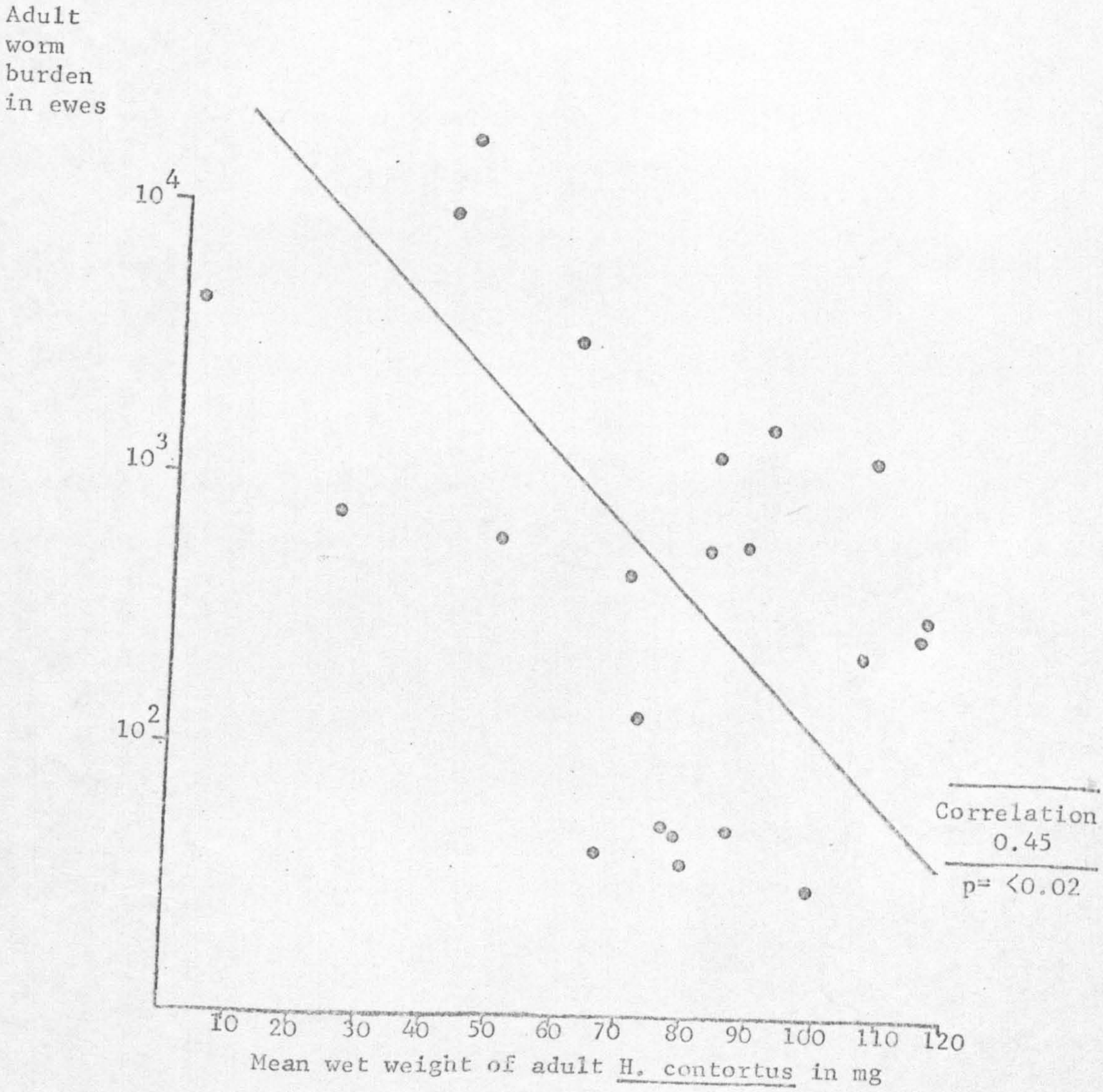

Fig. 3. The relationship between the wet-weight of individual *H. contortus* adults and the total number of adults present in the abomasum. Each point is the mean of a monthly parasitological examination of four ewes.

The mean monthly maximum and minimum temperatures illustrated in Fig. 1 show that there was relatively little seasonal variation over the experimental period. Mean monthly maximum temperatures generally ranged between 22°C (72°F) and 30°C (85°F) and the monthly minimum temperature, invariably recorded at night, between 6°C (43°F) and 15°C (60°F). The monthly relative humidity values ranged between a mean maximum of 73% and a mean minimum of 56% except during the period of two months February and March 1971, when the values fell to 28% and 29% respectively.

Pasture Analysis

During part of the experimental period, i.e., the 12 months from March 1970 to February 1971, monthly pasture analysis was performed in which iron content, crude protein, crude fibre and dry matter were estimated and the results are shown in Fig. 4 (see also Table 6 of Appendix B). The two most relevant features were, first, the variation in iron content throughout this period, which appeared to be approximately proportional to the monthly rainfall; thus during the last three months of the experimental period when rainfall was negligible the iron content fell to below 200 ppm; i.e., less than half of the previous monthly values. The second feature was the great variation in the crude protein content which differed during the experimental period by a factor of three.

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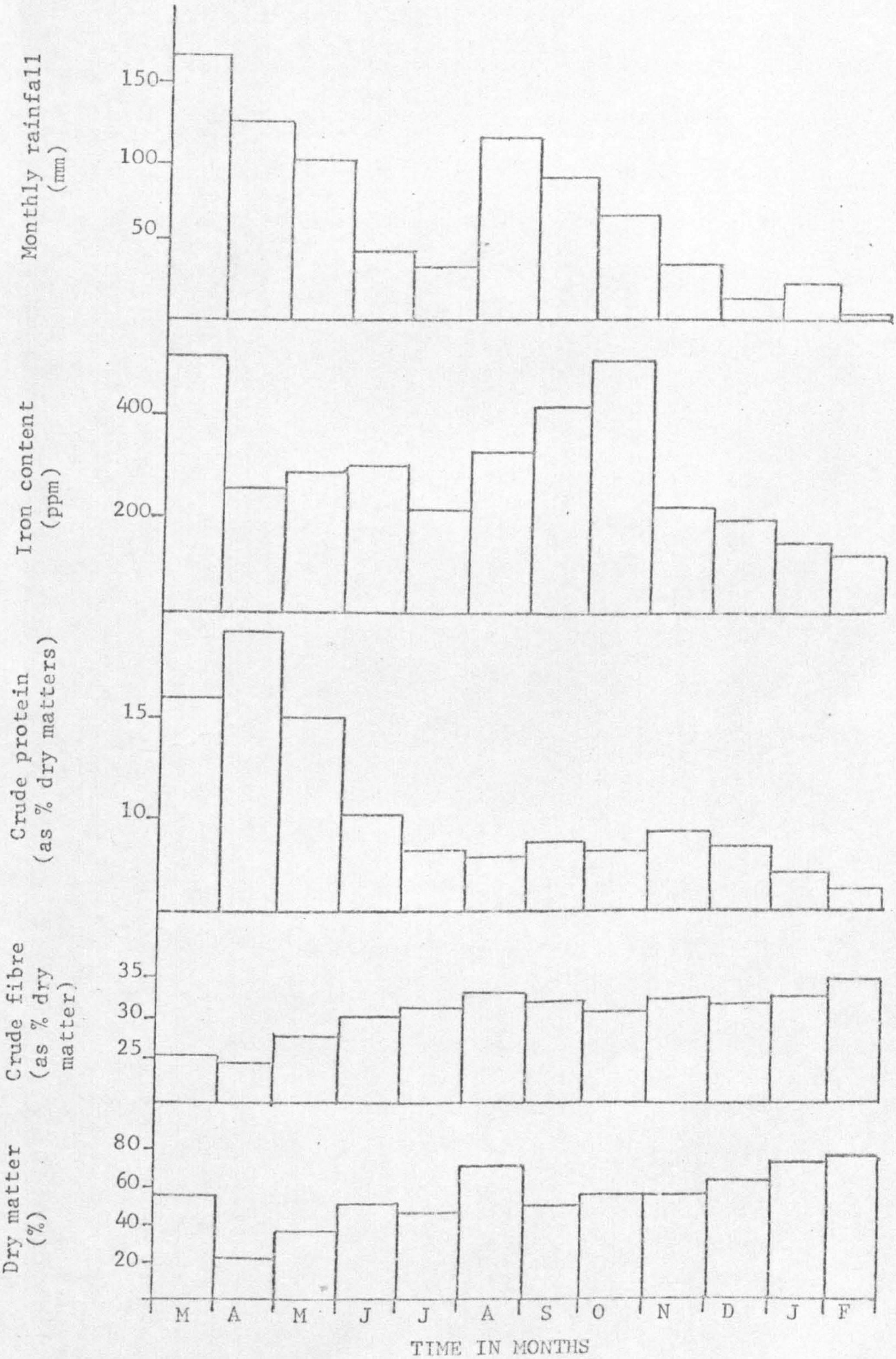

Fig. 4. The results of the monthly analysis of permanent pastures at Naivasha, Kenya from March 1970 to February 1971.

DISCUSSION

The results presented in this chapter were obtained in an effort to describe the course of *H. contortus* infections in a Merino flock grazing in an endemic area over a period of 21 months during which anthelmintics were not administered. The observations were also intended to elucidate the reasons for any fluctuations in worm burden which might occur and to provide an epidemiological background against which the onset and development of clinical signs might be correlated.

Perhaps one of the most striking features of the investigation was the sustained high level of faecal *H. contortus* egg counts despite apparently gross monthly variations in the abomasal worm burdens (particularly of the ewes) as determined by serial autopsies. Shortly after the commencement of the experiment when the worm burdens of the ewes were low (i.e., about 50 to 100 adults per ewe) the faecal egg counts rapidly reached around 4,000 epg. Thereafter except for two short periods the counts fluctuated between 4,000 and 7,000 epg despite mean abomasal worm burdens which averaged between 500 and 11,000 adults per ewe. It would therefore appear that faecal egg counts are not a reliable index of *H. contortus* burdens in natural infections. The reasons for this appear to be linked with the size and age of the adult worm population. Thus a heavy infection recently acquired seems to be associated with a relatively low faecal egg count. For example, during August

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and September 1970 the freshly acquired burdens of around 10,000 worms were not reflected by any increase in the faecal egg counts. By contrast, a light infection which has been established over several months appears to produce relatively large numbers of H. contortus eggs. Thus, during the period from December 1970 to April 1971, the worm burdens although relatively low (i.e., a mean of 1,000) maintained their former levels of egg production (i.e., between 4,000 to 7,000 epg). Additional evidence which supports these conclusions on the relative egg producing capacity of the two types of worm population is shown by comparison of the mean wet weights of the H. contortus adults from the two populations. Over the two month period when heavy infestations were observed the mean wet weight per H. contortus was 43 mg whereas over the five month period of small infestations the mean wet weight per H. contortus was 92 mg.

As mentioned above there were two short periods when the faecal egg counts were not sustained. During April 1970 and again in May 1971 the faecal egg counts of both ewes and lambs dropped sharply to means of 700 epg and 200 epg respectively. The second drop in faecal egg count which occurred following a period of heavy rainfall was clearly an example of the self-cure phenomenon as described in grazing sheep by Gordon (1948, 1950), since the mean abomasal worm burdens were 560 before the egg counts fell and 37 in the succeeding month.

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It is likely that the first drop in faecal egg counts which also occurred during a period of significant rainfall was likewise a manifestation of the self-cure phenomenon but involving only a proportion of the flock since the faecal egg counts of some of the sheep fell to zero (see Chapter 7).

The faecal egg counts of the lambs, although generally higher, followed the pattern of the ewes, self-cure occurring simultaneously with the ewes on both occasions. The observations on the relationship of the faecal egg counts to the adult worm burdens of the ewes also held good for the lambs. An additional feature of infection in the lambs was the time which elapsed between birth and the appearance and rise of faecal egg counts. In the first lamb crop eggs were only detected four months after birth while in the second lamb crop they appeared within two months. This disparity was presumably due to the near drought conditions prevailing up to the time of weaning of the first lamb crop compared with the abundant rainfall over the same period in the second season. Presumably during the second period many more infective larvae were present on the grass and this was substantiated by a comparison of the abomasal worm burdens in the tracer sheep.

As noted above, the unexpected uniformity of the faecal egg counts in the ewes and lambs over most of the period of observations was not paralleled by the abomasal worm burdens of H. contortus throughout the same period. The latter estimations were based on three types of sheep, i.e., ewes, lambs and a consecutive series of tracer sheep each of which grazed for two to four weeks with the ewes and lambs. Restrictions of finance and husbandry limited the number of sheep available for autopsy each month and in general approximately ten sheep were examined each month. Although gross individual variations were occasionally experienced it is believed that the numbers were sufficient to be representative of the flock in general.

In the ewes the mean monthly adult worm burden fluctuated widely; the lowest burden recorded was 20 and the highest 11,000. It is generally considered that heavy infestations of H. contortus in sheep are invariably dependent on high rainfall and temperatures above 18°C (65°F) (Dinaburg, 1944; Gordon, 1948, 1950). In the course of these observations such conditions existed for one to three consecutive months during four main periods, i.e., January 1970, March to May 1970, August to October 1970 and April to May 1971. On the third and fourth of these occasions massive infestations of 4,000 to 11,000 worms were indeed acquired by the grazing ewes and lambs but during the first and second period only minimal burdens of around 300 worms were recovered. This occurred despite the deposition of large numbers of eggs on the pasture during the preceding two months on both occasions particularly the second. However, as judged by the small burdens established in the tracer sheep during these two periods the H. contortus eggs apparently failed to develop to infective larvae or were not available in large numbers on the herbage. These

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observations indicate that factors other than rainfall and temperature are important in the development and availability of H. contortus larvae on herbage; this was the subject of further investigations which are described later in Chapter 3.

The course of the first massive infestation persisted for three months during which the mean monthly abomasal worm burdens were 11,000, 9,000 and 4,000 respectively. From an examination of the tracer results it would appear that these worm populations were composed of a decreasing number of worms which had survived over this period, partially replenished by freshly acquired infections. At the end of this time worm numbers dropped to about 500 adults per ewe and apparently persisted at this level for the ensuing six months, a dry period, during which the tracer intake was virtually negative.

One might, therefore, conclude that the classical self-cure phenomenon, i.e., a sudden drop in faecal egg counts associated with worm expulsion occurring during a period of heavy rainfall, is not the only way in which heavy infestations are eliminated and that, during periods when the intake of infective larvae is relatively low, worm numbers may be markedly reduced over a period of a few months. As mentioned earlier, however, this may not become apparent if faecal egg counts alone are used as the criterion.

In temperate countries a characteristic rise in faecal egg counts is commonly associated with parturition in ewes (Taylor, 1935; Crofton, 1958; Spedding and Brown, 1956; Field, Brambell and Campbell, 1960; Cvetkovic, Golosin and Kosanovic, 1971; Brunsdon, 1970; Gibbs, 1967) although its predictability in other areas of the world is not clearly defined (Lewis and Stauber, 1969; Donald and Waller, 1973). In these observations which extended over a single lambing period a peri-parturient rise was not observed; in fact the faecal egg counts remained relatively high both before and after lambing. Perhaps the absence of a peri-parturient rise is not surprising since the phenomenon is now considered to be primarily associated with the maturation of inhibited larvae. This in temperate countries has been shown to be associated with the previous exposure of infective larvae to low temperatures (see review by Armour and Bruce, 1974). The environmental temperatures recorded during this experiment never fell to levels known to be necessary to produce inhibition in temperate regions. However, other temperature factors may be more important in tropical areas although it should be pointed out that inhibited larvae were not found in the abomasum of the 12 ewes autopsied during the last three months of pregnancy in 1970.

Inhibited H. contortus larvae were, however, observed intermittently in both ewes, lambs and tracers, the proportion ranging between 1% and 13% and on one occasion 68% in lambs aged 5 months. No obvious factor was found to account for these numbers of inhibited larvae and the relative roles of the environmental effects on infective larvae and of immune responses on developing H. contortus larvae (Dineen and Wagland, 1966) require further investigation.

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In common with the Australian observations (Gordon, 1948, 1950) ewes did not appear to develop a significant degree of immunity to reinfection in that on the two occasions when large numbers of infective larvae were available on the pasture heavy infestations of adults were rapidly established. This observation may not imply a complete inability to develop immunity which may only be stimulated by the presence of a threshold number of recently acquired worms. Some evidence that this might be so can be seen in the progressive reduction of the adult worm population in the ewes over the four month period from 11,000 to 600 during August to November 1970. A similar pattern of infection, although less striking, was observed in the lambs.

The metereological data indicated that throughout the period of observations the temperatures were rarely unsuitable for the development of H. contortus eggs to infective larvae (Rose, 1963). The figures for relative humidity in relation to larval infectivity were less easy to interpret and are discussed more fully in Chapter 3.

Descriptions of clinical haemonchosis usually emphasise the sudden onset of anaemia, submandibular oedema, and the development of ascites coincident with the sudden acquisition of heavy infections. This in severe cases is followed rapidly by death. It is generally assumed that a "chronic haemonchosis" syndrome does not exist. The results obtained here indicate that in addition to acute clinical haemonchosis a condition of persistent anaemia without obvious clinical

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signs is possibly more characteristic of the naturally occurring disease.

Thus, from Table 1 it is apparent that in the ewes over the initial first six months of observation relatively light worm burdens of around 100 adult worms per ewe were recovered and were not associated with anaemia or clinical signs. During the subsequent four months moderate mean worm burdens of 330 were associated with a gradual fall in PCV from 32% to 22% and a similar fall in Hb from around 11 to 7. Following this, massive infestation of around 4,300 to 11,000 adults were acquired over the next three months and were accompanied by further significant drops in these indices to 17% and 5 g respectively. At this point clinical haemonchosis was obviously apparent, i.e., pale mucous membranes, submandibular oedema, lethargy and sporadic deaths.

Periods	Mean Worm Burden of Ewes	Mean PCV	Clinical Syndrome
Oct 69 - Mar 70	103±	32±	Nil
Apr 70 - Jul 70	336	22	Nil
Aug 70 - Oct 70	8173	17	Acute haemonchosis
Nov 70 - Feb 71	1010	21	Loss of weight, anaemia
Mar 71 - Apr 71	550	18	Chronic haemonchosis i.e. loss of weight and death
May 71	37	27	Self-cure and recovery
Jun 71 - Jul 71	6730	-	Hyperacute and acute haemonchosis.

Table 1. The relationship between worm numbers, PCV and clinical signs in a Merino flock set-stocked, on an *H. contortus* endemic pasture.

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Following the loss of these heavy infections there was a period of four months of moderate infection (November 1970 to February 1971), the worm burdens ranging from 590 to 1,540. During this time haematological values continued to be significantly reduced although somewhat above those seen during clinical haemonchosis, i.e., a PCV of around 21% and an Hb of around 7. The sheep appeared relatively normal on superficial observation in that pale mucous membranes were not apparent and oedema and deaths did not occur. However, in the ensuing two months (March to April 1971) before self-cure supervened the condition of the sheep deteriorated markedly. In spite of the absence of further infection and mean monthly worm burdens of 540 and 560 respectively the flock became lethargic, deaths occurred and, although weighing facilities were not available, it was obvious that most of the sheep had suffered severe weight loss. Haematological examination showed that in a proportion of the sheep the PCV and Hb dropped to levels comparable to those encountered during outbreaks of acute haemonchosis. Following the intervention of self-cure the clinical and haematological parameters improved rapidly and one month later the sheep were apparently normal. This was only temporary however and during the next two months hyperacute and acute haemonchosis developed.

The haematological and clinical data of the lambs followed a similar pattern in that the outbreak of clinically acute haemonchosis (August 1970) was followed by a period of chronic anaemia terminating in a "chronic haemonchosis syndrome", i.e., two to three months after birth.

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These results, which indicate that anaemia and loss of condition may exist in an infected flock over a period of months despite a relatively modest worm burden are, to the author's knowledge, not previously described. In this respect it is necessary to consider the possibility that the anaemia was largely or partly associated with a deficiency of iron in the pasture during the period of low rainfall. It was certainly the case that the amount of iron in the pasture over the twelve month period studied was directly proportional to the rainfall. While it is, therefore, unlikely that iron deficiency per se contributed to the anaemia associated with the acute syndrome which occurred during a period of high rainfall it is possible that, combined with the low crude protein content of the pasture, it may have played a part in the chronic syndrome which developed over a dry period of six months.

It may be concluded that this epidemiological study of haemonchosis in Kenya has confirmed some of the previously established features of this disease. It was found that the disease in its classical form occurred during periods of high rainfall and affected both ewes and lambs with equal intensity despite the former being reared throughout life in an endemic area. The occurrence of self-cure as a flock phenomenon involving both ewes and lambs was also confirmed.

However, this study has also revealed some aspects of haemonchosis, as it occurs in East Africa at least, which have not been previously recognised. The first of these is the uniformity of faecal egg counts over prolonged periods

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in both ewes and lambs despite gross fluctuations in the adult worm burdens. The second is the fact that self-cure may fail to occur despite the presence of all the known prerequisites, i.e., a period of high rainfall coincident with the intake of large numbers of infective larvae, the latter being superimposed on an existing adult worm population. The final point concerns the possibility that one of the most significant features of haemonchosis is not the acute syndrome so characteristically associated with the disease, but is the fact that a moderate infection of a few hundred worms persisting over a period of several months will produce severe loss of bodily condition, a degree of anaemia and deaths in ewes and lambs grazing on pasture of a poor quality.

SUMMARY

An epidemiological study was conducted over a period of 21 months on a flock of 80 set-stocked Merino ewes and their lambs grazing in an *H. contortus* endemic environment in Kenya.

The parameters studied included weekly fluctuations in the faecal egg counts, monthly variations in the abomasal worm burdens, the course of the haematological indices and the development of clinical signs. In addition tracer sheep were used to estimate the seasonal variation in levels of infective larvae on the pasture.

The results of the study confirm some of the previously established features of the disease. It was found that the disease in its classical form occurred during periods of high rainfall and affected both ewes and lambs with equal intensity. The occurrence of self-cure as a flock phenomenon in both ewes and lambs was also confirmed.

However, the study also revealed some aspects of haemonchosis which have not been previously recognised. The first of these is the uniformity of faecal egg counts over prolonged periods in both ewes and lambs despite gross fluctuations in the adult worm burdens. The second is the fact that self-cure may fail to occur despite the presence of all the known prerequisites, i.e., a period of high rainfall coincident with the intake of large numbers of infective larvae, the latter being superimposed on an existing adult worm population. The final point concerns the possibility that one of the most significant features of haemonchosis is not the acute syndrome so characteristically

associated with the disease, but the fact that a moderate infection of a few hundred worms persisting over a period of several months will produce severe loss of bodily condition, a degree of anaemia and deaths in ewes and lambs grazing on pasture of a poor quality.

CHAPTER 3

THE DEVELOPMENT OF AN IMPROVED TECHNIQUE FOR THE FORECASTING OF ACUTE HAEMONCHOSIS

INTRODUCTION

Considerable progress has been made in recent years in the establishment of useful relationships between weather conditions and the incidence and severity of various diseases of grazing livestock. This association has been shown to be particularly useful in forecasting the incidence of some helminth diseases since these usually involve life cycles which necessitate the exposure of the free-living stages to the vagaries of climate or, alternatively, a sojourn in a secondary host which may itself be influenced by the ambient weather conditions. The best examples of the former are ovine nematodiriasis (Ollerenshaw and Smith, 1966) and to a lesser extent bovine parasitic gastro-enteritis (Smith, 1969), and of the latter, fascioliasis in sheep and cattle (Ollerenshaw and Rowlands, 1959; Ollerenshaw and Smith, 1969). Generally speaking these forecasts are based on empirical formulae derived from various meteorological indices.

Several authors have suggested the use of various meteorological parameters in an attempt to forecast the seasonal incidence of clinical haemonchosis. The first of these was Dinaburg (1944) who suggested that a mean monthly minimum temperature of over 18°C (65°F) was necessary for the development of H. contortus larvae to the infective stage. Subsequently, in Australia, Gordon (1950) on the basis of his own correlations between meteorological data and field observations confirmed

Dinaburg's critical temperature of $18^{\circ}C$ ($65^{\circ}F$) but in addition proposed that rainfall in excess of 50 mm (2 ins) per month was also a necessary prerequisite for larvae to become available in sufficient numbers to initiate an outbreak of acute haemonchosis. Gordon's findings were largely substantiated by Hughes (1960) also in Australia, although the latter observed one period when the monthly rainfall temperature, although apparently favourable for the development of acute haemonchosis failed to produce even a significant increase in faecal egg counts. Subsequently, a similar observation was made by Swan (1970) in Queensland who found that on three occasions in three years, faecal egg counts did not increase despite suitable temperatures and rainfall as defined by Gordon. He attributed this to the wide diurnal fluctuations in temperature in his part of Queensland and suggested that in such areas the critical temperature for larval development and availability was a mean monthly maximum of above $10^{\circ}C$ ($50^{\circ}F$). He was unable to draw any conclusions as to the minimum amount of rainfall required in association with this critical temperature.

If one assumes on the basis of the observations reported elsewhere in this thesis (Chapter 2) that the development of inhibited larvae plays an insignificant part in the pathogenesis of haemonchosis in East Africa it follows that overt disease must depend on the ingestion of significant numbers of infective larvae over a period of 2 to 3 weeks before the onset of clinical signs. The most important single factor which determines the availability of large numbers of infective larvae is almost certainly the existence of a suitable microclimate in the pasture for a period of a few days at least. Because of variations in temperature, rainfall, soil moisture, and pasture height in the course of a month and between one month and the next, it is unlikely that this suitable microclimate can be defined merely in terms of mean monthly rainfall and temperature.

The two most important features of a microclimate are temperature and relative humidity. Of these the former is probably the least important when considering variations in availability of H. contortus larvae found in this study since the ambient mean daily temperature was always above 18°C (65°F).

The relative humidity of the microclimate in pasture cannot be extrapolated from the rainfall and ambient temperature alone and is, as far as is known, largely dependent on the interaction of a number of factors which include rainfall, evaporation, physical properties of soil, and the quality of the vegetative cover.

The contribution of rainfall to the microclimate is not only dependent on the total amount but is affected by other factors such as evaporation into the atmosphere, run-off from the soil surface and the degree of infiltration and retention by the soil profile. The rate of evaporation also varies and is dependent on such factors as wind speed, amount of sunshine, extent of cloud cover, relative humidity and ambient air temperature.

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Each soil, according to its type and depth, has a maximum water-holding capacity and this in turn affects the amount of water available for evaporation and for plant growth (Salter, 1967). The influence of the vegetative cover on the microclimate depends on its height, density, quality and conformation, and on the amount of transpiration from the foliage.

Although it is apparent from the above that the true assessment of the relative humidity of a micro-climate in pasture is extremely complex, several authors have produced formulae which attempt to provide information on some aspects of the problem. In this chapter an attempt has been made to find if any correlation existed between the various formulae and the availability of H. contortus larvae on the pasture over a period of 21 months as measured by the worm burdens of tracer lambs grazed at monthly intervals throughout this period. Although some correlation was established it was found that a more composite index which was developed provided better results.

MATERIALS AND METHODS

The endemic pasture, tracer sheep and the details of the parasitological techniques were those described in Chapter 2. The standing height of the pasture was measured monthly in cm by calculating the mean of numerous samples over representative areas of the paddock. The daily rainfall figures were obtained from rain gauges positioned on the pasture while the daily relative humidity values and the daily maximum and minimum temperatures were supplied by the official meteorological station at Naivasha about one mile distant.

Evaporation was calculated as daily potential evaporation (Eo) by the Penman (1948) formula. Eo is an abbreviation for "evaporation from open water" and is synonymous with daily potential evaporation. The figures for the Naivasha area were supplied by the Meteorological Officer in Nairobi. The Penman formula, by taking account of wind speed, sunshine, cloud cover, relative humidity and ambient temperatures gives the amount of evaporation from an open water surface. The justification for using this formula for evaporation from pasture is that Dagg (1965) found that this formula offered a good assessment of water loss by plants from the soil profile including that by transpiration.

Meteorological formulae with which correlations with *H. contortus* availability were sought.

These formulae, all expressed as monthly indices, were four in number:

1. The "Total Monthly Rainfall" - this was calculated from the daily rainfall.
2. The "Effective Rainfall" - this was calculated by subtracting the daily potential evaporation from the daily rainfall. This figure, at least in theory, represents the actual amount of rainfall that is available for replenishment of the soil profile for that day.

   The results were expressed as monthly effective rainfall.

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3. The "Soil Water Index" - this was calculated by the method of Woodhead (1970) and is essentially a hydrological balance sheet derived from a monthly estimate of soil water surplus or deficit based on the effective rainfall for the month added to the soil water index of the previous month. The method of calculation is illustrated in Appendix C.

4. "Soil Surface Moisture Index" - this formula was evolved in an attempt to provide a better meteorological correlation than the above with larval availability of *H. contortus* on pasture since it also takes into account pasture height; this has been shown to have a direct relationship with increased transpiration and thus humidity at the soil surface (Hudson, 1965; Nadi and Hudson, 1965). It was calculated from the formula:-

(Soil Water Index + 200) x Pasture Standing Height (metres).

RESULTS

Tracer Worm Burdens

The *H. contortus* worm burdens recovered from the tracer sheep grazed serially over the period of 21 months are shown in Fig. 5 (see also Appendix C). These have already been discussed in detail in Chapter 2 and for the purpose of this experiment it is only necessary to note that high worm burdens (>1200 adults) were observed only on two occasions, i.e., August 1970 and May to July 1971.

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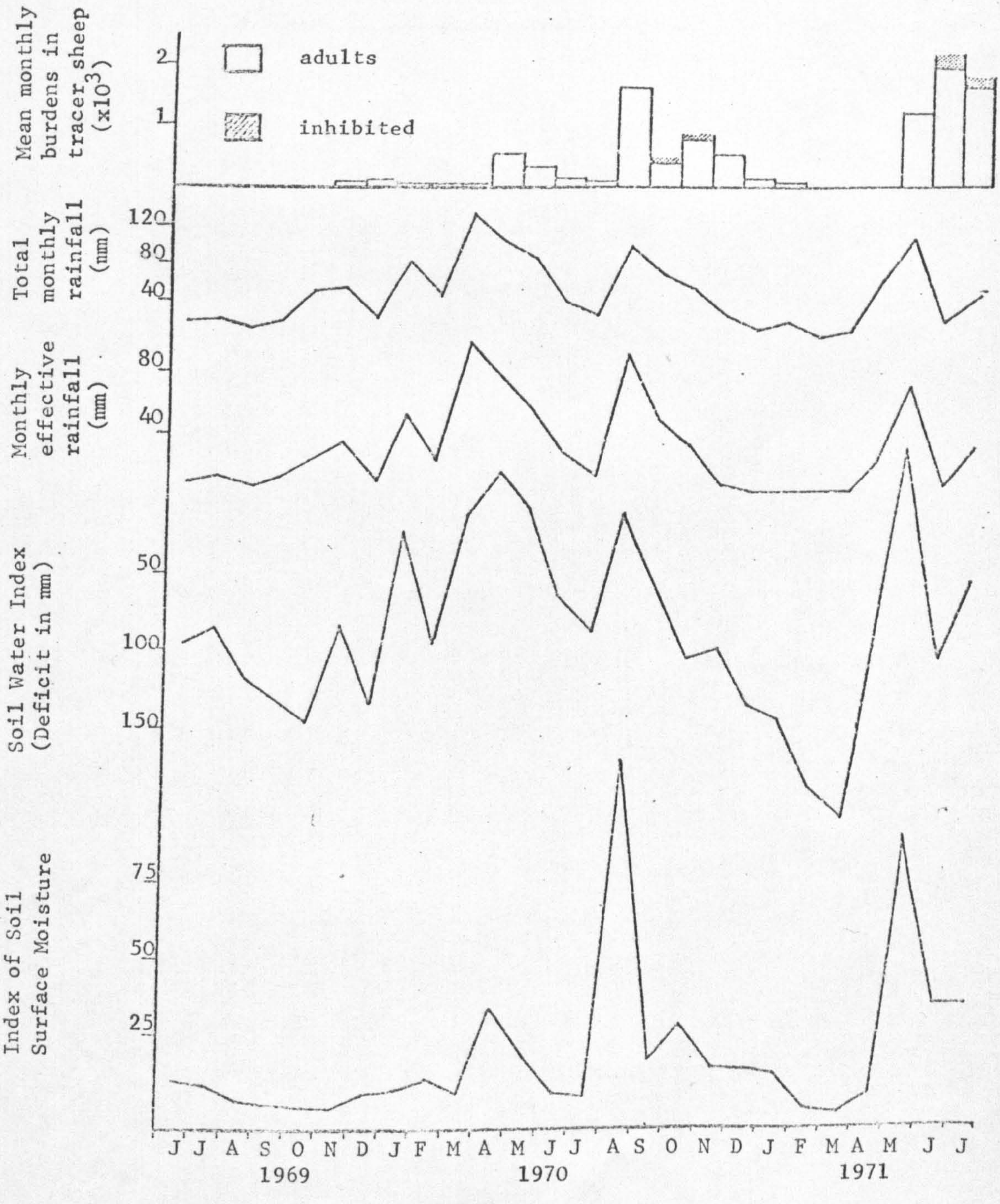

Fig. 5. The results of attempts to correlate four meteorological formulae with the availability of H. contortus larvae on pasture as measured by the mean worm burdens in tracer lambs.

Monthly Rainfall

This is also illustrated in Fig. 5 and shows that in general there were four marked peaks of 1, 3, 3 and 2 months duration respectively, when the total monthly rainfall was well in excess of the 50 mm (2 ins) proposed by Gordon as conducive to larval availability. Despite this, as mentioned above, high worm burdens, i.e., over 1200, were only observed on two of these occasions; also the highest rainfall peak recorded (162 mm: 6.4 ins) coincided with a period of minimal larval availability, i.e., a mean of 20 worms in the tracer sheep. However, it should be noted that when the monthly rainfall was less than 50 mm only negligible worm burdens were ever acquired.

Effective Rainfall

This is also shown in Fig. 5 and the net effect is similar to that of the monthly rainfall in that the same poor correlation with the mean tracer worm burdens was apparent.

Soil Water Index

This is illustrated in Fig. 5 and here the correlation with tracer worm burdens was slightly better in that during the three months when the mean tracer worm burdens were lowest, i.e., 5, 0, 5 adult H. contortus respectively, this index clearly was at its lowest. Furthermore during the month when the highest mean tracer worm burden was recorded the index was at its highest. Otherwise the correlation was poor, i.e., four high peaks.

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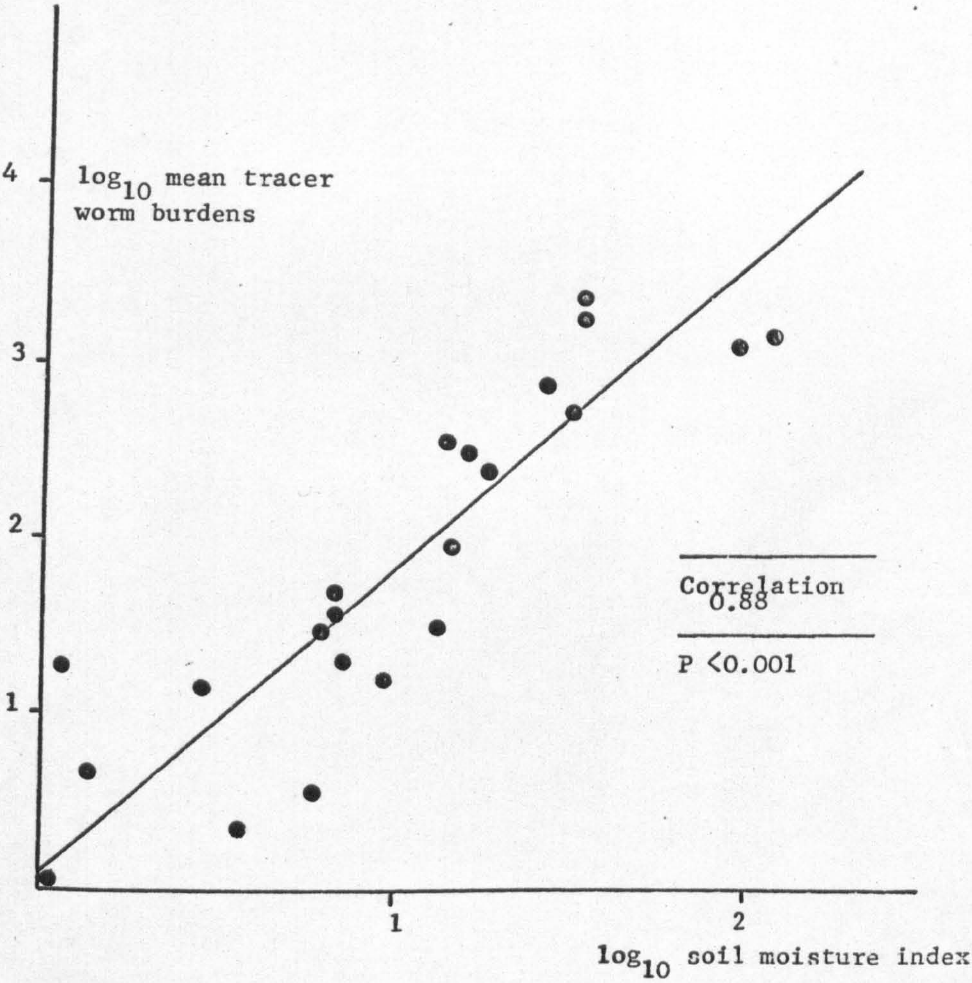

FIG. 6. The relationship between the mean monthly adult worm burdens of tracer sheep grazed on H. contortus endemic pasture and the soil moisture index of the pasture during the corresponding period.

Soil Surface Moisture Index

This is shown in Fig. 5 and appears to provide a good correlation in that the two periods of high mean tracer worm burden are clearly reflected in the two largest peaks of this index. The other two peaks in the other formulae during which negligible worm burdens were acquired were not evident. Instead, two other peaks are present at different months when the mean tracer worm burden reached their next highest levels, i.e., in April 1970 and October 1970. Furthermore the period of three months when larval intake was almost zero is represented by the lowest indices.

Because of this apparent close correlation between this index and the extremes of the mean tracer worm burdens a regression line ($Y = 1.5384\,x + 0.3589$) was constructed of the monthly soil surface moisture index and the mean tracer worm burdens. This is illustrated in Fig. 6 and is highly significant ($P < 0.001$).

DISCUSSION

The work described here was performed in an attempt to determine if outbreaks of acute haemonchosis in sheep in an endemic area might be forecast by an analysis of meteorological data. This was done by comparing the mean monthly worm burdens of tracer sheep grazed consecutively for a period of 21 months on an endemic pasture set-stocked with a permanent flock.

The first correlation examined was that of Gordon (1950), who proposed that a mean monthly rainfall of over 50 mm (2 ins) together with a temperature above 18°C (65°F) provided suitable conditions for larval availability and the subsequent initiation of an outbreak of acute disease. Actually only the significance of the first parameter could be examined since the mean maximum ambient temperatures of the experimental site was always above 18°C (65°F). As described in the results this provided a relatively poor correlation.

Of the other indices examined the effective rainfall provided an equally poor correlation and that of the soil water index was only marginally better. The soil surface moisture index appeared to provide an excellent correlation not only with the highest and lowest mean tracer burdens but throughout their entire range. Furthermore when related to the outbreaks of acute and hyperacute haemonchosis in the permanent flock the index reached its highest points throughout these periods. The basis for this correlation is unknown but it may be significant that this index is essentially that of a soil water index with an allowance made for the height of the pasture. As mentioned previously transpiration is almost directly proportional to plant height and it may be that the considerable variations in plant height observed during these observations caused significant variations in the microclimate. Although the validity of this hypothesis requires to be tested in a variety of environments over an extended period of time the results reported in this chapter suggest that it may be of real practical value.

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Finally there are three minor points which merit brief discussion. The first is essentially parasitological and concerns the phrase "larval availability". The observations recorded here and those described on larval longevity in Chapter 4 were not designed to clarify whether larval availability, as shown by high worm burdens in tracer sheep, was due to a rapid development to the infective stage of eggs deposited during the period when the microclimate was most suitable or alternatively whether the microclimate merely provides ideal conditions for infective larvae of various degrees of longevity to move towards a position on herbage where they are likely to be ingested. The question is an important one and should be resolved by further experimentation.

The second and third points concern the limitations of forecasting acute haemonchosis by meteorological observation. No matter how efficiently this may be developed it is likely that the interval between the forecast and the onset of disease will always be very short and perhaps of the order of two weeks. Also even in the absence of acute haemonchosis the economic significance of the "chronic haemonchosis" syndrome which is associated with relatively small worm burdens as described in Chapter 5 is important enough to merit attention and treatment. However, neither of these limitations significantly detract from the value of the forecast for acute haemonchosis which is so often associated with sudden high mortality.

SUMMARY

In this chapter four meteorological formulae were examined with regard to their respective value in forecasting the seasonal incidence of acute haemonchosis in an area of Kenya. Three of these were standard formulae i.e., total monthly rainfall, effective rainfall and soil water index. The fourth, "soil surface moisture index", was evolved by the author and was based on the soil water index and the pasture height.

Each of these formulae, month by month, was compared with the H. contortus worm burdens in a series of tracer sheep grazing on endemic pasture together with a set-stocked permanent flock.

The total monthly rainfall, presently regarded as of practical significance in the forecast of acute haemonchosis, showed the poorest correlation in that high worm burdens occurred on only two of four occasions when the total monthly rainfall was well in excess of the accepted 50 mm level.

The "soil surface moisture index" provided the best correlation, not only with the highest and lowest mean tracer burdens but also throughout their entire range. Furthermore, when related to the outbreaks of acute and hyperacute haemonchosis in the permanent flock the index reached its highest points throughout this period.

The potential value and limitation of such a formula in forecasting outbreaks of acute haemonchosis was discussed.

CHAPTER 4

AN INVESTIGATION OF THE DURATION OF INFECTIVITY OF *HAEMONCHUS CONTORTUS* FREE-LIVING STAGES ON PASTURE

INTRODUCTION

The environmental conditions necessary for the successful embryonation of Haemonchus contortus eggs, their development through the 1st and 2nd larval stages (L_1 and L_2) to the infective 3rd stage larvae and their subsequent longevity has been studied by many workers under both field and laboratory conditions. Unfortunately there is some disparity in these findings due perhaps to the cursory nature of some of the investigations conducted under a wide range of climatic conditions; it is also possible that some of the inconsistencies depend on the existence of strains of H. contortus which have different optimal conditions for their development from eggs to infective larval stages. Since the survival of infective larvae is not necessarily a reliable indication of infectivity the validity of some workers' conclusions, based entirely on larval motility, may also be somewhat suspect when applied to epidemiological studies.

For infective larvae to be available to grazing animals three conditions must be fulfilled. First the egg must have developed successfully to the infective larva. Secondly the infective larva must survive until the time of ingestion. Thirdly it must be in a suitable position on the herbage to be available for ingestion. It is apparent therefore that under field conditions the bionomics of larval infectivity is a complex study, and it is proposed to review the relevant literature under the three headings described above.

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The Development of Eggs to Infective Larvae

The general consensus is that desiccation is unfavourable to larval development. Although both Veglia (1915) and Berberian and Mizelle (1957) observed that developed eggs were relatively more resistant to the effects of desiccation than freshly passed eggs, Rose (1963) concluded that the effects of desiccation are such that few if any *H. contortus* eggs in faecal pellets develop into infective larvae. This view is supported by Donald (1968) and Waller and Donald (1970) who concluded that only the infective juvenile stage of *H. contortus* is desiccation-resistant.

The advantage of the retention of the L_2 sheath by the L_3 stage was demonstrated by Ellenby (1968) who showed that the ensheathed larvae were relatively more resistant to the desiccating effect of 43% relative humidity than were the exsheathed stages.

A conclusion somewhat at variance with those quoted above has been expressed by Silverman and Campbell (1959) who reported that well developed eggs containing larvae survived at room temperature for six weeks in dried faecal pellets.

Of particular relevance here are the findings of Dinnik and Dinnik (1958) who found that in the Highlands of Kenya a diurnal fluctuation in temperature of between a mean minimum of 11°C (52°F) and a mean maximum of 24°C (75°F) with at least one inch of rainfall evenly distributed within a ten day experimental period were the most favourable for larval development. These optimal conditions are similar to those

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originally described by Dinaburg (1944) who concluded that a temperature of above 18°C (65°F) was a primary requirement for the development of H. contortus larvae provided there was adequate humidity.

The Longevity of Infective Larvae on Pasture

Despite the numerous reports on this important aspect of H. contortus infection it is difficult to draw precise conclusions. This is partly due to the different conclusions reached by various workers and partly to the impossibility of extrapolating these observations to the daily fluctuations in temperature and relative humidity encountered in the field.

Under laboratory conditions a range of optimal temperatures for longevity has been reported from 11°C (52°F)(Silverman and Campbell, 1959; Rose, 1963) to 21°C (68°F) (Shorb, 1943, 1944). The subject has recently been reviewed by Levine (1963) and Todd (1970). The former, on the basis of his own work, concluded that H. contortus larvae survived for 8½ months in faecal pellets at room temperature and in water at 4 to 5°C (39 to 41°F) for up to 22 months. Only in the first of these experiments was the infectivity of the surviving larvae confirmed by animal experiment. Todd's conclusions were rather different in that he suggested that the optimal conditions for maximal survival were humidity of 75% and a temperature of 20°C (68°F); under these conditions larvae remained motile for 3 months.

In field conditions the longevity of infective larvae on pasture has been found to show considerable variation probably due to the different climatic conditions to which the larvae were exposed. Reports range from 3 weeks (Spindler, 1936) to

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22 months (Boughton and Hardy, 1936); this subject has been reviewed by Kates (1950).

The Availability of Infective Larvae on Pasture

It is generally assumed that high relative humidity and temperature aid the movement of infective larvae towards the tips of pasture thus facilitating their ingestion. For *H. contortus* larvae this has been demonstrated by Rees (1950) and Silangwa and Todd (1964) who concluded that a high relative humidity of 93% and temperatures of around 25 to 27°C (77 to 81°F) produced maximal vertical migration of infective larvae. However, the former showed that, even under these circumstances, the percentage of larvae recovered from tne gress tips was very low compared to that recovered from the total herbage and the contribution of active migration towards larval infectivity on pasture remains doubtful.

Larval availability in a more general sense has been shown by Gordon (1948) in Australia to be associated with a temperature of 18°C (65°F) and rainfall in excess of 50 mm (2 ins) per month. Swan (1970), working in another part of Australia which had wide diurnal fluctuations in temperature, concluded that a mean monthly minimum of over 8°C (46°F) was a more useful criterion. However, it should be noted that the index which both these workers used to estimate larval availability was an increase in the faecal egg counts of set-stocked sheep. Even if one assumes that an increased egg output is always a consequence of increased infection (see Chapter 2) it does not show whether the increased

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availability of infective larvae on the pasture is due to their activation by moisture or simply the rapid development to the infective stage of eggs which had been passed a few days previously.

In summary it would appear that in areas where haemonchosis is endemic the most favourable conditions for the development of eggs to infective larvae are temperatures of 11 to 21^{o}C (52 to 68^{o}F) and adequate moisture (93 to 95% relative humidity.) The subsequent survival of infective larvae in endemic areas is thereafter probably more dependent on relative humidity than on temperature since mean monthly maximum and minimum temperature are usually relatively consistent and within the optimal range in tropical and sub-tropical areas.

The purpose of the experiment described in this chapter had the essentially practical objective of estimating the length of time during which pasture might remain contaminated with significant numbers of H. contortus larvae.

MATERIALS AND METHODS

The method of rearing the tracer sheep and the parasitological and meteorological techniques employed were as described in Chapter 2.

Five ewes naturally infected with H. contortus were introduced at different points in time into four paddocks each of one eighth of an acre. The respective times were

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in June 1970, December 1970, April 1971 and May 1971. The ewes remained for seven days in each of the paddocks their faecal egg counts being estimated daily. After their removal the paddocks remained empty until the introduction of the tracer sheep. The periods during which each paddock remained free of livestock are shown in Table 2.

A pair of tracer sheep was introduced into each of the four paddocks on the 1st June 1971 remaining there for 15 days. At the end of this period they were returned indoors and maintained under parasite-free conditions for 2 weeks prior to autopsy and subsequent enumeration of their *H. contortus* worm burdens.

Pasture conditions in each of the four paddocks were similar and consisted of permanent pasture of the type described in Chapter 2. Each paddock was situated on level ground of open aspect which offered minimal protection to faecal pellets on the soil surface.

RESULTS

These are shown in Table 2.

DISCUSSION

The results of this work showed that H. contortus eggs survived on pasture in an area where the disease is endemic for at least twelve months despite the fact that there was a period of four months i.e., December to March 1971 during which rainfall was low i.e., a mean monthly rainfall of 10 mm

Table 2. The results of an experiment to determine the duration of infectivity of *H. contortus* larvae on pasture in an endemic area.

Paddock Number	Month during which eggs were deposited on the pasture	Mean faecal egg count of ewes used to infect pasture (epg)	Length of time paddock left vacant	Tracer* sheep No.	*H. contortus* worm burden at autopsy: Male	Female	L_4	Total	Mean±SE
1	June 1970	1000	12 months	1	0	10	0	10	15±5
				2	0	20	0	20	
2	Dec. 1970	7500	6 months	3	210	130	0	340	244±96
				4	18	100	30	148	
3	Apr. 1971	5000	2 months	5	1140	1730	50	2920	2390±530
				6	1030	830	0	1860	
4	May, 1971	2000	1 month	7	50	48	20	118	153±35
				8	93	64	30	187	

* One pair of tracer sheep was grazed from the 1st to the 15th June, 1971, on each of the four paddocks.

and a high potential evaporation, i.e. a mean monthly index of 170 mm (see Chapter 3). For all practical purposes it would appear that this period might be approaching the ultimate level of larval survival since only 10 and 20 worms respectively were found in the two tracer sheep. However, this conclusion would require to be substantiated since only two tracer sheep were used, the faecal egg counts of the ewes used to contaminate the pasture were relatively low and no investigation was made of the potential of infective larvae to survive beyond twelve months.

In the contaminated paddock which was left vacant for 6 months larval survival was improved i.e., worm burdens of 148 and 340, despite the fact that the monthly rainfall from the period when the eggs were first deposited until four months later was always low i.e., 10 mm, 21 mm, 1 mm and 8 mm respectively. Since laboratory studies described earlier have shown that only the infective larval stage is resistant to desiccation it is likely that the pasture micro-climate after the deposition of the eggs was sufficiently humid to permit rapid development of a significant proportion of these to the infective stage.

These results show that a small though significant number of H. contortus larvae may survive on pasture for as long as six or even twelve months despite prolonged periods of aridity. These, although not sufficient in themselves to produce acute haemonchosis, would establish adult infections with sufficient biotic potential to

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initiate clinical disease in the next generation.

In the paddock left vacant for 2 months, larval survival was relatively high, worm burdens of 1860 and 2920 respectively being found in the two tracer sheep. This is perhaps not surprising since the monthly rainfall during these two months was 74 mm and 127 mm respectively, presumably producing conditions ideal for larval development and survival. The practical significance of this finding is that a contaminated paddock, even if left vacant for two months, may still be capable of producing acute haemonchosis immediately after the re-introduction of sheep i.e., no further cycling of the infection is necessary.

The results obtained from the paddock left vacant for one month are somewhat puzzling in that burdens of only 118 and 187 worms were recovered from the tracer sheep. Since the total monthly rainfall during this period was 127 mm one might have assumed that conditions were near optimal for larval development, which from laboratory studies has been shown to be as little as 4 days (Rose, 1963).

This apparent anomaly is difficult to understand particularly since during the same period other tracer sheep used in the experiments described in Chapter 2 acquired the largest worm burden recorded in the entire 21 months. Some possible explanations are, first that it may have been that the two tracer sheep were naturally resistant to H. contortus infection. Secondly, it is

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conceivable that under field conditions the development of H. contortus eggs to infective larvae may be very much more prolonged than established under laboratory conditions due, for example, to gross diurnal fluctuations in temperature and relative humidity. A third possibility is that the environmental conditions during the period when the eggs were deposited were unfavourable in some unrecognised fashion, so that the majority of the eggs failed to develop. Finally a contributory factor may have been the relatively lower faecal egg counts of the ewes used to contaminate the paddock in May 1971 i.e., 2000 epg compared to 5000 epg of the ewes used in April 1971.

SUMMARY

In this experiment each of four paddocks were grazed by two sheep reared worm-free for a period of two weeks, after which they were autopsied and their worm burdens estimated. The paddocks had been last grazed by H. contortus infected ewes for monthly periods 1, 2, 6 and 12 months previously.

The results showed that a small but significant number of H. contortus larvae had survived on pasture for as long as 6 and 12 months despite prolonged periods of aridity. These, although not sufficient in themselves to produce acute haemonchosis, would presumably establish adult infections with sufficient biotic potential to initiate clinical disease in the next generation.

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When a contaminated paddock was left empty for a shorter time i.e., two months, the numbers of larvae subsequently acquired by the tracer sheep indicated that acute haemonchosis was a likely sequel. Finally, and inexplicably, a paddock grazed one month after being heavily contaminated produced a relatively light infection in tracer sheep despite the fact that the intervening period seemed ideal for larval development and survival.

CHAPTER 5.

THE CLINICAL ASPECTS OF HAEMONCHOSIS IN MERINO SHEEP

INTRODUCTION

Despite the importance of haemonchosis as the prevalent endoparasitic disease of sheep and goats in most tropical and sub-tropical countries and the fact that the course of experimental infection has been extensively studied (Veglia, 1915; Fourie, 1931; Andrews, 1942; Baker et al., 1959; Baker and Douglas, 1966; Clark et al., 1952; Pradhan and Johnstone, 1972) the sequence of clinical signs as they develop during disease outbreaks under natural conditions in an endemic area has not been adequately described. This chapter describes the clinical findings and gross pathology observed during an epidemiological study of endemic haemonchosis from 1969 to 1971 in a commercial flock of Merino sheep reared and maintained in a semi-arid region of East Africa.

In the discussion an attempt is made to interpret these various findings against the background of epidemiological findings important in haemonchosis. These include the size and rate of larval challenge, the age and nutritional status of the host and the effect of the self-cure phenomenon. Difficulties encountered in the correlation of the various clinical syndromes with established parameters of laboratory diagnosis are also discussed and criteria suggested as a basis for field diagnosis.

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MATERIALS AND METHODS

Sheep and Goats

The bulk of the observations described here was made on the flock of Merino sheep untreated with anthelmintics, which were the subject of the epidemiological studies reported in Chapter 2.

Parasitological Techniques

Faecal egg counts, haematological and parasitological examinations were performed as described earlier in Chapter 2.

RESULTS

Haemonchosis is usually described as a disease of sporadic occurrence and relatively sudden onset. If untreated its course is acute, the characteristic signs of anaemia and oedema frequently terminating in death; occasionally it may be hyperacute and mortality is the first sign of disease, (Soulsby, 1965). Although these types were both observed in this study a third syndrome of "chronic haemonchosis" also became apparent and is described, it is believed, for the first time.

To facilitate description each outbreak is not described chronologically. Instead, the three syndromes are dealt with separately, the results being based on a composite of the relevant outbreaks. The description of the clinical signs and gross pathology of each syndrome is also accompanied by a brief account of the epidemiological background and the subsequent parasitological findings.

A) ACUTE HAEMONCHOSIS

Epidemiological Background

This syndrome was observed simultaneously in both ewes and lambs on two occasions i.e., August-September 1970 and July 1971. It occurred during periods when the intake of infective larvae was high as judged by the worm burdens of the ewes and lambs and by tracer sheep autopsies over these periods (see Chapter 2). During both of these periods the soil surface moisture index, as described in Chapter 3, was very high being exceeded only on one occasion i.e., during the outbreak of hyperacute haemonchosis in June 1971.

Clinical Signs

On each occasion the clinical signs were evident in the entire flock of both ewes and lambs although there was considerable individual variation. First indications of disease in the ewes were those of lethargy and loss of condition; this occurred despite the fact that pasture quality was rapidly improving. Clinical examination revealed pale mucous membranes and evidence of increasing sub-mandibular oedema. Later this tissue became more dependent and developed into the well recognised "bottle jaw" syndrome (Fig. 7). In severely infected sheep this tissue became very dependent and marked facial and peri-orbital oedema developed so that the whole head became swollen with the lips thickened and swollen and the muzzle much broadened (Fig. 8). In some cases the faeces were darker than normal and were occasionally formed in the shape of large rouleaux.

As the ewes lost weight the abdomen became more prominent and the presence of ascites was sometimes detectable clinically. The progressive loss of weight was accompanied by increasing weakness, exemplified by lethargy and a slow swaying walk and by affected animals trailing behind the remainder of the flock. Some difficulty in rising from the lying position was noticed at this stage. Loss of wool in patches was common and occasionally sheep lost their entire fleece. The udder was pale and indurated and suckling lambs sometimes died as a result of the subsequent agalactia.

Terminal acute cases developed from 1-4 weeks after the initial onset of clinical signs and were characterised by extreme weakness, marked loss of weight and gross clinical anaemia. Some ewes were dyspnoeic and showed lip-curling and flared nostrils. Respiratory rates of up to 120 per minute were recorded and heart rates reached 150 per minute in some cases. In severe infections faeces were seen to be dark reddish-brown in colour. Anorexia was observed only as a terminal feature of the disease. The mortality on each occasion averaged around 14%.

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The development of clinical signs in the lambs which were aged 2-4 months old was generally similar to that of the ewes although a fatal outcome was usually more rapid. "Bottle jaw" and pale mucous membranes were common (Fig. 9). The young lambs of severely affected and agalactic ewes became emaciated and died without clinical evidence of anaemia.

Gross Pathology

At post-mortem examination acute haemonchosis was characterised by a pale, thin, oedematous carcase. The blood was thin and watery while the mucous membranes were very pale coloured. The reflection of the skin revealed the presence of widespread subcutaneous oedema and a marked accumulation of hard, chalkwhite abdominal fat. This was particularly noticeable in the omentum which was frequently developed into a thickened, white solid structure lying ventrally in the abdomen (Fig. 10).

All the abdominal organs, particularly the liver, were pale coloured and were bathed in up to 4 litres of straw-coloured ascitic fluid. The spleen was usually enlarged and on sectioning was dark red in colour and of softer consistency than normal. The adrenals were often enlarged and oedematous on section. The mesenteric and abomasal lymph nodes were usually grossly enlarged, especially in severe infections in lambs (Fig. 11). Pleural and pericardial effusion was common and the heart was often enlarged, flabby and pale (Fig. 12). On incision

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the ventricles were thin-walled. The lungs were filled with pink frothy exudate.

Examination of the abomasal mucosa revealed a series of gross pathological changes which could be related to the length and severity of infection. In the early stages of the disease multiple red petechiae, 1-2 mm in diameter, were found, presumably caused by the blood-sucking habits of the adults. Generally the petechiae were present in the highest concentrations on those abomasal folds adjacent to the main central area of the fundus and produced large reddish patches on an otherwise light yellow abomasum (Fig. 13). The majority of the adult parasites were normally found adjacent to the mucosal surface and were often clumped together in the pyloric region. In severe infections the entire mucosa was found to be congested and red in colour and the folds of the abomasum were grossly thickened by submucosal oedema.

The abomasal contents, whose pH was usually between 4.5 to 6.5, were often found to be coarse and very dark in colour, probably due to the presence of earth since pica was observed commonly in the terminal stages of the disease. This may also have been a cause of irregular black areas which were sometimes found on the abomasal folds in terminal cases (Fig. 14). In more longstanding infections, i.e., sub-acute haemonchosis, circular ulcerations 1-2 mm in diameter were found scattered over the body and folds of the

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abomasum in addition to the petechiae described above. The former had the appearance of small "pin pricks" or punctures in the mucosa (Fig. 15). At what appeared to be a later stage these became surrounded by circular areas of hyperplastic mucosa from 2-4 mm in diameter (Fig. 16). Eventually the central pin prick disappeared leaving conspicuous white raised lesions scattered over the mucosa (Fig. 17). In addition a generalised hyperplastic reaction of the abomasal mucosa was often seen which produced an irregular wavy pattern on the mucosal surface (Fig. 18).

Frequently, ewes which had a record of sustained high faecal egg counts until a few days before autopsy were found to contain few or no adult parasites at postmortem. In such cases the abomasum was usually very congested and oedematous, contained a small volume of clear mucilaginous fluid and often a small volume of coarse green ingesta. The mucosal surface was very slimy to the touch but was readily washed clean in tap water. Any adult H. contortus still present in the abomasum were dark coloured and slow moving. Examination of the duodenum and small intestine revealed the presence of moribund H. contortus in most cases. A point of some diagnostic importance when examining the ocular conjunctivae of sheep which have died a few hours previously is illustrated in Figs. 19 and 20 and is self-explanatory.

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Haematology

As described in Chapter 2 the mean values for haematocrit and haemoglobin during acute haemonchosis were 20% and 5 g respectively although great individual variation was experienced depending on the severity of the disease. Thus in 6 of the 20 ewes which suffered fatal infections the terminal PCV values ranged from 8% to 21% and Hb from 2 to 6g. Figures for the lambs suffering fatal infections were usually somewhat higher.

Bone Marrow

Figs. 21 and 22 show two stages of erythropoietic response in acute haemonchosis. A normal femur is shown for comparison (Fig. 23).

In the normal femur, erythropoiesis is confined to the proximal and distal extremities of the medullary cavity and the intervening marrow is pinkish white in colour. During the initial response to heavy H. contortus infection the red marrow extended some distance along the medullary cavity (Fig. 21). When the syndrome was established the white marrow disappeared completely and was replaced by cellular red marrow throughout; frequently this also involved the epiphyses (Fig. 22).

Faecal Egg Counts

The onset of the clinical signs of acute haemonchosis was usually associated with high faecal egg counts which ranged in the ewes between 20,000 and 100,000 epg and in the lambs between 5,000 and 50,000 epg. As the disease progressed the faecal egg counts of the surviving animals showed great individual variation. As mentioned earlier low faecal egg counts were frequently observed in moribund animals while in others the egg count remained high until death suddenly supervened. In the survivors the faecal egg count gradually decreased i.e., to about 2,000 epg over a period of 2-3 months.

Worm burdens

In the ewes, worm burdens of fatal cases usually ranged between 5,000 and 10,000 worms, mostly adults with a proportion of fifth stage larvae; in the lambs 1,000 to 5,000 were usually recovered. In the ewes in which terminal expulsion of the worm burden occurred there were, of course, few or no adult worms. Abomasal digests usually revealed several hundred fourth larval stages.

HYPERACUTE HAEMONCHOSIS

Epidemiological Background

This syndrome occurred on a single occasion, i.e., in June 1971. During this period the second highest level of soil surface moisture index was recorded and larval intake as judged by worm burdens in tracer sheep reached its maximum.

Clinical Signs

This syndrome was observed in both ewes and lambs and was characterised by almost immediate death. In all, 5 ewes and 4 lambs succumbed. Sheep which were in good condition suddenly became lethargic and, following progressive weakness, died within 1 or 2 days. Prior to death the ewes and lambs showed slight oedema, hyperpnoea and increasingly pale mucous membranes (Fig. 24). They were otherwise in good condition and had no history of inappetence or pyrexia.

Gross Pathology

At post-mortem the carcase was usually in good condition but generally pale. On opening the abdomen the omental fat had a normal pinkish-white colour and consistency. Little ascitic fluid was present. The adrenals were grossly enlarged and oedematous. The spleen was small and shrunken with a white wrinkled capsule; on section it was a very pale pinkish colour with conspicuous white interstitial tissue. The abomasum contained very large numbers of immature or young adult H. contortus, which often made up the larger part of the contents and were usually seen as a writhing dark reddish coloured mass. Whole blood and lumps of dark brown blood were commonly found (Fig. 25). The pH of the abomasal fluid ranged between 5.5 and 7.5.

On gentle removal of the abomasal ingesta the mucosa was seen to be covered in multiple red petechiae which, when numerous, converged to produce a single large erythematous area. The abomasal mucosa had a generally light orange or salmon-pink colour. Sub-mucosal oedema was usually evident. The mucosa was not thickened and in severe cases the mucosal surface was almost entirely eroded. this was apparently due to the coalescence of the punctate lesions caused by the feeding habits of the parasites which could only be seen

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grossly by a careful examination of the mucosa held in reflected light when a finely stippled surface became evident (Fig. 26).

Haematology

In the sheep which died of this syndrome the PCV values and Hb concentrations were similar to those of acute haemonchosis.

Bone Marrow

Femoral sections were similar to those illustrated in Fig. 21; the apparent absence of erythroid response is presumably due to the rapidly fatal outcome.

Faecal Egg Counts

Although counts of as high as 400,000 epg were recorded in some sheep, in others the counts ranged from a few hundred to a few thousand epg.

Worm Burdens

These ranged between 6,000 and 35,000 worms, almost entirely composed of young adults.

CHRONIC HAEMONCHOSIS

Epidemiological Background

The initial observations which led to the recognition of this syndrome were not made on the commercial flock, but on a small group of sheep, also naturally infected with *H. contortus*, grazing in an adjacent field. These sheep

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were part of an experiment described in Chapter 6 designed to determine the basis of self-cure under field conditions. The design of the experiment was such that sheep with a light infection of H. contortus were grazed together with worm-free sheep for a period of several months. To prevent infection of the latter the entire group were folded daily over worm-free pasture of a similar quality to that of the commercial flock.

During three of these months, i.e., February, March, April 1971, the larval intake of the commercial flock was negligible as judged by the worm burdens of the tracer sheep, the weather was generally hot and dry and the soil-surface moisture index was the lowest recorded during the period of observations. Because of this the pasture quality gradually deteriorated, becoming dried and brown, although always in ample supply. Towards the beginning of March 1971, it was observed that the bodily condition of the group of sheep lightly infected with H. contortus and the ewes and lambs in the commercial flock had started to deteriorate while that of the worm-free sheep was unaffected. It was therefore apparent that the loss of condition was not due to poor quality pasture but was evidently associated with H. contortus infection.

Clinical signs

The syndrome was not one normally associated with clinical haemonchosis and was characterised by a progressive loss in weight over a period of two months leading in the adults to emaciation (Figs. 27 and 28) and in the lambs to marked stunting of growth (Fig. 29). All of the sheep were affected to some degree and ultimately eight ewes and five lambs became moribund and died. Pale mucous membranes indicative of severe anaemia were only a terminal feature of the disease, although haematological values were in fact always below normal (see Chapter 2). Transient submandibular oedema appeared intermittently. Loss of wool was not a feature of this syndrome. As the condition progressed the sheep became increasingly weaker, walking with a swaying gait and standing with legs crossed or splayed (Fig. 30).

Terminally there was extreme weakness and inability to rise, although even at this stage animals which were sternally recumbent continued grazing on the surrounding pasture or on cut pasture brought to them (Fig. 31). Pica was common. In contrast to the infected sheep the worm-free group continued to thrive during these three months of relative drought, to increase in weight and to remain free from clinical signs of disease (see Figs. 28 and 29).

Gross Pathology

At post-mortem each affected carcase was pale, emaciated and slightly oedematous and subcutaneous fat was found to have been largely replaced by clear gelatinous tissue. On opening the abdomen, the omentum was a translucent pinkish colour and consisted of clear gelatinous tissue (Fig. 32). The abdominal organs were pale and the spleen was shrunken with a wrinkled whitish capsule. Little ascitic fluid was present in the abdomen.

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The gross pathological lesions in the abomasum varied according to the length and severity of the infection but evidence of mucosal hyperplasia was a constant finding. This was characterised by an opacity and thickening of the abomasal folds to 2 to 3 times the normal, with a widespread roughening of the mucosal surface (Fig. 33) resembling the "Morocco leather" of O. ostertagi infections in calves described by Anderson et al (1965). This was such that the blood vessels of the underlying muscularis were not visible as was observed in the abomasum of sheep reared parasite-free (Fig. 34). In chronic disease of long duration there was often gross distortion of the free edges of the abomasal folds.(Fig. 35). The abomasal contents had an elevated pH (5.0 to 7.4), were usually dark green in colour and contained coarse ingests.

Haematology

Despite the moderate infections (see below) encountered in this syndrome the PCV and Hb values were relatively low i.e., a mean of 18% and 7 g respectively. Latterly the severely affected sheep, thirteen in number, showed further significant drops in these indices which fell to as low as 6% and 2 g respectively.

Bone Marrow

A typical section of a femur from a case of chronic haemonchosis is illustrated in Fig. 36. The red marrow is confined to two abnormally small areas at the proximal and distal ends of the medullary cavity. The typical white marrow has disappeared, being replaced by a yellow gelatinous substance. The medullary shaft has thinning and irregularities of its inner surface due to absorption while the medullary bone adjacent to the epiphyses shows rarefaction.

Fig. 37 illustrates a femur from a rather more complex case of chronic haemonchosis. Following chronic infection this animal survived until the onset of the rains when the faecal egg count suddenly dropped to zero i.e., self-cure occurred. It then seemed to recover and was autopsied a few weeks later. Examination of the femur still showed some of the features typical of chronic haemonchosis, i.e., disappearance of red marrow at the distal epiphysis associated with absorption of the medullary bone and thinning of the cortical bone. However, recovery is indicated by the presence of abundant red marrow at the proximal epiphysis and atypically localised in an area in the shaft and by the presence of normal white marrow.

Faecal Egg Counts

These ranged within normal limits for a flock grazing in an endemic area i.e. up to 2,000 epg.

Worm Burdens

Estimates of worm burdens were obtained both from sheep dying *in extremis* and from clinically affected sheep selected for autopsy. Worm burdens ranged from a maximum of 1,000 adults down to as few as 100; almost all of these were adults.

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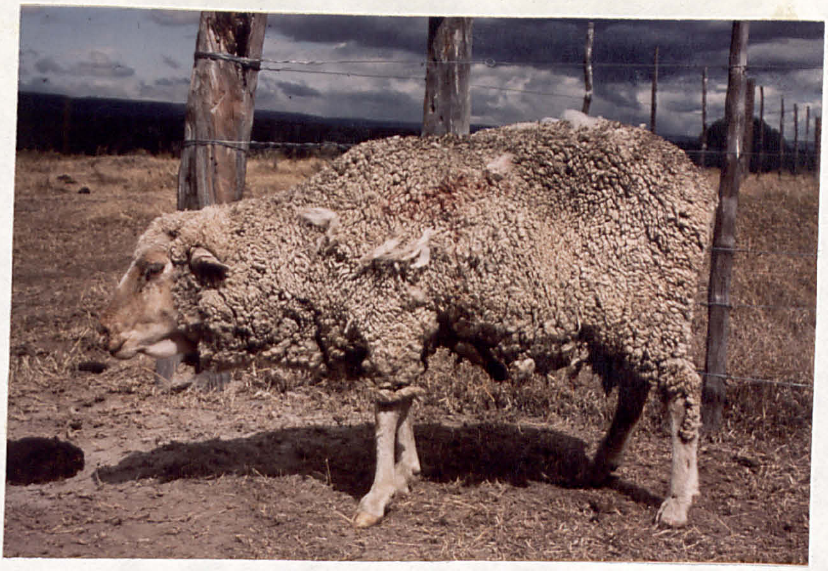

Fig. 7. An adult Merino ewe with classical symptoms of acute haemonchosis; pale mucous membranes and submandibular oedema ("bottle jaw") and ascites. Lethargy, falling wool, loss in weight and agalactia are also characteristic.

Fig. 8. Merino ewe with severe acute haemonchosis. Note the extensive broadening of the face with oedema.

Fig. 9. Acute haemonchosis in a Merino lamb aged 2 months. Facial and submandibular oedema, pale mucous membranes and failure to thrive are characteristic symptoms.

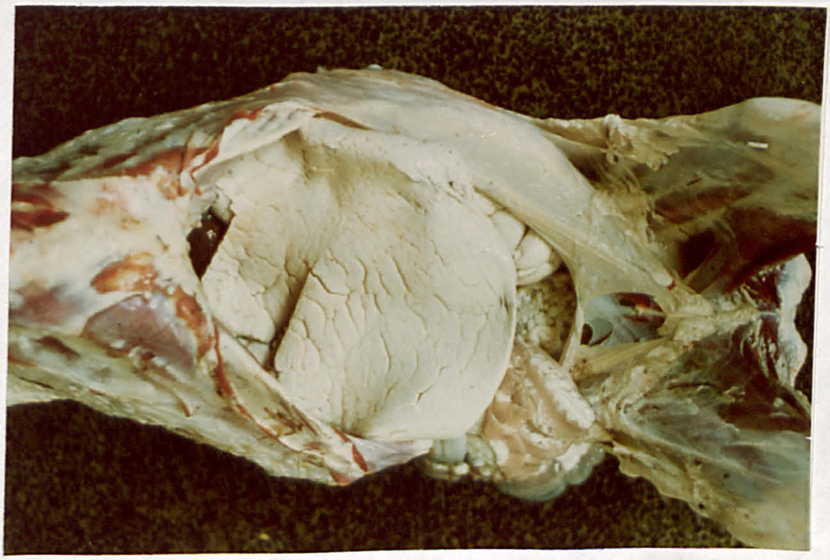

Fig. 10. The thickened white solid omentum characteristic of terminal acute haemonchosis.

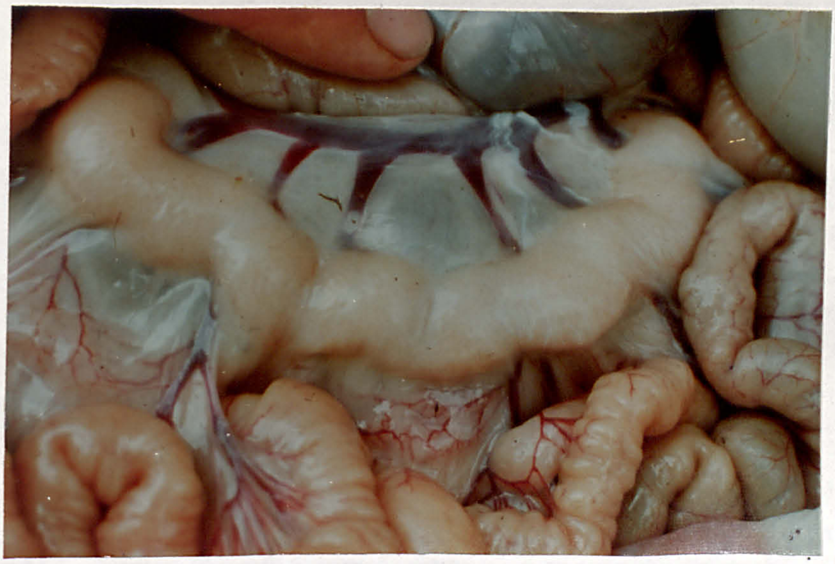

Fig. 11. Marked enlargement of mesenteric lymph nodes in acute haemonchosis in a Merino lamb. The abomasal lymph nodes are usually also enlarged.

Fig. 12. Acute haemonchosis. Typical findings in the thorax at post-mortem are large volumes of serous effusions in the pleura and pericardium; the heart is enlarged, pale and flabby. Note evidence of increased erythropoiesis in sternal marrow.

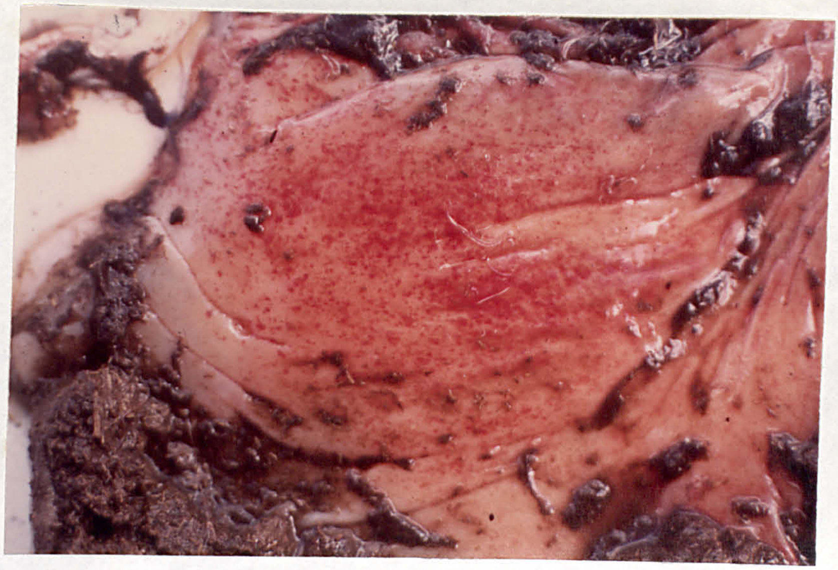

Fig. 13. Acute haemonchosis. The gross appearance of the abomasal mucosal surface at post-mortem. Numerous small, circular petechiae have coalesced to form large erythematous areas. Adult parasites are visible on the mucosa. The abomasal folds are extensively thickened as a result of submucosal oedema.

Fig. 14. Acute haemonchosis. Conspicuous, irregular dark coloured areas are sometimes found in the folds and pyloric region of the abomasal mucosa.

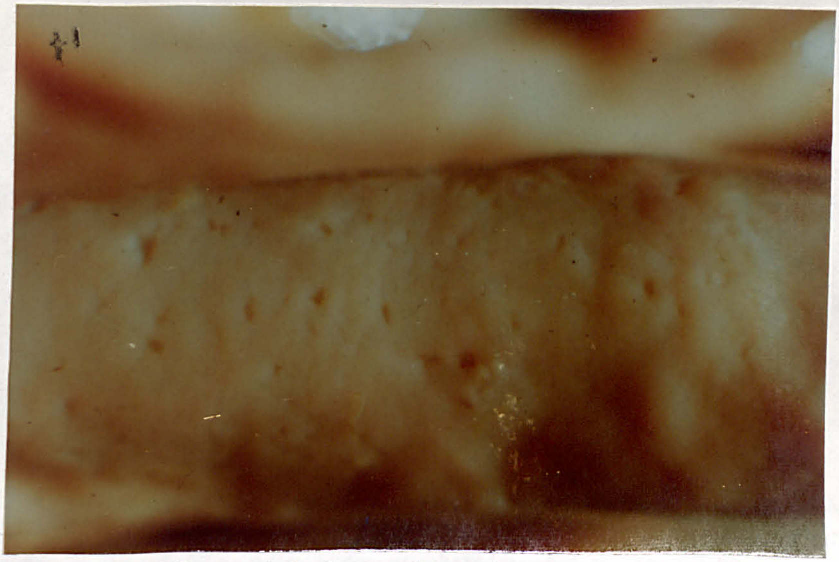

Fig. 15. Mucosal surface with irregular ulcerated lesions presumably caused by the feeding activities of the developing adult parasites.

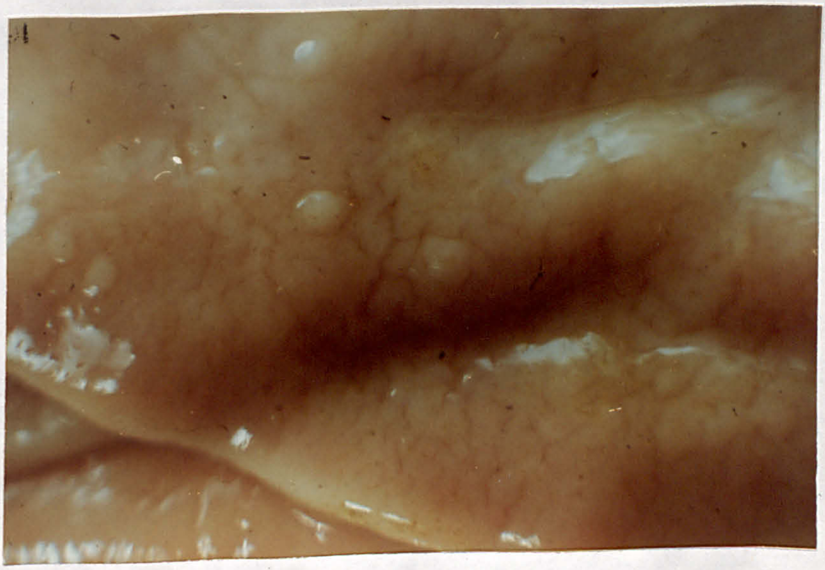

Fig. 16. Circular hyperplastic mucosal lesions. These appear to result from the lesions shown in Fig. 15.

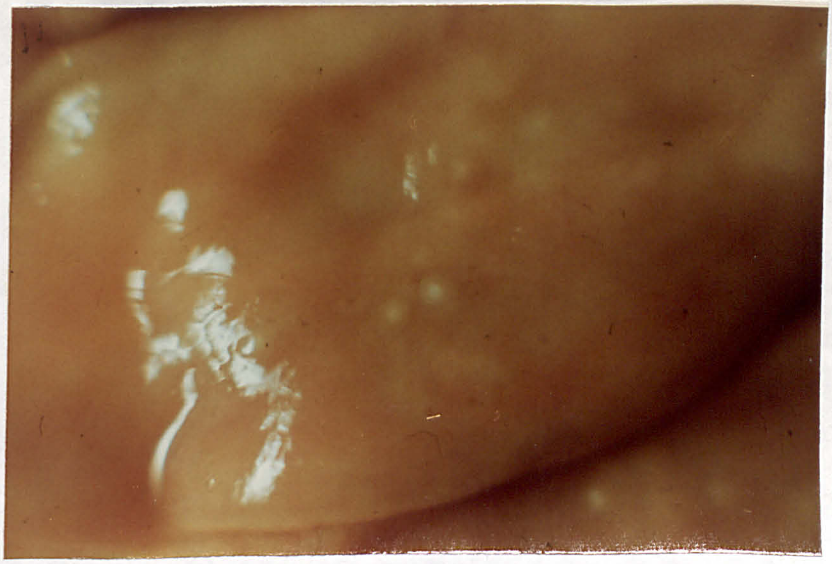

Fig. 17. White raised lesions apparently the end result of the process shown in Figs. 15 and 16.

Fig. 18. Thickened hyperplastic abomasal mucosa characteristic of sub-acute haemonchosis.

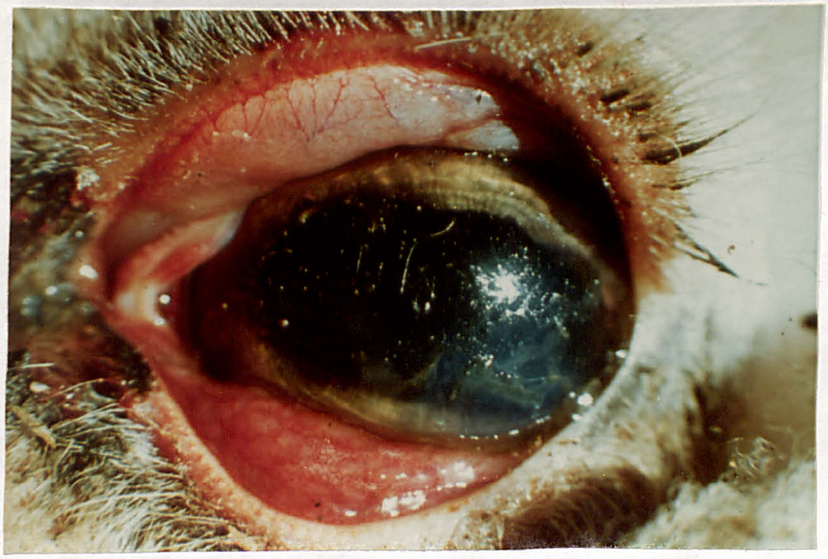

Fig. 19. Acute haemonchosis; ocular mucous membranes on the lower side of the carcase of a Merino ewe twelve hours after death.

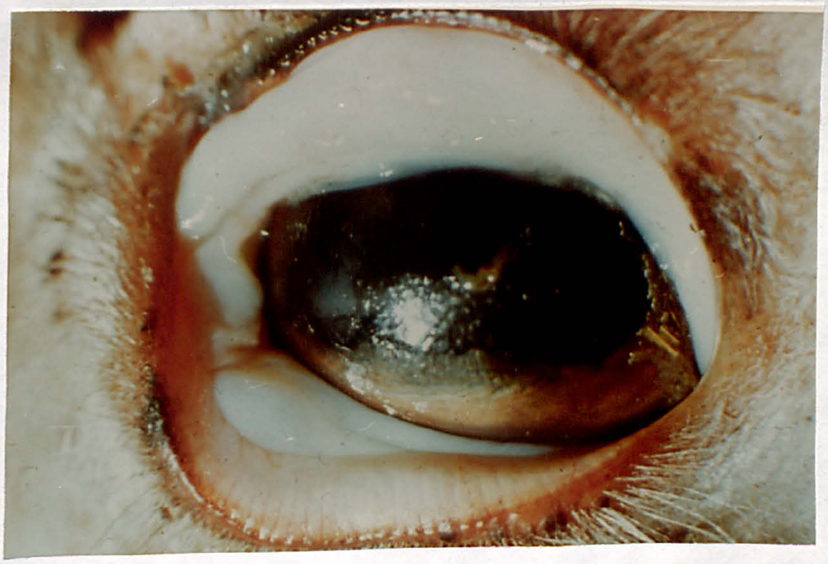

Fig. 20. Acute haemonchosis; ocular mucous membranes on the upper side of the same Merino ewe twelve hours after death.

Fig. 21. Acute haemonchosis; note the initial expansion of the red marrow along the medullary cavity in response to the stimulus of blood loss caused by haemonchosis.

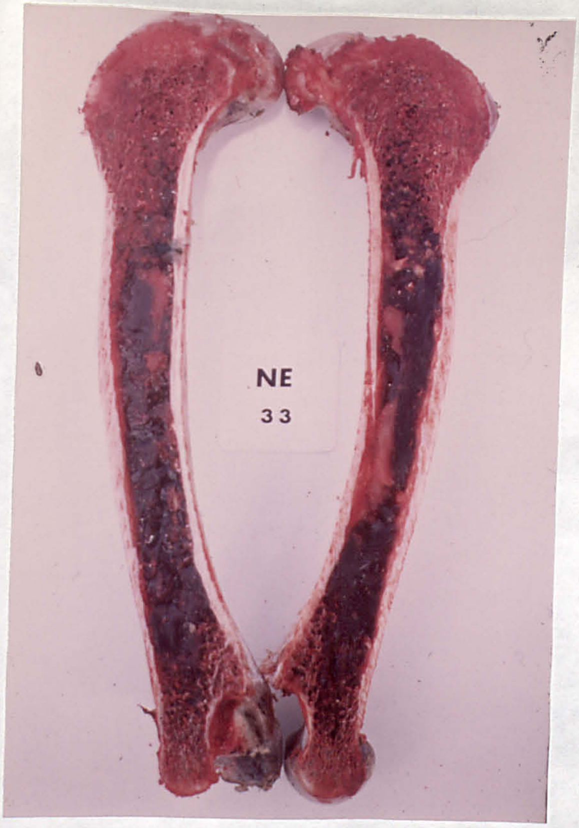

Fig. 22. Acute haemonchosis; note the maximal expansion of the red marrow throughout the entire femoral cavity.

Fig. 23. A femur from a worm-free ewe sectioned longitudinally to show the normal distribution of red marrow.

Fig. 24. Hyperacute haemonchosis in a Merino ewe. Severe anaemia, slight facial oedema and lethargy appearing within a few days in a healthy sheep and a rapidly fatal outcome are the diagnostic features of this syndrome.

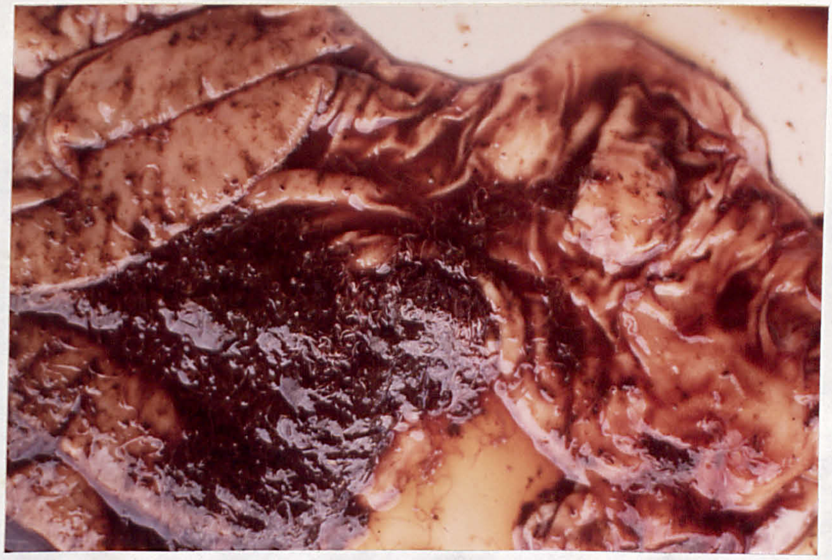

Fig. 25. The post-mortem appearance of the abomasal mucosa and contents of a Merino ewe which died of hyperacute haemonchosis. A large mass of young adult parasites can be seen amongst the abomasal contents which consist largely of a mass of dark coloured clotted blood and reddish whole blood. Extensive submucosal oedema is also present.

Fig. 26. The gross appearance of the mucosal surface of the abomasum of a Merino ewe which succumbed to hyperacute haemonchosis. The extensive multiple erosions on the surface can be seen grossly only by careful examination against reflected light.

Fig. 27. The clinical appearance of a Merino ewe with the "chronic haemonchosis" syndrome. Progressive loss of weight, weakness and lethargy are characteristic.

Fig. 28. The clinical effect of "chronic haemonchosis" in a Merino ewe (left) in comparison to a non-infected control ewe (right), initially comparable in weight, size and age and subsequently grazed together over parasite-free pasture.

Fig. 29. The clinical effect of chronic infection with low numbers of H. contortus in a Merino lamb when pasture conditions were poor. Both lambs are 6 months old and were grazed together on parasite-free pasture for 4 months, only the nearer lamb carrying a low level chronic infection.

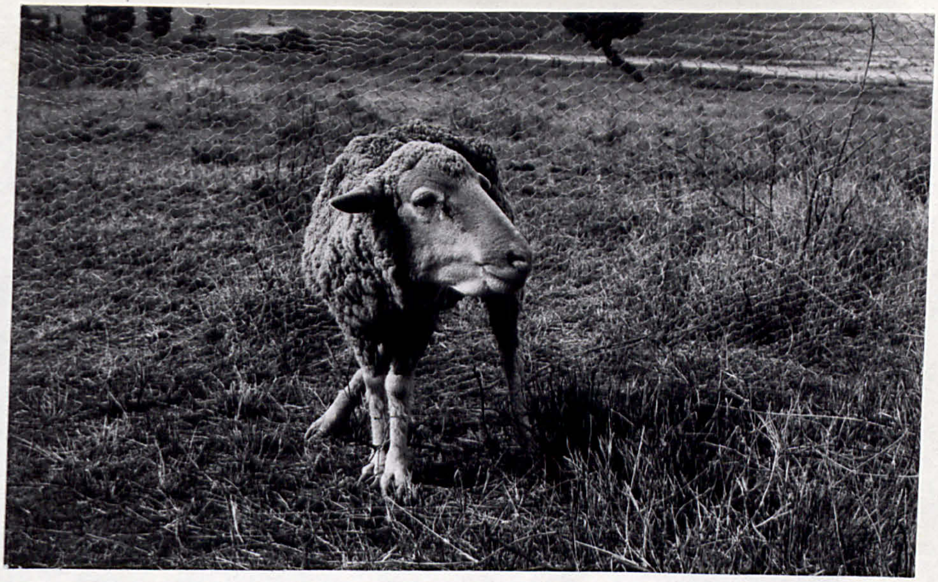

Fig. 30. The later clinical stages of chronic haemonchosis in a Merino ewe showing the characteristic splay-legged stance. Slow movement, lethargy and transient slight submandibular oedema are also characteristic of this stage. The mucous membranes are not markedly pale.

Fig. 31. The clinical picture of terminal chronic haemonchosis in a Merino ewe characterised by extreme loss of weight, great weakness, pica and eventually anorexia.

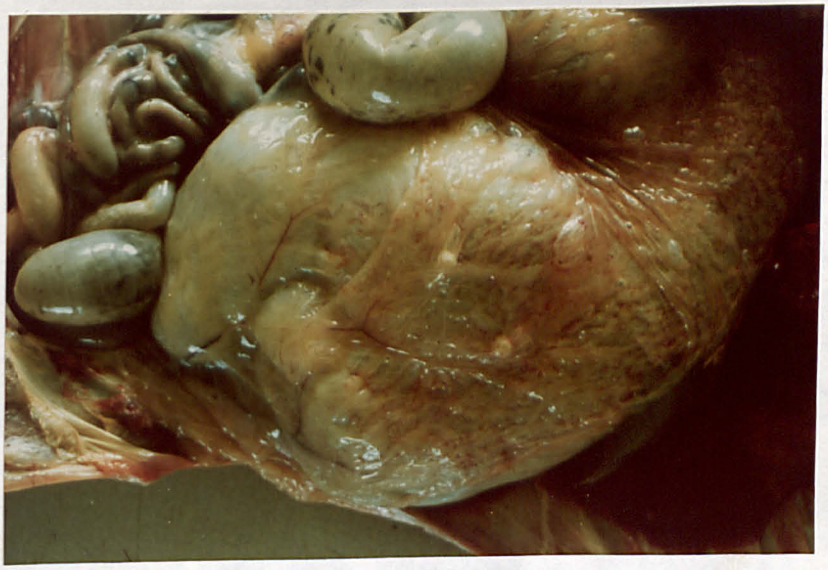

Fig. 32. The characteristic post-mortem appearance of the abdominal organs of a Merino ewe with chronic haemonchosis. The carcase is emaciated, pale and oedematous and the abdominal fat is replaced by pinkish, translucent gelatinous tissue.

Fig. 33. The irregular mucosal surface of an abomasal fold of a Merino ewe following chronic haemonchosis. The widespread hyperplasia has resulted in a roughening of the mucosal surface, an increase in fold thickness and an elevated abomasal pH.

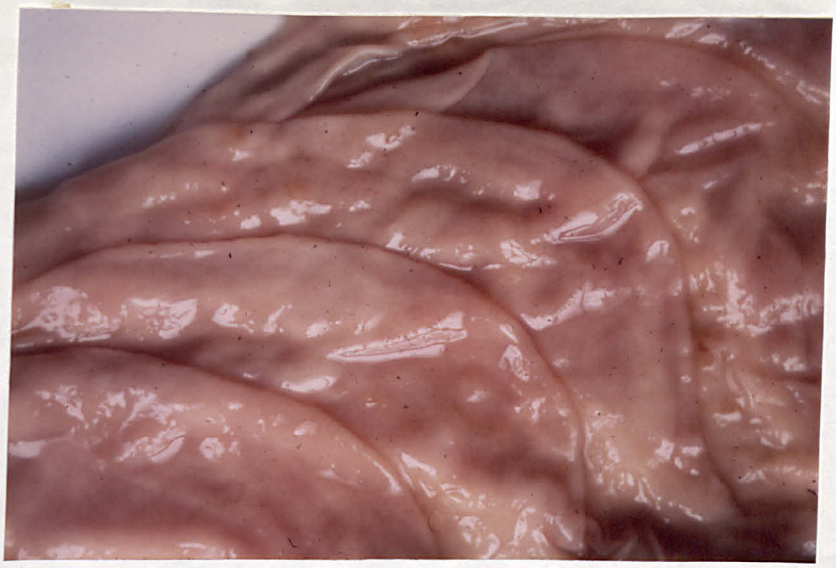

Fig. 34. The abomasal fold of a Merino ewe reared under parasite-free conditions since birth. The colour, translucent appearance and smooth texture of the abomasal mucosa is characteristic of parasite-naive sheep. The generalised deposit of white fat in the submucosal region can be seen through the thin translucent mucosa.

Fig. 35. The free edge of an abomasal fold grossly distorted following naturally acquired chronic infection with H. contortus. The abomasal folds are markedly thickened (3-4 mm) and noticeably opaque in appearance.

Fig. 36. This femur, from a case of chronic haemonchosis shows almost complete disappearance of red marrow while the white marrow is replaced by a gelatinous substance. The medullary shaft is thin and irregular on its inner surface and the medullary bone adjacent to the epiphyses is rarefied.

Fig. 37. A femur showing the recovery of red marrow activity following self-cure after chronic haemonchosis. Note the irregular distribution of the red marrow, the absorption of the medullary bone in the epiphyses and the thinning of the cortical bone.

DISCUSSION

Haemonchosis is usually described in text books (Marsh, 1958; Monnig, 1962; Blood & Henderson, 1963) as an acute or occasionally hyperacute syndrome characterised by anaemia, oedema and death and the reader is generally left to assume that in the absence of these features H. contortus is of little consequence. The results of this study have indicated that this is far from the case and that H. contortus infection in an endemic area is a phenomenon of almost continuous significance and great complexity.

Generally speaking, the results showed that clinically significant haemonchosis could be conveniently described under the three headings of acute, hyperacute and chronic syndromes, the main features of which are summarised in Tables 3 and 4. Although each of these was usually recognised as a distinct clinical entity there were considerable individual variations in severity and occasional cases occurred which shared some of the features of two syndromes.

As described in Chapter 2, acute haemonchosis was only observed on two of the four occasions when it might have been anticipated, i.e., periods of heavy rainfall and temperatures above 18°C (65°F). The possible reason for this i.e., the level of the soil surface moisture index, is discussed in Chapter 3. The clinical signs found were generally similar to those recorded by other workers, although four points merit discussion. First, facial oedema occurred frequently and was often more easily recognised than submandibular oedema in individuals among a flock. Second, the characteristic appearance of the omentum as a sheet of hard white fat in almost every fatal case has not, to my knowledge, been previously described. The pathogenesis of this is unknown. Third, it was not uncommon to find that in ewes which had died of acute haemonchosis few or no adult worms were found in the abomasum although examination of the small intestine usually revealed the presence of moribund parasites. Whether this was due to the raised pH of the abomasal fluid or to anoxia caused by a combination of abomasal oedema, anaemia and cardiac failure or to some other cause is unknown. Finally, contrary to some reports (see review by Soulsby 1965) diarrhoea was not a feature of acute haemonchosis at any time, the only abnormality observed being the darkened colour and rouleaux formation of the faeces. The only time diarrhoea was observed in sheep infected with H. contortus was during the full expression of self-cure as described in Chapter 6.

Hyperacute haemonchosis was usually readily differentiated from the acute disease by the sudden lethargy and death within a few days of sheep which were apparently previously normal. This syndrome was only observed on one occasion and was associated with high rainfall and, possibly more significantly, the second highest soil surface moisture index

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recorded during the observations.) Although peak egg counts of up to 400,000 epg were recorded with this syndrome, this was not always so and counts of several hundred epg were occasionally found. Presumably the low egg count was associated with the presence of a high worm burden composed largely of fifth stage larvae or young adults. It is perhaps worth noting that the omental fat was not altered as in the acute haemonchosis syndrome and that the abomasal mucosa was not thickened and on superficial examination appeared relatively normal. Only close examination in reflected light revealed the characteristically confluent eroded surface.

The third syndrome referred to as chronic haemonchosis does not appear to have been previously described and only came to light in this study because a group of continuously worm-free sheep were grazed together with infected sheep during the time over which the syndrome developed. Otherwise, because of the relatively modest worm burdens of the affected sheep and the negative intake of the tracers, the marked loss in condition would almost certainly have been ascribed to malnutrition since the pasture at this time was of very low quality (see Chapter 2). In contrast to the two previous syndromes the clinical signs developed insidiously over a period of two to three months. For most of this period anaemia and oedema were not prominent clinical features and the characteristic clinical signs in ewes were those of gradual loss of weight leading ultimately to emaciation; in lambs a marked stunting of growth was observed. Since from the results of serial autopsies the moderate burdens of around 500 adult worms evidently remained static over this period, the explanation for this syndrome, in view of the performance of the worm-free sheep, is almost certainly associated with the deteriorating quality of the pasture. Presumably, the nutritional value of the pasture although just adequate for maintenance of ewes and modest growth of lambs was not sufficient to support in addition the accelerated rate of erythropoiesis and the synthesis of plasma proteins in the face of chronic blood loss (Dargie and Allonby, in preparation). The maintenance of these processes, at levels necessary to ensure survival of the host, presumably occurred at the expense of increased body weight and normal growth. Ultimately, when the animals had become cachectic, the PCV values dropped sharply and the animals died within a week or so. These preliminary observations on chronic haemonchosis suggest that the syndrome may be extremely significant in commercial flocks where similar grazing conditions are experienced and should be the subject of further study.

As mentioned above there were occasions where the affected sheep could not be placed into any of the three categories, but shared some of the features of two of these. This typically occurred on two occasions. First when moderate worm burdens were being acquired whilst grazing on pasture of good quality, e.g. the period from March to July 1970. During this time the sheep remained in relatively good physical condition and anaemia and oedema were not apparent on superficial clinical examination. Nevertheless, the PCV and Hb values gradually fell from 30% to 22% and from 11 gm to 8 g respectively. The haematological parameters resemble those recorded during the development of chronic haemonchosis but because of good pasture conditions the weight loss characteristic of the latter did not occur. The second occasion occurred as a sequel to the outbreak of acute haemonchosis experienced in August and September 1970. During the ensuing two months the worm burdens of the surviving sheep, as shown by the random autopsies, dropped considerably, i.e., from 11,000 to 590 adults. However, there was no improvement in haematological indices and the syndrome could only be regarded as one of sheep on the threshold of chronic haemonchosis.

These observations indicate that the haematological indices and physical condition of a sheep infected with H. contortus at any one point in time are not merely a consequence of the worm burden at that time, but are a reflection of the larval intake, worm burden and pasture conditions over the preceding few months.

Perhaps one of the most useful parameters in diagnosis, in that it provides some of this otherwise unobtainable history, is the information obtained by longitudinal section and visual examination of the femoral bone marrow. Thus, acute haemonchosis was associated with characteristic

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changes in the distribution and extent of the red marrow leading eventually to a complete replacement of the white marrow. In hyperacute haemonchosis because of the short course of the disease only the initial stages of this erythropoietic hyperplasia were seen. The femoral marrow picture presented by chronic haemonchosis was quite different, i.e., scanty red marrow, the replacement of the red marrow by a yellow gelatinous substance, rarefaction and absorption of the medullary bone combined with thinning of the shaft and pitting of its inner surface. The diagnostic use of this technique, in combination with other criteria, is summarised in Tables 3 and 4.

TABLE 3

A summary of the incidence, aetiology and clinical findings of the three syndromes of ovine haemonchosis in East Africa.

	Hyperacute	Acute	Chronic
Incidence	Uncommon	Common	Widespread
Duration	0 - 7 days	1 - 6 weeks	2 - 6 months
Aetiology	A sudden massive challenge of infective larvae	Prevailing burden of adult parasites with continued reinfection	A relatively low burden of adults without necessarily any reinfection.
Associated Climate	Warm, humid	Warm, intermittent rain	Independent of climate but effects are worse in dry weather when pasture is of poor quality.
Morbidity	Low	Medium - high	Very high
Pathogenesis	Severe haemorrhagic gastritis and rapidly fatal severe anaemia	Acute gastritis leading to anaemia, hypoproteinaemia and generalised oedema	Chronic parasitic gastritis with chronic blood loss and abomasal dysfunction leading to progressive weight loss and stunting of growth.
Clinical Signs	Sudden death in previously healthy sheep. Severe anaemia and dark-coloured faeces are main signs prior to death. No diarrhoea	Pale mucous membranes, generalised oedema, especially "bottle-jaw" in sheep in poor condition. Lethargy, falling wool and dark-coloured faeces are also common. No diarrhoea	Progressive insidious loss of weight resembling malnutrition. No gross anaemia or oedema making diagnosis difficult in the absence of non-infected controls; ultimately extreme weakness and anorexia. No diarrhoea.
Outcome	Sudden death in previously healthy sheep	Agalactia leading to death of lambs; weakness and loss in condition in ewes which is often fatal. Self-cure may give temporary alleviation at any stage of infection	Outcome depends largely on nutritional status of host. Loss of production on good pastures; severe loss of production on poor pastures. Many deaths during extended spell of dry weather. Self-cure may alleviate condition even after the sheep has become recumbent.

TABLE 4

Summary of the post-mortem findings in the three common syndromes of ovine haemonchosis in East Africa.

	Hyperacute	Acute	Chronic
Carcase	Few gross changes	Thin, pale with generalised oedema	Pale carcase with generalised emaciation; little oedematous changes.
Abomasum	Large numbers of immature or young adult parasites present. Multiple petechiae and wide-spread erosion of mucosa	Progressive series of lesions originating from the continued feeding activities of parasites and the host tissue reaction: mucosal lesions and sub-mucosal oedema dependent on length and severity of infection	Generalised hyperplastic metaplasia of abomasal mucosa leading to increased thickness and opacity of abomasal folds; this results in increased abomasal pH.
Bone Marrow	Evidence of initial expansion of red marrow in most cases	Marked expansion of red marrow throughout medullary cavity and eventually into epiphyses	Evidence of chronic expansion of red marrow through absorption of cancellous and cortical bone. Reversion to secondary white marrow when iron deficiency is marked.
Number of parasites in abomasum	10,000-35,000 L_5 or immature adults	1,000-10,000 adults $\pm$ immature adults and larval stages	100-1,000 adults $\pm$ immature adults.
Total blood loss over period of infection	7 days at 200-600 ml/day	7-40 days at 50-200 ml/day	40-100 days at 5-50 ml/day.
Serum iron levels at post-mortem	Normal	Low	Very low.
Nature of blood loss*	Young adults ingest 0.05 ml/day, loss from damage caused by developing larvae and frank blood loss from grossly damaged abomasal mucosa	Adults ingest 0.05 ml/day, loss from damage caused by developing larvae and seepage of blood from petechiae caused by feeding activities of the adults	Adults ingest >0.05 ml/day because of feeding activities of small numbers of adults which develop to greater individual size.

* Dargie, J.D. and Allonby, E.W. (1974). Inter. J. Parasitol. In press.

SUMMARY

This chapter describes the clinical findings and gross pathology observed during an epidemiological study of endemic haemonchosis over a period of two years in a set-stocked flock of Merino sheep.

The results showed that H. contortus infection of sheep in an endemic area is a phenomenon of almost continuous significance and great complexity. Generally speaking the clinical syndromes of haemonchosis were recognised as three forms i.e., hyperacute, acute and chronic.

The observations of the clinical signs of hyperacute and acute haemonchosis although generally similar to those recorded by other workers i.e., anaemia and oedema, revealed some new features of diagnostic importance.

The third syndrome i.e., chronic haemonchosis, does not appear to have been specifically described before. Essentially it is characterised by a marked loss of weight in ewes and severe stunting of growth in lambs rather than as an acute haemorrhagic event. It is associated with modest worm burdens of around 500 adult worms in sheep grazing on pasture of poor nutritional value and only the fortuitous presence of healthy sheep, maintained entirely worm-free on the same pasture, revealed the true significance of the H. contortus infection; otherwise, the syndrome of progressive weight loss, not normally associated with haemonchosis, would have been ascribed to malnutrition.

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It was found that a particularly useful parameter in diagnosis was the gross examination of the femoral bone marrow in that it reflected the larval intake, worm burden and pasture conditions over the preceding few months.

CHAPTER 6

STUDIES OF THE SELF-CURE OF *H. CONTORTUS* INFECTIONS UNDER FIELD CONDITIONS.

INTRODUCTION

"Self-cure" is currently one of the most contentious of helminthological topics. The term was first introduced by Stoll (1929) to describe a sudden dramatic fall in faecal egg counts which occurred in two lambs subjected to continuous natural reinfection with Haemonchus contortus larvae from pasture. The great epidemiological significance of the phenomenon was subsequently shown by Gordon (1948) who demonstrated that self-cure occurred regularly and consistently in entire flocks of sheep grazing in haemonchosis-endemic areas in Australia. Gordon also observed that not only was self-cure associated with a dramatic fall in faecal egg counts but that it was also associated with the expulsion of the adult H. contortus burdens of the affected sheep. The occurrence of self-cure was generally recorded once or twice yearly and invariably occurred shortly after the onset of a period of rainfall which led Gordon to suggest that the phenomenon was possibly attributable to an ' anthelmintic factor' in freshly growing pasture. In a later series of experiments however, Stewart (1950a, b, 1953) showed that an experimental challenge with H. contortus frequently produced a similar fall in faecal egg count and the expulsion of a pre-existing adult infection. From these observations Stewart concluded that the mechanism of self-cure depended on an immediate-type hypersensitivity reaction in the abomasal mucosa triggered by the antigenic stimulus of the

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newly acquired larvae. It has been subsequently assumed that self-cure as a flock phenomenon depended on a similar mechanism and that the significance of the rainfall was that large numbers of infective larvae became available to the sheep.

There are however, certain features of the naturally occurring flock self-cure which are inconsistent with an immunological explanation. Self-cure occurs at exactly the same point in time in mature ewes and young lambs, is expressed equally in sheep with high or low adult worm burdens and is not usually followed by effective resistance to reinfection (Gordon, 1948, 1967; Lopez and Urquhart, 1967). In view of these apparent anomalies surrounding the naturally occurring self-cure phenomenon as it occurs in endemic areas, an investigation into its possible aetiology was undertaken. The primary object of the following three experiments was to determine whether the fresh acquisition of infective larvae was required in order to initiate self-cure or whether climatic or pastoral changes were in themselves a sufficient stimulus.

MATERIALS AND METHODS

Types of Experimental Animals

The following three experiments were performed at the National Husbandry Research Station, Naivasha, the location and environmental conditions of which have been described earlier in Chapter 2.

The sheep were pure-bred Merinos reared on the station and divided into three categories 'permanent', 'experimental' and 'tracer'. The 'permanent' sheep were the adult ewes of the resident breeding flock described in Chapter 2 and were set-stocked on permanent pastures known to be endemic for haemonchosis.

The 'experimental' sheep were two-year old ewes which were withdrawn from the permanent flock 6 months before the start of each experiment. These sheep were dosed with thiabendazole at 80 mg/Kg and subsequently maintained indoors during which time the anthelmintic treatment was repeated several times. Their parasite-free status was checked by regular faecal examinations and confirmed by the autopsy of 2 tracers at the start of each experiment.

The 'tracer' sheep were born and reared under parasite-free conditions until approximately 18 months old. Again their parasite-free status was confirmed by regular faecal examination and sample autopsies.

Experimental Techniques.

The *Haemonchus contortus* eggs from which the larvae were obtained for the experimental infections were intially recovered by the maceration of adult *H. contortus* obtained locally. Faecal culture and the recovery of infective larvae were performed by the methods of Roberts and O'Sullivan (1950) and the strain subsequently passaged in sheep which had been born and reared in worm-free conditions indoors.

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Faecal samples were collected individually from the rectum and the faecal egg count estimated by the modified McMaster method (Whitlock, 1948). During experiment 3 the faecal samples were examined grossly and given a "consistency value" according to a scale drawn up from 1 (liquid) to 10 (hard) to give an approximate indication of the water content. The sheep were clinically examined at the time of faeces collection for evidence of diarrhoea from the condition of the perineum and fleece around the hind-quarters.

Experimental Design and Results

Three experiments were carried out. The first two were of a preliminary nature and the third was a more detailed study.

Experiment 1

Four groups of three experimental sheep were individually infected with 10,000 infective larvae as shown in Table 5.

The first group were set-stocked on a small permanent pasture and were therefore exposed to reinfection. The remaining three groups were fitted with faecal bags and folded every third day over parasite-free pasture in order to avoid reinfection. The faecal egg counts of each sheep were determined daily and the mean results are shown in Fig. 38. Sixty-five days after the start of the experiment and at the onset of a period of rainfall one sheep from each group was autopsied and the H. contortus worm burden was determined. Self-cure, as judged by a marked fall in faecal egg counts, occurred about 15 days later. After a further

Table 5. The simultaneous occurrence of self-cure in groups of sheep originally infected on different dates and subsequently grazed on infected or parasite-free pasture. (Exp. 1).

Worm numbers in individual sheep

Group	Regimen	Before self-cure (Day 65)	After self-cure (Day 90)	Expulsion of worm burden (%)
1	Infected with 10,000 larvae on day 0 and subsequently grazed on infected pasture	3380	240 170	94
2	Infected with 10,000 larvae on day 0 and subsequently grazed on parasite-free pasture	6570	1260 800	82
3	Infected with 10,000 larvae on day 10 and subsequently grazed on parasite-free pasture	2940	30 30	99
4	Infected with 10,000 larvae on day 20 and subsequently grazed on parasite-free pasture.	4200	40 180	97

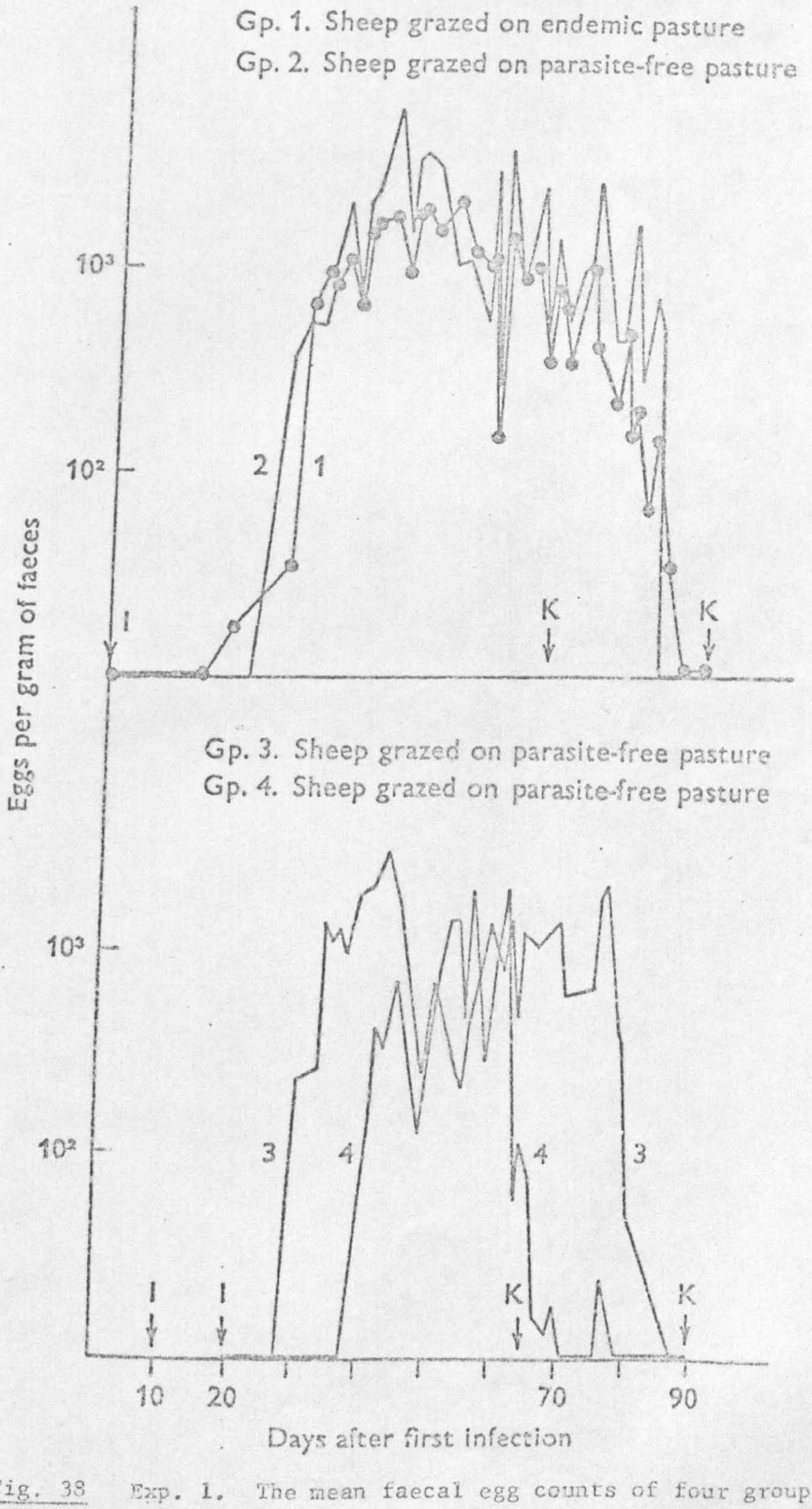

Fig. 38 Exp. 1. The mean faecal egg counts of four groups of grazing sheep only one of which (group 1) was exposed to reinfection with H. contortus before the onset of self-cure.

10 days the remaining sheep were autopsied.

From the results (Fig. 38 and Table 5) it is apparent that self-cure as judged by a fall in faecal egg counts and a marked loss of adult H. contortus occurred simultaneously in three of the four groups irrespective of re-exposure to infective larvae and at a time when a period of rainfall had resulted in the growth of new pasture. Self-cure of the 4th group, which harboured the youngest infection, had occurred 2 weeks previously, shortly after the onset of the rain. Coincident with the occurrence of self-cure in the experimental sheep, a similar fall in faecal egg counts occurred in the permanent flock.

Experiment 2

Three groups of three experimental sheep were individually infected with 100,000 larvae as shown in Table 6. The same system of maintaining groups 2 and 3 on parasite-free pasture was added while the control animals (group 1) were grazed on infected pasture together with the permanent flock.

Ninety days later self-cure, as judged by faecal egg counts, again occurred simultaneously in all three groups (Fig. 39), and in the commercial flock shortly after the onset of a period of major rainfall and irrespective of re-exposure to infective larvae. All of the experimental sheep were autopsied on day 116, when low worm burdens, i.e., a mean of 175 H. contortus, were found.

Table 6. The residual burdens of *Haemonchus contortus* after self-cure had occurred simultaneously in groups of sheep originally infected on different dates and subsequently grazed on infected or parasite-free pasture. (Exp. 2).

Group	Regimen	Residual burdens of *H. contortus* in sheep after self-cure
1	Infected with 10,000 larvae on day 0 and subsequently grazed on infected pasture	120 60 310
2	Infected with 10,000 larvae on day 0 and subsequently grazed on parasite-free pasture	490 180 140
3	Infected with 10,000 larvae on day 14 and subsequently grazed on parasite-free pasture	120 60 110

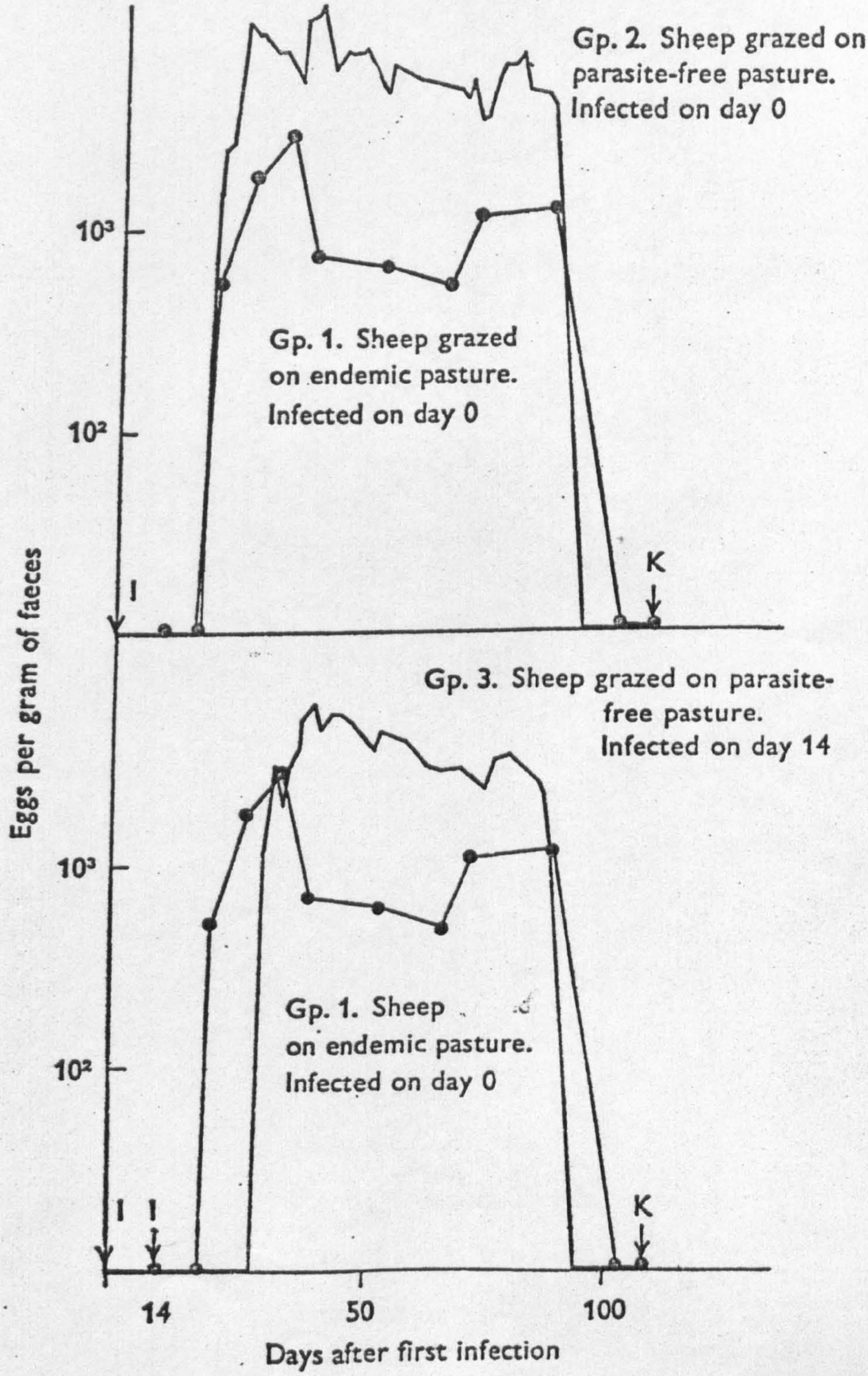

Fig. 39. Exp. 2. The mean faecal egg counts of three groups of grazing sheep only one of which (group 1) was exposed to reinfection with *H. contortus* before the onset of self-cure.

Experiment 3

The design of this experiment is shown in Table 7. Two large permanent pastures of over 50 acres were used. On the first, the endemic pasture, the permanent flock of 70 naturally infected sheep (group 1) had grazed for several months and continued to do so for the duration of the experiment. The second pasture, which was immediately adjacent to the first, was parasite-free; confirmation of its parasite-free status was obtained by grazing a pair of tracer sheep for a period of 1 month before the start of the experiment. H. contortus worms were not found in either of these sheep at autopsy.

Two groups of six sheep (groups 3 and 5) each experimentally infected with 10,000 H. contortus larvae were introduced into the endemic pasture and grazed alongside the permanent flock for 210 and 240 days respectively. Each month five sheep were withdrawn and autopsied to obtain an estimate of the H. contortus burden present during that month. These were selected to include both the experimental and the permanent sheep. In addition, eight separate pairs of tracer sheep were grazed at monthly intervals throughout the experiment to assess the infectivity of the endemic pasture.

In the parasite-free pasture, two groups of six experimentally infected sheep (groups 4 and 6) were introduced into a small fenced area together with six naturally infected sheep drawn from the permanent flock

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Table 7. The design of Exp. 3 in which the onset of self-cure was studied in groups of infected sheep grazing comparable pastures, one of which remained parasite-free throughout the experiment.

Group	Endemic Pasture	Group	Parasite-free Pasture*
1	Naturally infected sheep (70)	2	Naturally infected ewes (6)
3	Sheep, experimentally infected on day 0 (6)	4	Single experimental infection day 0 (6)
5	Sheep experimentally infected on day 30 (6)	6	Single experimental infection day 30 (6)
	'Tracer' sheep (8 x 2)		'Tracer' sheep (4 x 1)

* All of the sheep in this pasture were confined to a paddock which was moved to a new parasite-free area every other day.

(group 2). Subsequently during the entire duration of the experiment the fenced area containing the sheep was repeatedly moved to an ungrazed part of the pasture; initially this was carried out every second day and ultimately twice daily. Each month one or two sheep were withdrawn and autopsied as described for the sheep on the endemic pasture. As a check on its continuing parasite-free status a total of four tracer sheep were grazed serially with the infected sheep.

The mean faecal egg counts and adult worm burdens throughout the experiment are shown in Figs. 40 and 41 and Table 8.

For a period of 200 days from the start of the experiment the faecal egg output of all the groups was sustained at a high and relatively consistent level. Thereafter, immediately after the onset of a period of significant rainfall, self-cure, as measured by a dramatic fall in faecal egg counts and worm burdens, occurred spontaneously in all six groups. Thus, the faecal egg counts dropped from a mean of 10,000 epg to zero between day 200 and day 240. The expulsion of the adult worm burden was equally dramatic falling by 98% in the sheep grazing both the endemic and parasite-free pastures (see worm burdens between April and May in Table 8.)

The changes in the mean faecal consistency of the rectal samples collected daily from groups 1 to 6 during the period of field self-cure are shown in relation to the

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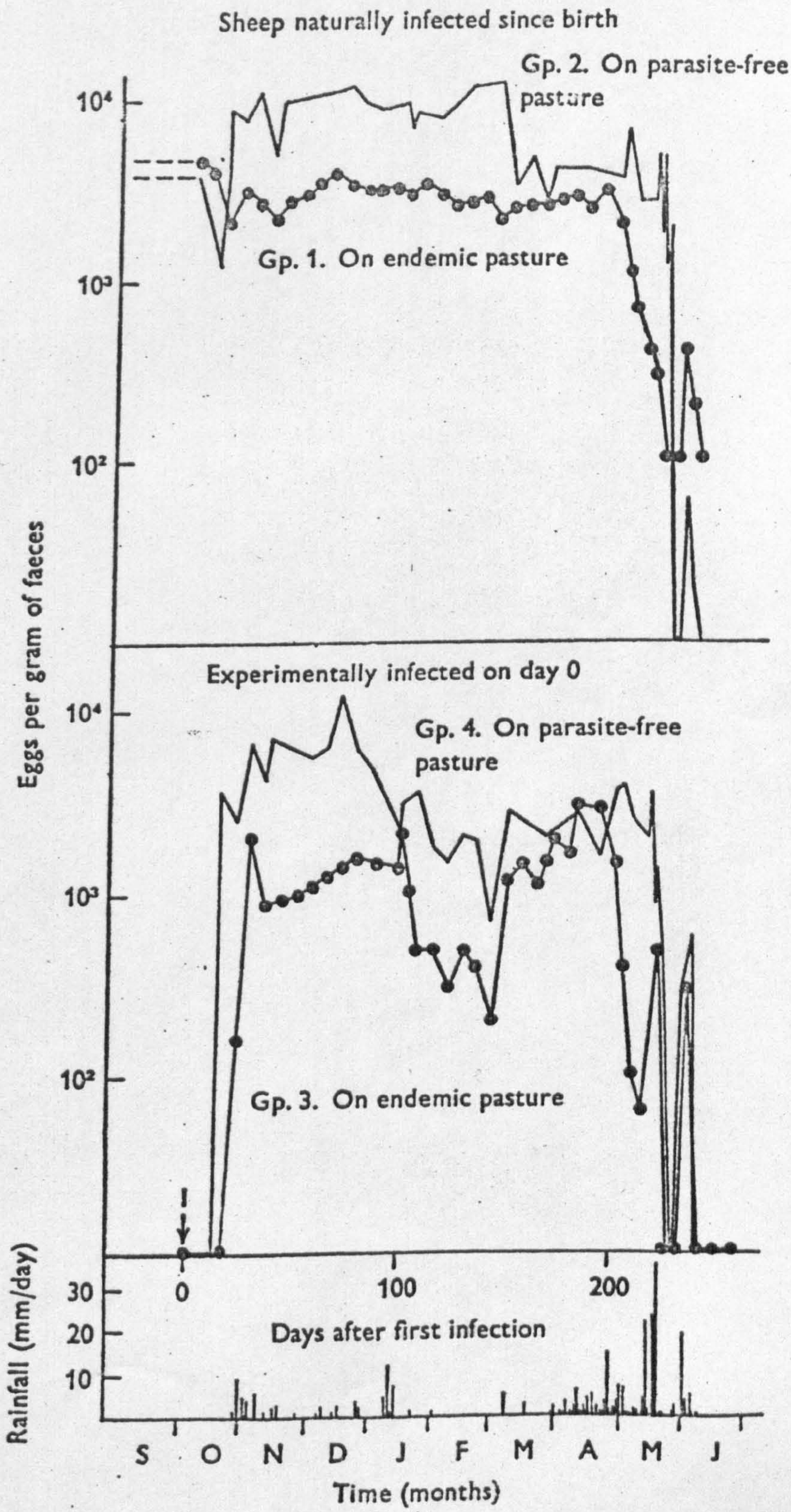

Fig. 40. Exp. 3. The simultaneous occurrence of self-cure in groups of sheep naturally and experimentally infected with H. contortus and grazing on parasite-free pastures.

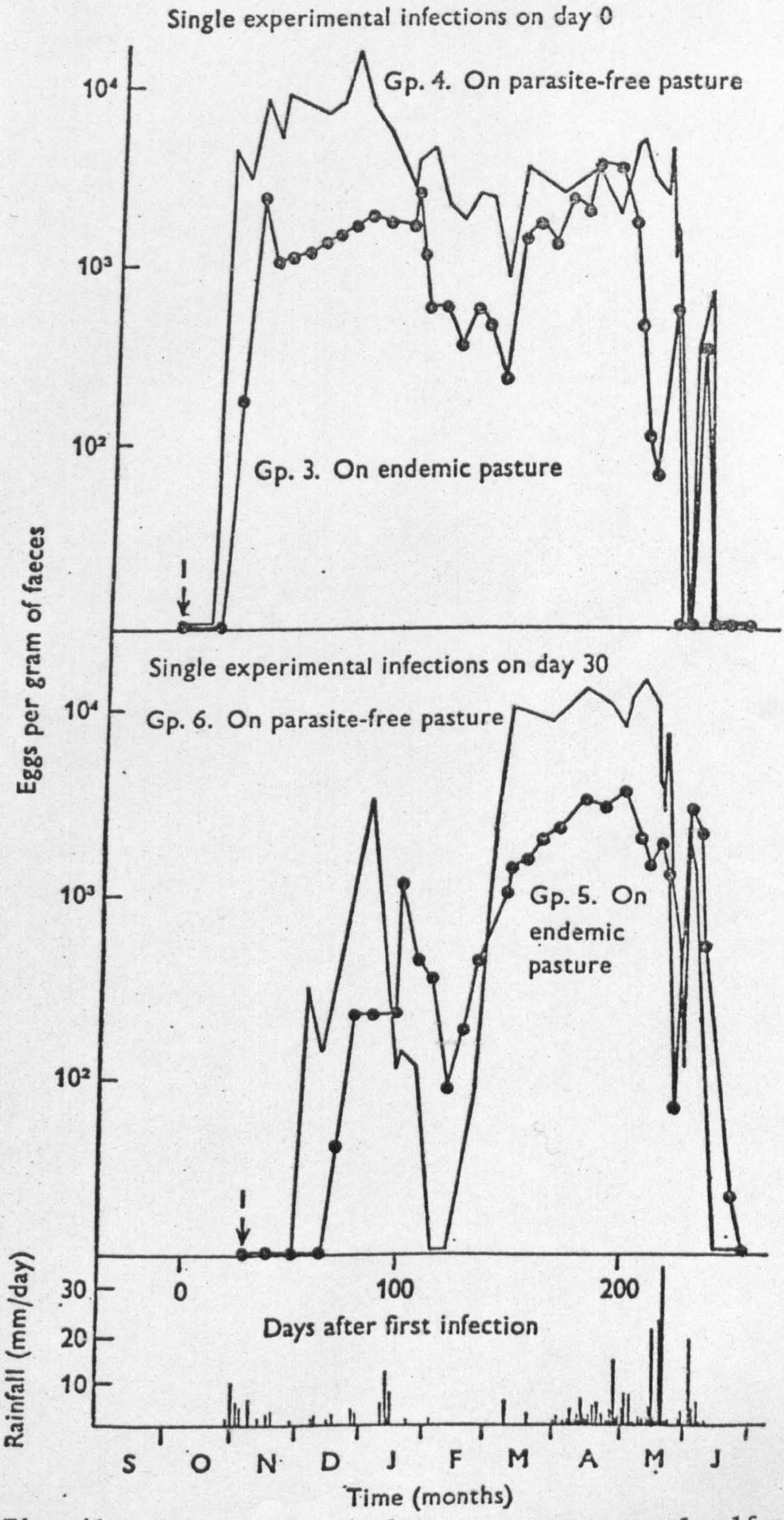

Fig. 41. Exp. 3. The simultaneous occurrence of self-cure in four groups of sheep irrespective of the duration of infection with H. contortus and the infectivity of the pasture.

Table 8. The mean monthly adult worm burdens at autopsy of sheep naturally and experimentally infected with *Haemonchus contortus* and grazed on endemic and parasite-free pasture. (Exp. 3).

	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.*	May*	June
Sheep grazing endemic pasture	590	1200	1540	1180	540	560	12	3080
Tracer sheep on endemic pasture	483	90	30	5	0	5	1044	1876
Sheep grazing parasite-free pasture	720	3100	1520	1087	590	690	13	18
Tracer sheep on parasite-free pasture	0		0		0		0	

* Self-cure occurred between April and May with 98% worm expulsion in both groups of sheep.

changes in mean daily faecal egg counts in Fig. 42. An increase in faecal water content and the onset of clinical diarrhoea were coincidental in all six groups with the fall in mean faecal egg counts in May 1971. These changes occurred in all groups of sheep irrespective of exposure to infective larvae from pasture.

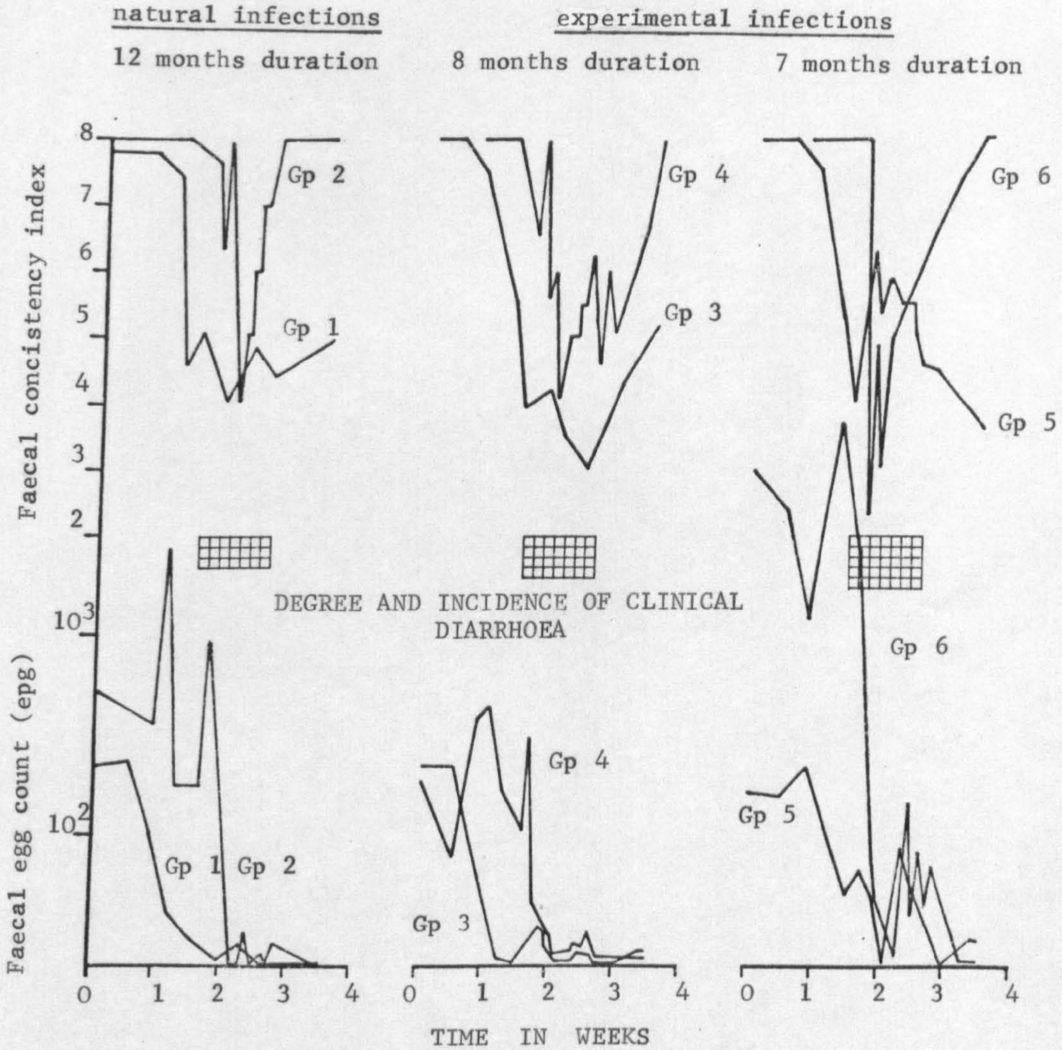

Fig. 42. Exp. 3. The simultaneous change in faecal consistency accompanying the self-cure of *H. contortus* infections under field conditions in Merino ewes; groups 1, 3 and 5 on endemic pasture and groups 2, 4 and 6 on parasite-free pasture.

DISCUSSION

A major practical difficulty in any field study of the aetiology of self-cure is the problem of maintaining the sheep on parasite-free pasture, environmentally comparable to the endemic pasture, for an indefinite period of weeks or months until the event occurs. This was overcome in the two preliminary experiments described here by the use of faecal bags on groups of sheep which were folded every third day over a series of small paddocks which had not been grazed by sheep for 18 months. In the third experiment which extended for 240 days, a mobile paddock of approximately 2,500 square feet was moved progressively over pasture immediately adjacent to the endemic pasture; it had not been grazed by sheep for 18 months and its continuing parasite-free status was monitored by the use of worm-free tracer sheep during the course of the experiment.

As described earlier, the experiments here were designed so that a study of self-cure in the experimental sheep could be made over the period normally associated with the occurrence of this phenomenon in the commercial flock. In the latter, this occurred on three occasions, each being characterised by a drop in faecal egg count of the entire flock, usually to zero. On each of these three occasions self-cure, as measured by the same parameter, occurred at exactly the same point in time in the experimental sheep. It was therefore highly probable that the mechanism of self-cure in the experimental sheep in these three experiments was the same as that of the natural phenomenon in the commercial flock.

If this is the case it is apparent that self-cure as a flock phenomenon was characterised by four features. First, it occurred with equal vigour in sheep subjected to reinfection and in sheep grazing parasite-free pasture. Second, it did not depend on the sheep possessing a naturally acquired infection as opposed to a single experimental infection. Third, as judged by the experimental infections, it did not depend on the duration of the *H. contortus* infection. Finally, self-cure was always associated with a period of significant rainfall.

These observations confirm and extend the original epidemiological observations of Gordon (1948). However, Stewart's subsequent explanation of the phenomenon (1950a,b, 1953), i.e., that it depends on an immunological response to reinfection, is not applicable to the results reported here.

The stimulus which initiates self-cure as observed in these experiments is unknown. Since a common factor in all three experiments was the growth of fresh pasture after the onset of rain, three possibilities might be considered. First, that the shoots of freshly growing pasture contain a chemical which is toxic to adult *H. contortus*. Second, that certain physiological alterations in the abomasum induced by sudden dietary change, e.g. pH, make existence intolerable for adult *H. contortus*. Third, that some factor in new pasture induces an anaphylactoid state of the abomasal mucosa which, in terms of mucus secretion and mucosal oedema, replicates the hypersensitivity phenomena described by Stewart (1953) as being responsible for the expulsion of

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the H. contortus adults.

The results and suggestions discussed here do not invalidate the experimental work described by Stewart. Indeed, from past experience (unpublished results) it has been possible to produce self-cure by experimental reinfection of sheep maintained indoors. However, it does appear that the mechanism of self-cure, as it occurs in naturally infected flocks, is not necessarily associated with reinfection.

A point which deserves comment is the premature self-cure exhibited by group 4 in Exp. 1 (established by faecal egg counts and autopsies) and the 'transient' self-cure of group 6 in Exp. 3. The latter was characterised by a marked but temporary suppression of egg count, was apparently not accompanied by expulsion of adults and involved single experimental infections of relatively short duration, i.e., about 40 days. This suggests that young adult H. contortus are more susceptible to the factors which precipitate self-cure and that the spectrum of self-cure may range from merely the temporary suppression of egg-laying to complete expulsion of the adult worm burdens.

There is a general lack of agreement in text-book descriptions of haemonchosis with regard to the relationship between the occurrence of clinical diarrhoea and the incidence of disease. As described earlier in Chapter 2 the observations during the epidemiological studies showed that during outbreaks of acute haemonchosis the faeces were usually of normal

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consistency. Faecal droppings were sometimes a dark reddish colour however, and were occasionally, in severely affected animals, passed in the form of rouleaux. In chronic haemonchosis the faecal pellets were occasionally darker in colour and usually of a harder consistency than normal. The only occasions when softening of the faeces and clinical diarrhoea was recorded was following periods of significant rainfall when pasture conditions were rapidly improving and when the mean faecal egg output was falling.

The results of this experiment show that the onset of diarrhoea and self-cure was simultaneous in all groups and indicate that the ingestion of larvae was not required either to induce self-cure nor for the development of diarrhoea. It is interesting to note that in Stoll's original report of self-cure in 1929 he records that there was a softening of faeces at the time of the fall in the faecal egg output of the two grazing lambs. In 1953 Stewart was able to induce "self-cure" in sheep, experimentally infected with H. contortus and maintained indoors, by the administration of Staphylococcal organisms and noted violent diarrhoea as a side-effect. Reports have not linked diarrhoea with self-cure in the field however, and the relationship between the two phenomena has apparently not been associated hitherto.

SUMMARY

The phenomenon of self-cure, as it occurred under field conditions in East Africa, was studied in Merino sheep infected with Haemonchus contortus. The onset of self-cure, as judged by a dramatic fall in faecal egg counts, was found to be simultaneous in sheep grazing on infected pasture and in sheep grazing on parasite-free pasture. Furthermore the results of autopsies carried out before and after self-cure showed that a marked and equal loss of adult worm burdens had also occurred. These results indicate that self-cure of H. contortus infections under natural conditions occurs in the absence of reinfection and is apparently non-immunological in origin. Since the phenomenon was always associated with a period of significant rainfall, it is suggested that new growth of pasture may be a significant aetiological factor.

CHAPTER 7.

A POSSIBLE RELATIONSHIP BETWEEN HAEMOGLOBIN POLYMORPHISM AND HAEMONCHOSIS IN SHEEP IN KENYA

INTRODUCTION

Sheep and goats show a clear polymorphism for haemoglobin. Two alleles (A and B) and three phenotypes (A, AB and B) are commonly found (Harris and Warren, 1955; Evans, King, Cohen, Harris and Warren, 1956; Helm, van Vliet and Huisman, 1957; Huisman, van Vliet and Sebens, 1958a, b) their inheritance being controlled by a simple allelic pair (Evans *et al.*, 1956; Huisman, van Vliet and Sebens, 1958a, b). The distribution of these haemoglobin types varies markedly between breeds of sheep (Evans, Harris and Warren 1958a, b; Meyer, 1963) although similarities have been noted between certain breeds indigenous to broad geographical regions. Initially, this was associated with altitude for two reasons; first, Huisman *et al.*, (1958a, b) had shown that red cells of haemoglobin A (HbA) have a higher affinity for oxygen than haemoglobin B (HbB) cells; second, Evans *et al.*, (1958a, b) found that sheep breeds living at high altitudes in Northern Britain and Northern Europe were predominantly HbA whilst those at low altitudes in Southern Britain, Northern Africa and the Middle East were largely HbB.

Later reports did not substantiate this association between haemoglobin type and altitude. Dassat (1964) found that HbB was common in sheep living at high altitudes in Cuneo Province of Italy, Chan (1968) found that HbB was commonest among the Corriedale, Jonin and Criollo breeds in the Peruvian Andes, and Dassat and Sartore (1963) found a homogeneous distribution of haemoglobin types among Sardinian sheep living in lowland, hill and mountain regions. In India, Agar and his colleagues

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(Agar, 1968; Agar, Rawat and Roy, 1969) found that the native sheep of hilly Rajasthan were largely HbB type. In the mountainous Himalayan region both the indigenous Rampur Busheir and the Polwarth, which are three-quarter Merino and one-warter Lincoln, were predominantly HbB types (Agar and Seth, 1971). From Meyer's results (1963) it can also be seen that various breeds of European sheep which are usually regarded as lowland breeds have a high frequency of HbA.

An interesting and possibly significant observation in this context was made by Evans and Blunt (1961) who showed that the gene frequencies of the haemoglobin types of Romney Marsh and Southdown sheep in Australia were predominantly HbA whereas the original stock in their native British environment were predominatly HbB. This suggested that HbA might be, in some way, associated with an adaptation to the Australian environment.

One possible basis for this adaption was subsequently suggested by Evans, Blunt and Southcott (1963) who experimentally infected both HbA and Hb AB sheep with H. contortus. The trend in faecal worm egg counts and worm burdens at autopsy suggested that the former were more resistant to the establishment of adult infections of this nematode, although there were no significant differences in the haematological indices of the two types. However, the following year Evans and Whitlock (1964) were able to demonstrate a significant association between the seasonal maximum haematocrit of grazing sheep and their Hb type and

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again suggested that this might be related to a varying degree of resistance to natural infection with *H. contortus*.

Five years later Jilek and Bradley (1969) divided a flock of Florida Native Ewes into "high" and "low" resistance groups, based on their Hb concentration over a period of two years on *H. contortus* endemic pasture. They found no significant difference between these groups and their Hb types unless certain allowances, in their view justifiable, were made for the poor performance of aged HbA sheep. Finally Radhakrishman, Bradley and Loggins (1972) in a study of the relationship of Hb type, again in Florida Native Sheep, to experimental *H. contortus* infections concluded that there was "no definitive data to support the theory that Hb types in Florida Sheep were indicators of resistance to *H. contortus* or that HbA sheep were less susceptible". Nevertheless, it was generally the case that the HbA sheep had higher PCV values throughout the course of infection and they appeared to undergo self-cure more readily. In a subsequent re-analysis of this data (Loggins, Radhakrishnan, Bradley and Franke, 1973) they also reported that HbA type lambs, subject to experimental infection with *H. contortus*, had significantly higher L_4 counts at autopsy 3 to 4 weeks after infection which they attributed to an innate resistance to the normal development of *H. contortus* worms.

Since all three Hb genotypes were present in the Merino flock studied in this work it was thought worthwhile to find if any relationship existed between these three types of sheep

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and their performance in an H. contortus endemic environment. The parameters examined were:- faecal egg counts; the ability of each Hb type to undergo self-cure as described in Chapter 6; haematological indices and total body weights. Much of the data used was collected for other experiments previously reported in this thesis and for this reason some of the analyses lack the significance which would have been provided by larger numbers of sheep. Nevertheless, it was thought that the results were of sufficient interest to merit inclusion in this thesis.

MATERIALS AND METHODS

Experimental Animals

The sheep which were the subject of these investigations were derived from two sources. First, the permanent Merino flock, described in Chapter 2 and referred to here as Flock 1. Second, a flock of 40 Merino Ewes (Flock 2) purchased locally and introduced into a paddock adjacent to the permanent flock in June 1970.

Haematological and Parasitological Techniques

Haematological examinations and parasitological techniques were conducted as described earlier in Chapter 2.

Haemoglobin Typing

The separation and identification of the haemoglobin types was carried out by starch-gel electrophoresis using Huisman's (1963) modification of the procedure developed by Smithies (1955). This technique is described in Appendix D.

RESULTS

Faecal Egg Counts and Self-cure

The fluctuations in weekly mean faecal egg counts of the three Hb phenotypes for Flock 1 and Flock 2 are shown in Figs. 43 and 44 respectively. In Flock 1, which was studied over a period of 15 months the faecal egg counts of all three types were sustained at a high and generally similar level. However, using an arbitrary definition of self-cure as an 80% drop in the faecal egg count within two weeks or less, it is apparent that self-cure occurred in the ewes on four occasions in the HbA type and not at all in type Hb AB and HbB. In the lambs only Hb AB and HbB types were present and in these self-cure occurred on only one occasion i.e., April 1971 in the Hb AB group. At this time a fall in the mean faecal egg count was also recorded in the ewes of this group but this was not sufficiently marked (i.e., a fall of 66%) to be classified as self-cure.

In Flock 2 which was studied over a period of 13 months the mean faecal egg counts of the three groups of ewes were again generally similar. However, self-cure occurred on one occasion i.e., April 1971, coincidentally with Flock 1. All three Hb types were involved, the percentage fall in mean faecal egg count being 100, 90 and 86 in groups HbA, Hb AB and HbB respectively. When compared with Flock 1 the only other time when self-cure might also have occurred was in September 1970; the possibility of this happening in Flock 2 was probably pre-empted by thiabendazole treatment two weeks previously.

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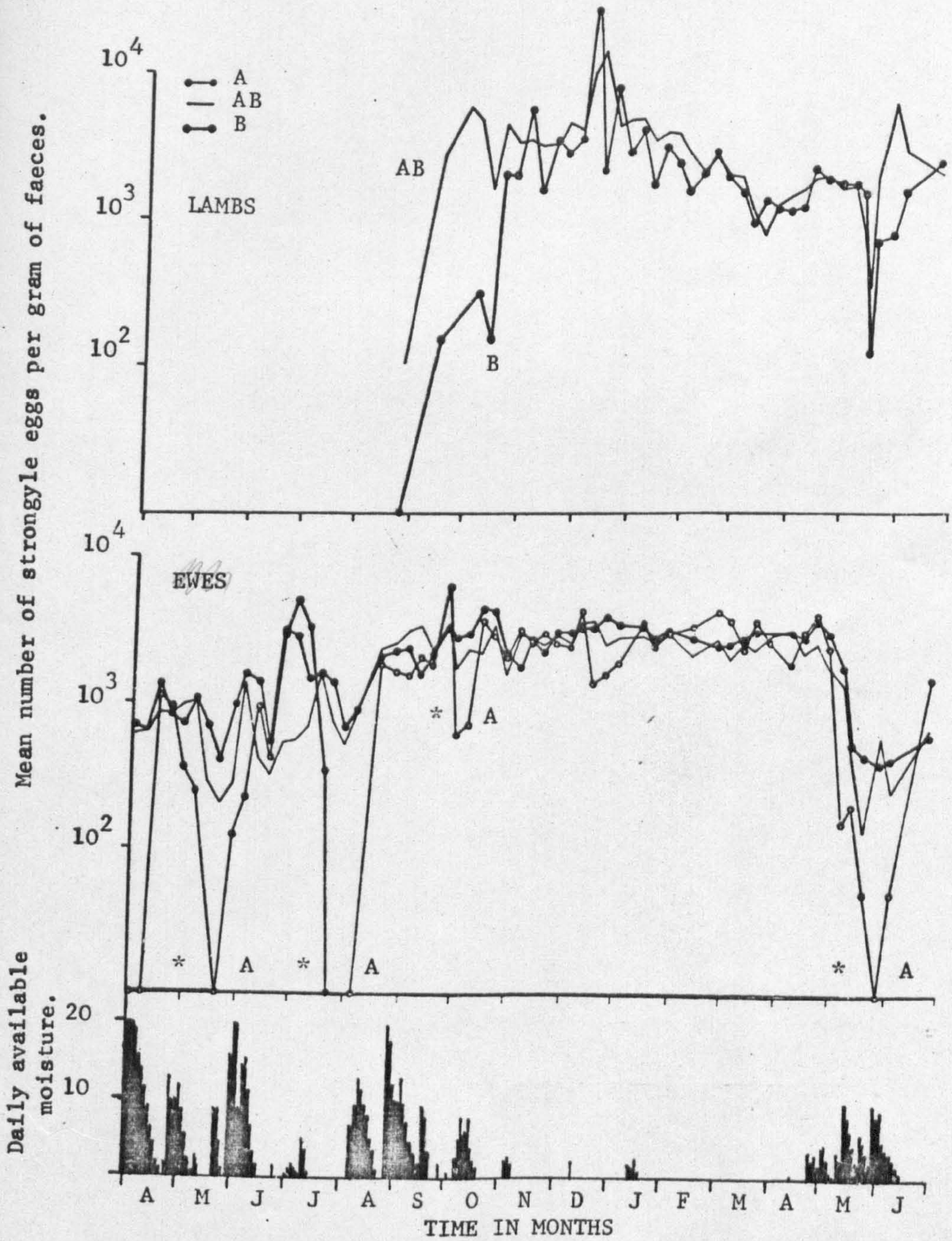
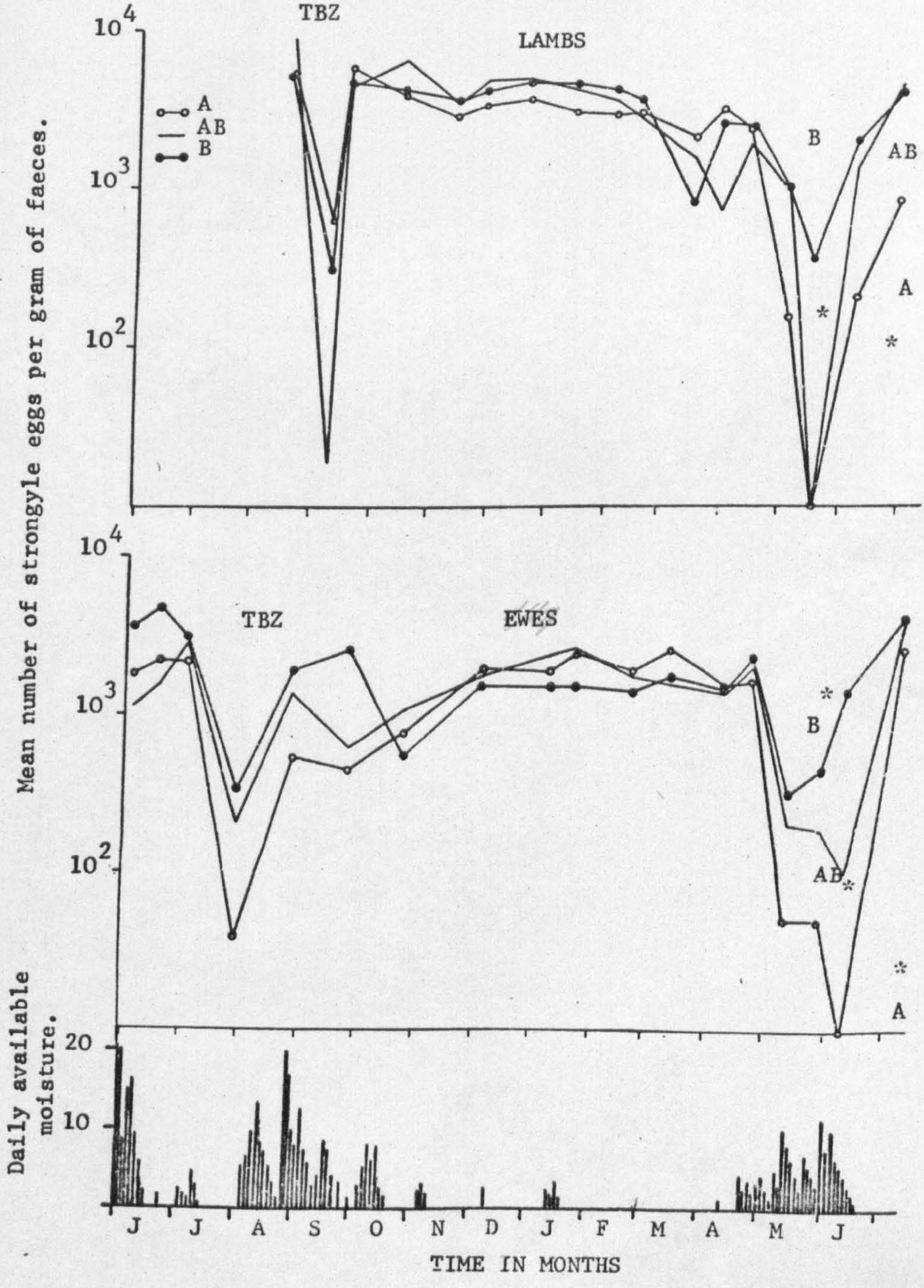


Fig. 43. The fluctuations in mean faecal egg counts of the three common haemoglobin phenotypes of Merino sheep (Flock 1 A) exposed to natural infection with H. contortus.

* This indicates a drop in mean faecal egg count of more than 80% in less than two weeks.



<u>Fig. 44.</u> The fluctuations in mean faecal egg counts of three common haemoglobin phenotypes of Merino sheep (Flock 2) subject to natural infections of <u>Haemonchus contortus.</u>

In the lambs self-cure occurred on the same occasions as the ewes but involved only groups HbA and Hb AB.

Haematology

To provide a basis for comparison the mean PCV values and Hb concentrations of a number of sheep of the three phenotypes, drawn from both flocks, were examined. These were prepared from haematology results accumulated over the period January to March 1971 during which time both flocks were suffering from chronic haemonchosis (see Chapter 2). The results are shown in Table 9, from which it is apparent that the PCV values and Hb concentrations of the HbA ewes were significantly higher than the HbB ewes. The values of the Hb AB ewes were intermediate. A similar trend was observed in the lambs although this was not statistically significant.

Total Body Weights

The mean total body weights were prepared from data accumulated over the same period and are also shown in Table 9. Again the HbA ewes showed significantly higher body weights than the HbB ewes. The lambs also showed a similar trend, the HbA type being 40% heavier than the HbB type, although the significance of this is limited by the small number of HbA lambs. Again in each case the Hb AB types were intermediate in value.

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		Haemoglobin Type		
		A	AB	B
Ewes	Total numbers	15	55	40
	PCV (%)	23.7^{+}	21.4	19.2
	Hb (g/100 ml)	10.2^{+}	8.6	8.3
	Total B.W. (Kg)	41.5^{+}	40.6^{+}	36.8
Lambs	Total numbers	4	31	20
	PCV	24.0	24.7	20.7
	Hb (g/100 ml)	10.4	10.3	8.7
	Total B.W. (Kg)	24.0^{+}	18.8	18.5

Table 9 The numbers of each common haemoglobin phenotype of Merinos in Flocks 1 and 2 and the results of the mean PCV values, Hb estimations and total body weights of each group for the period Jan-March 1971. Figures marked with $^{+}$ indicate that these are significantly different ($P<0.05$) from the corresponding value of the HbB type.

DISCUSSION

The results of this preliminary study appear to provide evidence which supports a theory that the degree and frequency of self-cure of H. contortus infection is associated with the Hb type of the host. In Flock 1 the HbA ewes showed self-cure, defined as an 80% drop in the faecal egg count occurring within two weeks or less, on a total of four occasions in 15 months. In the other two Hb types a limited fall in the mean faecal egg count occurred on three of these occasions; in each case the HbB ewes showed the least fall, the Hb AB ewes being intermediate. In the lambs of this flock which contained only Hb AB and HbB types, self-cure occurred on only one occasion being exhibited by both types.

In Flock 2, which was studied for a shorter period, self-cure occurred in all three Hb types of ewes and lambs on one occasion which was contemporaneous with the last self-cure of Flock 1. In the ewes it was exhibited most markedly by the HbA types and least so by the HbB and with Hb AB intermediate. In the lambs it occurred with equal vigour i.e., the mean faecal egg counts fell to zero, in both HbA and Hb AB groups.

These results provide some preliminary evidence which suggests that in Merino sheep at least, those with HbA type show self-cure more frequently and effectively than those with Hb AB and HbB types; there is also some indication that Hb AB sheep self-cure more readily than HbB sheep.

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Examination of the PCV values, Hb concentrations and total body weights of the ewes and lambs of both flocks over a period of 3 months revealed a similar trend i.e., the HbA type had the highest haematological parameters and the heaviest body weights, while the HbB types were consistently lowest. Unfortunately only 15 HbA ewes and 4 HbA lambs were available for study and the significance of these observations requires to be validated by further study.

If confirmed these results may have considerable academic and practical significance. The association of Hb type with the exhibition of phenomena which indicate an improved ability to survive and thrive in a haemonchosis-endemic environment is difficult to explain. It is well established that HbA type sheep under conditions of severe haemorrhagic stress have the ability to produce a new type of Hb (HbC) which has an improved oxygen-carrying capacity, (Gabuzda, Schuman, Silver and Lewis, 1968), and it would be interesting to see if this phenomenon occurs in sheep made severely anaemic by *H. contortus* infection. However, even if this was so, it would be difficult to account for the occurrence of self-cure on this basis. The practical significance of these observations do not require elaboration and it is suggested that new ventures into sheep husbandry in *H. contortus* endemic areas should take account of Hb type in the selection of rams and ewes or, at the least, should incorporate studies on the relative performance of the three Hb phenotypes.

SUMMARY

It is known that sheep show polymorphism for Hb in that three phenotypes (HbA, HbB and Hb AB) are commonly found. Since it has been suggested, on the basis of haematological values, that HbA type sheep are more able to survive in *H. contortus* endemic environments, a preliminary study was conducted on the performance of set-stocked Merino ewes and lambs in relation to their Hb phenotypes.

The results provided some evidence that in Merino sheep at least, those with HbA type show self-cure more frequently and effectively than those with HbB and Hb AB types.

Examination of the PCV values, Hb concentrations and total body weights of both ewes and lambs showed a similar trend i.e., the HbA types had the highest haematological parameters and the heaviest body weights, while the HbB types were consistently lowest.

It would appear that the practical significance of the results justifies an investigation on a larger scale.

APPENDIX A

The Life-Cycle of *H. contortus*

The adult *H. contortus* occurs in the abomasum of sheep, goats, cattle and many other ruminants, and is among the largest of those species commonly found in the abomasum. The adult female measures about 20-30 mm and has the appearance of a "barber's pole" because of the white ovaries which are spirally wound around the blood-filled intestine. The adult male is about half the length of the female and has a well developed bursa which is characterised by an asymmetrical dorsal lobe, supported by a Y-shaped dorsal ray.

Both male and female adults feed on whole blood which is taken from the sub-mucosal capillaries several times each day. The female is very prolific and produces up to 10,000 eggs daily. These pass out of the abomasum with the intestinal contents and are deposited in the faeces on the pasture.

H. contortus eggs hatch in a minimum of one day and the first stage larva is released; but depending on the conditions of temperature and humidity this may be delayed for several weeks. Under optimal conditions the development of the first larval stage to the second and third infective stage occurs within a further two or three days but may be prolonged by adverse weather conditions. The first and second larval stages feed on bacteria and, like the eggs, are susceptible to desiccation. The third larval stage, which is the infective stage, is unable to feed because it retains the sheath of the second stage. This double sheath gives the infective larva increased resistance to desiccation and is evidently largely responsible for the prolonged survival of this stage on pasture.

Following ingestion by the host from the pasture, the outer sheath is lost in the rumen and the exsheathed third larval stage passes into the abomasum where it becomes enveloped by the mucous secretion. This is the site of the first parasitic moult i.e., the third ecdysis, which occurs after a further two days and results in the formation of the fourth larval stage which is of comparable size. Following this, however, growth is rapid and, with the development of the mouth apparatus, the larva becomes attached to the mucosa by means of the buccal cavity, and as a result of its blood-sucking activities a small haemorrhage develops and the subsequent coagulum surrounds the worm.

The second parasitic moult i.e., the fourth ecdysis, occurs about 10 days after infection at which time the male and female larvae measure about 5 mm and 7 mm respectively. At this stage the early fifth stage larvae show distinct development of the genital organs and continue their rapid growth to reach about 12 mm for males and 17 mm for females, by the 17th day. The worms are now sexually mature and eggs usually begin to appear in the faeces during the next day or so, i.e., 18-20 days following infection.

APPENDIX B.

Table 1. The mean monthly numbers of *H. contortus* recovered at autopsy. Four ewes and two lambs were withdrawn from the flock each month and maintained indoors for 2 weeks prior to autopsy.

	Month	EWES Adults	L_4	Total±SE.	LAMBS Adults	L_4	Total±SE
1969	Oct.	50	5	55±45	0	0	0±0
	Nov.	20	10	32±12	8	5	13±13
	Dec.	48	5	55±26	205	3	208±97
1970	Jan.	51	0	51±49	258	0	258±12
	Feb.	128	0	128±107	118	0	118±42
	Mar.	220	0	220±46	-	-	- -
	Apr.	740		740±381	-	-	- -
	May.	41	0	41±13	-	-	- -
	Jun.	300	0	300±63	-	-	- -
	Jul.	263	0	263±100	-	-	- -
	Aug.	11000	0	11000±4203	50	0	50±50
	Sep.	9210	170	9380±287	2120	0	2120±20
	Oct.	4320	56	4376±752	968	33	1001±120
	Nov.	590	67	657±279	698	1500	2198±1134
	Dec.	1200	0	1200±294	1060	0	1060±40
1971	Jan.	1540	0	1540±540	233	0	233±12
	Feb.	1180	200	1380±543	690	0	690±90
	Mar.	540	0	540±20	510	150	660±290
	Apr.	560	205	765±160	800	0	800±100
	May.	37	0	37±19	0	0	0±0
	Jun.	3080	400	3480±2522	3000	200	3200±200
	Jul.	9980	0	9980±1480	7010	0	7010±2990

APPENDIX B.

Table 2. The mean numbers of H.contortus recovered at autopsy of 'tracer sheep' which had been grazed for successive two or four week periods on the permanent pasture and were subsequently maintained indoors for 2 weeks prior to autopsy.

	Month	Tag Nos. of Tracer Sheep	Worm Burden at Autopsy L_4	Male	Female	Total+ SE	Male/ Female Ratio
1969	Oct.	1-4	0	2	1	3 ± 2.44	1:0.5
	Nov.	5-8	0	1	2	3 ± 2.50	1:2.0
		9-12	0	5	20	25 ± 9.57	1:4.0
	Dec.	13-16	0	8	25	33 ± 12.50	1:3.1
		17-20	0	20	33	53 ± 23.22	1:1.7
1970	Jan.	21-24	0	12	13	25 ± 25.00	1:1.1
		25-28	0	5	3	8 ± 4.78	1:0.6
	Feb.	29-30	0	10	10	20 ± 0	1:1.0
	Mar.	33-34	0	10	10	20 ± 0	1:1.0
	Apr.	37-40	0	205	280	485 ± 182.05	1:.14
	May.	41-44	0	130	145	275 ± 154.13	1:1.1
	Jun.	45-48	0	25	48	73 ± 57.06	1:1.9
	Jul.	49-52	0	20	35	55 ± 35.23	1:1.8
	Aug.	53-56	0	750	830	1580 ±1070.95	1:1.1
	Sep.	57-60	20	210	200	430 ± 125.83	1:1.0
	Oct.	61-64	30	350	413	793 ± 174.57	1:1.2
	Nov.	65-68	0	206	277	483 ± 154.83	1:1.3
	Dec.	69-70	0	25	65	90 ± 80.0	1:2.6
1971	Jan.	73-74	0	15	15	30 ± 0	1:1.0
	Feb.	77-78	0	0	5	5 ± 5.0	0:5.0
	Mar.	81-82	0	0	0	0 ± 0	0
	Apr.	85-86	0	0	0	0 ± 0	0
		89-90	0	0	10	10 ± 10	0:10
	May.	93-94	0	80	170	250 ± 220	1:2.1
		97-100	0	1090	1080	2170 ± 424.33	1:1.0
	Jun.	101-4	0	1433	1660	3093 ±1203. 6	1:1.2
		105-8	445	335	398	1178 ± 371.96	1:1.2
	Jul.	109-12	50	335	420	805 + 210.67	1:1.3
		113-4	200	1080	1380	2660 + 1155	1:1.3

APPENDIX B.

Table 3. The mean weekly or two-weekly haematological values of 20 Merino ewes of the permanent flock.

Date	PCV	Hb	MCHC
15.10.69	28.4	8.5	29.9
29.10.69	23.4	10.8	46.2
12.11.69	25.7	11.3	44.0
19.11.69	26.4	11.4	43.2
26.11.69	27.1	11.5	42.5
28.11.69	27.7	11.2	40.2
10.12.69	28.3	10.9	38.5
17.12.69	29.7	10.7	36.0
31.12.69	31.1	10.5	33.8
14. 1.70	30.6	10.2	33.3
28. 1.70	31.6	10.4	32.9
11. 2.70	31.6	10.8	34.2
25. 2.70	33.3	10.6	31.8
11. 3.70	31.5	11.1	35.2
25. 3.70	28.4	10.9	38.4
1. 4.70	27.3	9.9	36.3
15. 4.70	28.9	10.1	35.0
22. 4.70	27.4	9.2	33.6
29. 4.70	28.9	9.9	34.3
6. 5.70	27.9	8.3	29.8
13. 5.70	28.0	9.0	32.1
20. 5.70	28.0	8.7	31.1
27. 5.70	27.0	8.5	31.5
3. 6.70	26.9	8.4	31.2
10. 6.70	24.7	8.5	34.4
17. 6.70	24.6	8.0	32.5
1. 7.70	22.9	7.7	33.4
22. 7.70	22.3	7.3	32.7
5. 8.70	20.1	6.8	33.8
26. 8.70	17.3	5.2	30.1

Table 3 (cont'd.)

Date	PCV	Hb	MCHC
2. 9.70	19.3	6.2	32.1
17. 9.70	21.9	7.1	32.4
30. 9.70	16.5	6.5	39.4
14.10.70	20.4	6.8	33.3
28.10.70	21.3	6.8	31.9
4.11.70	20.0	6.7	33.5
21.11.70	19.6	6.5	33.2
23.12.70	19.5	6.7	34.4
13. 1.71	21.1	6.9	32.7
20. 1.71	20.1	7.0	34.8
3. 2.71	20.5	7.3	35.6
17. 2.71	21.1	7.4	35.1
24. 2.71	21.6	7.4	34.3
3. 3.71	21.1	7.3	34.6
7. 3.71	20.8	7.2	34.6
18. 3.71	20.5	7.2	35.1
24. 3.71	19.4	7.1	36.6
3. 4.71	16.1	7.0	43.5
6. 4.71	16.2	7.1	33.3
14. 4.71	16.8	7.1	37.6
21. 4.71	16.4	7.1	43.3
28. 4.71	16.5	7.1	43.0
8. 5.71	22.6	8.1	35.8
11. 7.71	23.9	8.1	33.9
15. 5.71	25.1	8.3	33.1
25. 5.71	26.3	9.0	34.2
28. 5.71	25.5	8.2	32.2

APPENDIX B.

Table 4. The mean two-weekly haematological values of 20 Merino lambs of the permanent flock.

1969 Lambs.

Date	PCV	Hb	MCHC
22.10.69	31.9	9.7	30.4
5.11.69	29.0	9.7	33.5
19.11.69	32.4	10.9	33.6
3.12.69	31.5	12.1	38.4
17.12.69	33.0	13.1	39.7
24.12.69	31.9	8.3	26.0
7. 1.70	33.0	8.6	26.1
21. 1.70	32.3	8.9	27.6
5. 2.70	31.8	9.3	29.3
18. 2.70	31.3	10.2	32.6
4. 3.70	29.8	8.8	29.8

Table 4 (Cont'd.)

1970 Lambs

Date	PCV	Hb	MCHC	RBC	MCV
2. 9.70	37.2	13.4	36.0	11.2	33.2
9. 9.70	36.0	12.4	32.6	10.7	35.5
17. 9.70	34.9	11.4	32.7	10.2	34.2
24. 9.70	31.0	10.6	34.2	10.0	31.0
30. 9.70	30.3	10.4	34.3	10.1	30.0
7.10.70	30.0	9.4	33.6	9.5	29.5
14.10.70	29.8	8.7	29.2	6.9	43.2
20.10.70	30.0	8.5	32.7	7.2	36.1
28.10.70	30.2	8.5	28.2	7.2	41.9
4.11.70	25.6	8.5	33.3	7.2	35.6
21.11.70	21.7	7.1	32.6	7.1	30.6
23.12.70	23.2	7.3	31.5	7.1	32.7
13. 1.71	24.3	7.5	31.0	7.1	34.2
20. 1.71	22.8	7.6	33.3	4.9	46.5
27. 1.71	22.2	7.7	34.7	5.3	41.9
3. 2.71	21.6	7.8	36.1	5.7	37.9
17. 2.71	23.2	7.9	34.1	5.5	42.2
24. 2.72	22.3	7.8	35.0	5.7	39.1
3. 3.71	21.3	7.6	35.6	5.3	40.2
7. 3.71	21.0	7.5	35.7	5.4	38.9
18. 3.71	21.0	7.4	35.2	5.5	38.2
24. 3.71	20.9	7.3	34.8	5.5	38.0
3. 4.71	21.8	7.4	34.1	5.3	41.1
6. 4.71	22.7	7.6	33.5	5.2	43.7
14. 4.71	25.3	8.5	33.6	5.1	49.6
21. 4.71	26.6	8.8	33.1	5.0	53.2
28. 1.71	27.0	9.1	33.7	4.7	57.4
8. 5.71	27.8	9.4	33.8	4.4	63.2
11. 5.71	28.3	9.5	33.6	4.3	65.8
15. 5.71	28.1	9.6	34.2	5.8	48.4
25. 5.71	30.2	10.1	33.4	6.5	46.5
1. 6.71	29.0	9.0	31.1	6.6	43.9

APPENDIX B.

Table 5. The mean monthly wet-weights of individual H.contortus adults in relation to the mean monthly total number of worms recovered from the abomasum of four ewes.

	Month	Mean monthly wet weights of individual adult H. contortus	Mean monthly total number of adult H.contortus
1969	Oct.	0.00086	50
	Nov.	0.00099	20
	Dec.	0.00078	48
1970	Jan.	0.00076	51
	Feb.	0.00072	128
	Mar.	0.00106	220
	Apr.	0.00025	740
	May.	0.00066	40
	Jun.	0.00116	300
	Jul.	0.00115	263
	Aug.	0.00044	11,000
	Sep.	0.00041	9,210
	Oct.	0.00070	4,320
	Nov.	0.00050	590
	Dec.	0.00083	1,200
1971	Jan.	0.00091	1,540
	Feb.	0.00117	1,180
	Mar.	0.00082	540
	Apr.	0.00088	560
	May.	0.00079	37
	Jun.	0.00061	3,080
	Jul.	0.00045	9,980

APPENDIX B.

Table 6. The results of the monthly analysis of the permanent pasture at Naivasha from March 1970 to February 1971.

	Month	Monthly rainfall (mm)	Dry matter (%)	Crude protein (as % dry matter)	Crude fibre (as% dry matter)	Iron content (ppm)
1970	Mar.	162	54.1	15.8	24.7	517
	Apr.	125	22.5	19.2	24.5	250
	May.	104	35.7	14.6	27.1	285
	Jun.	44	50.8	10.0	29.6	317
	Jul.	30	47.5	9.7	30.9	220
	Aug.	120	68.9	7.8	31.8	359
	Sep.	82	50.0	8.5	31.0	425
	Oct.	63	55.0	8.0	30.0	525
	Nov.	30	55.0	9.0	31.0	225
	Dec.	10	62.0	8.2	30.8	200
1971	Jan.	21	70.1	6.9	31.5	150
	Feb.	1	73.2	6.0	32.5	120

APPENDIX C

Table 7. The mean monthly worm burdens in the tracer sheep in relation to monthly rainfall, monthly effective rainfall, mean monthly pasture height and indices of soil water status and soil surface moisture.

		Mean monthly total worm burden	Monthly rainfall	Monthly effective rainfall	Mean monthly		
					pasture height (metres)	soil water index (+200)	soil surface moisture index
1969	J	-	21	8.6	.10	106	10.6
	J	-	25	11.8	.08	114	9.1
	A	-	13	5.8	.05	79	4.0
	S	-	22	11.1	.05	66	3.3
	O	3	60	21.5	.06	53	3.2
	N	14	66	32.5	.15	17	2.6
	D	43	24	6.7	.10	60	6.0
1970	J	16	99	52.7	.05	177	8.9
	F	20	53	19.6	.11	100	11.0
	M	20	162	105.7	.08	85	6.8
	A	485	125	76.0	.15	216	32.4
	M	275	104	56.3	.10	192	19.2
	J	73	44	24.9	.05	133	6.7
	J	55	30	9.7	.06	106	6.4
	A	1580	120	91.5	.61	189	115.3
	S	430	82	48.3	.46	36	16.6
	O	793	63	31.8	.30	91	27.3
	N	483	30	6.5	.15	98	14.7
	D	90	10	0	.23	60	13.8
1971	J	30	21	10.4	.25	51	12.8
	F	5	1	0	.15	8	1.2
	M	0	8	0	.10	0	0
	A	5	74	20.9	.05	124	6.2
	M	1210	127	70.2	.40	222	88.8
	J	2136	21	3.4	.38	89	33.8
	J	1733	55	28.4	.24	143	34.3

APPENDIX C

Tables 8,9 and 10

The method of calculation of 'Soil Water Index ' from meteorological and hydrological data at Naivasha, Kenya. Over the period studied the mean monthly index was always in deficit.

Table 8. 1969	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	MEAN
<u>Mean monthly</u>													
faecal egg counts Lambs	1278	-	-	-	-	-	-	-	-	9	33	394	429
Ewes	130									-51	84	165	108
adult worm burden Lambs	1713									0	8	205	482
Ewes	60									50	20	48	45
total worm burden Tracers	-									3	14	43	20
<u>Mean Monthly</u>													
Maximum temp. (^{o}F)	81	79	79	83	77	75	77	78	79	83	77	82	79
Minimum temp. (^{o}F)	51	55	55	55	55	55	55	52	54=	55	53	53	54
Relative Humidity a.m.	78	86	84	77	79	72	92	79	92	90	74	99	84
Relative Humidity p.m.	53	68	84	46	56	48	69	75	75	81	79	81	68
<u>Mean Daily</u>													
Wind run (miles)	-	-	-	-	-	44	38	52	108	130	95	93	80
Radiation (Langleys)						501	415	505	473	562	416	533	486
Potential Evaporation						38	36	43	52	67	50	53	48
<u>Mean Monthly</u>													TOTAL
Rainfall (mm)	87	66	34	52	101	21	25	13	22	60	66	24	571
Potential Evaporation (mm)	168	124	148	163	112	115	111	133	155	207	149	164	1749
Rainfall - (Eo)	-81	-58	-114	-111	-11	-94	-86	-121	-134	-147	-83	-140	-1180
Soil Water Change	0	0	0	0	0	0	0	0	0	0	0	0	-
Storage	0	0	0	0	0	0	0	0	0	0	0	0	0
Surplus	0	0	0	0	0	0	0	0	0	0	0	0	0
Deficit	81	58	114	111	11	94	86	121	134	147	83	140	1180

Table 9.	1970	Jan	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	MEAN
Mean monthly														
faecal egg counts	Lambs	2306	3173	1551	-	-	-	-	0	2800	5817	5264	10,771	3960
	Ewes	1029	1749	1521	851	1134	2877	2180	2872	3298	3606	2709	3437	2272
adult worm burden	Lambs	258	118	-	-	-	-	-	50	2120	968	698	1060	753
	Ewes	51	128	220	740	40	300	263	11000	9210	4320	590	1200	2339
total worm burden	Tracers	16	20	20	485	275	73	55	1580	430	793	483	90	360
Mean Monthly														
Maximum temp. (^{o}F)		82	85	85	84	80	77	76	76	77	78	78	82	80
Minimum temp. (^{o}F)		51	47	60	50	48	46	44	48	43	46	45	47	48
Relative Humidity a.m.		71	59	68	77	63	89	79	68	66	61	72	53	69
Relative Humidity p.m.		57	42	37	38	55	56	44	50	46	50	50	57	49
Mean Daily														
Wind run (miles)		70	87	85	54	53	55	68	76	87	109	76	92	76
Radiation (Langleys)		434	527	477	426	422	444	431	401	461	490	436	476	452
Potential Evaporation		39	55	57	36	36	37	40	42	49	56	44	48	45
Mean Monthly														TOTAL
Rainfall (mm)		99	53	162	125	104	44	30	120	82	63	30	10	922
Potential Evaporation (mm)		122	153	177	109	113	112	124	131	146	172	131	150	1640
Rainfall - (Eo)		-23	-100	-15	+16	-8	-67	-94	-11	-164	-109	-102	-140	-717
Soil Water	Change	0	0	0	16	-8	-8	0	0	0	0	0	0	-
	Storage	0	0	0	16	-8	0	0	0	0	0	0	0	24
	Surplus	0	0	0	0	0	0	0	0	0	0	0	0	0
	Deficit	23	100	15	0	0	67	94	11	64	109	102	140	525

Table 10. 1971

		Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	MEAN
Mean monthly														
faecal egg counts	Lambs	4646	4099	2740	1688	948	4232	5471						3401
	Ewes	3226	2820	2595	2692	363	217	1850						1966
adult worm burden	Lambs	233	690	510	800	0	3000	7000						1748
	Ewes	1540	1180	540	560	37	3080	9980						2417
total worm burden	Tracers	30	5	0	5	1210	2136	1733						731
Mean Monthly														
Maximum temp.	(°F)	84	85	85	80	75	74	72						79
Minimum temp.	(°F)	59	54	57	50	49	47	45						52
Relative Humidity a.m.		50	52	54	70	70	83	81						66
Relative Humidity p.m.		46	28	29	75	59	61	60						51
Mean Daily														
Wind run (miles)		93	106	102	82	66	88	79						88
Radiation (Langleys)		501	518	461	459	431	478	438						469
Potential Evaporation		57	69	71	50	37	44	36						52
Mean Monthly														TOTAL
Rainfall (mm)		21	1	8	74	127	21	55						307
Potential Evaporation (mm)		176	193	220	150	105	132	112						1088
Rainfall- (Eo)		-149	-192	-213	-76	22	-111	-57						-776
Soil Water	Change	0	0	0	0	22	-22	0						-
	Storage	0	0	0	0	22	0	0						22
	Surplus	0	0	0	0	0	0	0						0
	Deficit	149	192	213	76	0	111	57						798

APPENDIX D

HAEMOGLOBIN SEPARATION

The haemoglobin separation was performed by starch-gel electrophoresis from a modification of Smithies' technique (1955) using TRIS-EDTA Boric acid buffer adjusted to pH 8.7. Following triple washing of the red blood cells by the addition of physiological saline (8.5g NaC1/1) and centrifugation at 2,000rpm for 10 mins, the red blood cells were haemolysed by the addition of distilled water.

Stock buffer was made up by the addition of 20.2g TRIS (Trihydroxymethyl Methylamine (Puriss*), 2.0g sodium EDTA and 1.5g boric acid to one litre of distilled water and the pH adjusted to pH 8.7. Fifty-three ml of stock buffer was added to 347 ml of distilled water and heated to boiling point before being added to a solution of 100 ml distilled water and 62g of hydrolysed starch.** After adding the two solutions together in a Beuchner funnel, the mixture was shaken vigorously and the air bubbles removed by prompt connection to an electric suction pump. The starch solution was poured onto a glass plate to a depth of about 3 mm and confined to a rectangular area by siliconed perspex strips (Fig.1). A second glass plate was placed over the top of the strips in order to express any excess solution and the gel was then left to cool for about one hour. The top glass was removed after loosening it from the underlying gel by passing a fine wire between them. The rectangular gel was divided into horizontal strips about 5 cm wide by the careful use of a palette knife so as to allow insertion of the samples in three serial rows.

* Koch-Light Laboratories Ltd., Colnbrook, Bucks, England.

** Connaught Medical Res. Labs., Unv. of Toronto, Canada.

A small square of blotting paper (approx. 3mm x 3mm) was dipped into the haemolysed blood sample and excess fluid removed by placing it briefly on the absorbent tissue. The square was then placed in a vertical position at one end of the first cut edge of the gel and further samples were placed along the edge of the gel, each about 1 mm apart. When the line was filled the middle section of gel was carefully eased upwards so as to close the initial cut in the gel, and produce another trench below for the insertion of more samples. When all the blood samples had been inserted into the gel it was arranged for electrophoresis so that the upper and lower edges were in contact with stock buffer solution by means of pieces of absorbent tissue. A sheet of cellophane was then placed over the upper surface of the gel in order to minimise evaporation. A direct electric current of 450 volts and 20 amps was connected for 1 minute and then the small sample blotting papers withdrawn. The current was restored and maintained for between $1\frac{1}{2}$ to 2 hours until a good separation of haemoglobin was obtained.

The top surface of the gel was then removed by drawing a taught fine wire along the perspex side-strips. The starch gel was then statined by pouring Amido Black (5% solution) over the surface for two minutes. The gel was then placed in destaining fluid (100 ml glacial acetic acid 250 ml methanol and 250 ml distilled water) for one to two days. The gels were then examined for identification of Hb types.

APPENDIX D

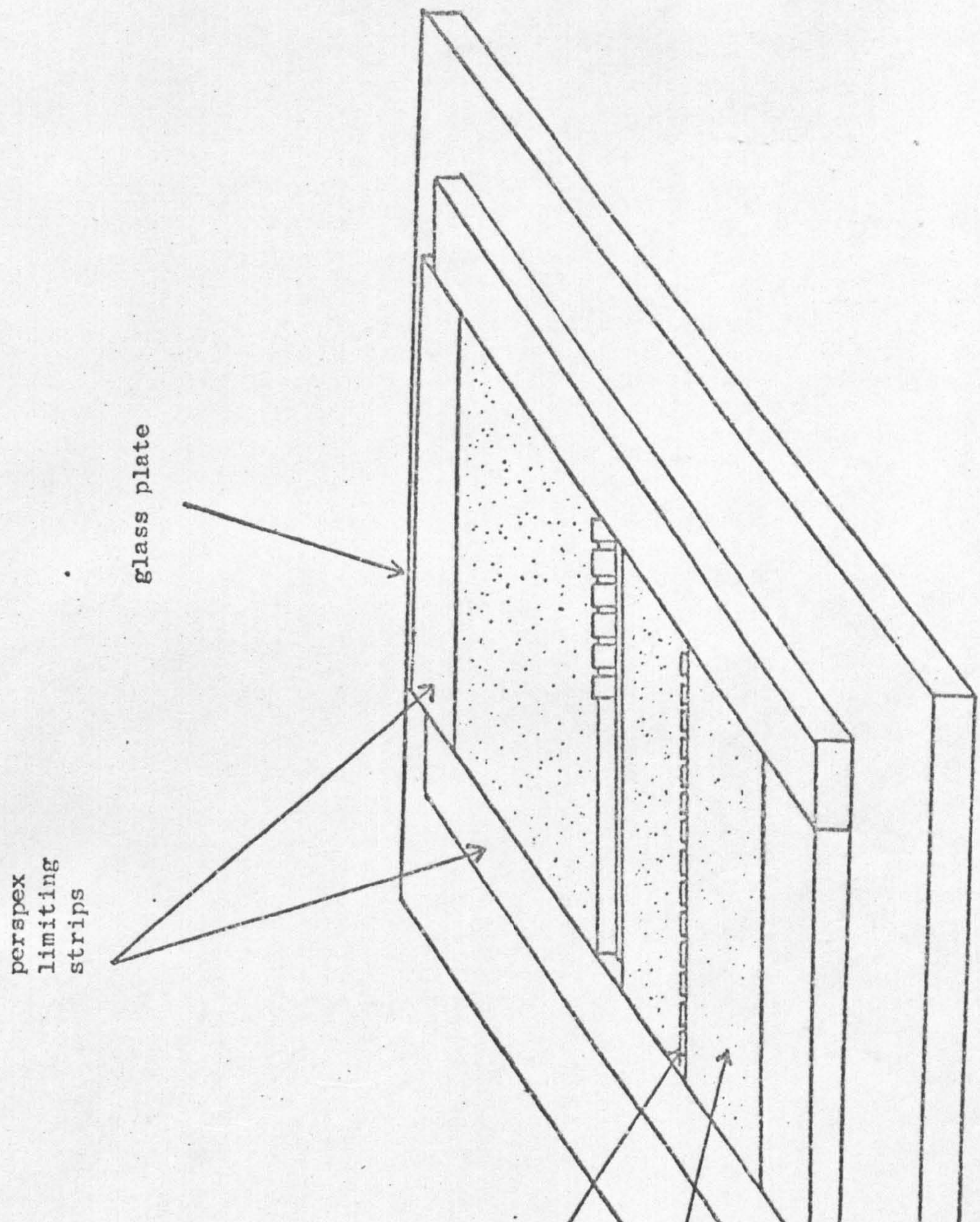

Fig. 1. Diagram to illustrate the positioning of the blood samples in the starch gel apparatus prior to the electrophoretic separation procedure.

References

Agar, N.S. (1968). Experimentia, 24, 1274.

Agar, N.S., Rawat, J.S. & Roy, A. (1969). Anim. Prod., II, 274.

Agar, N.S. & Seth, O.N. (1971). Amer. J. Vet. Res., 32, 361.

Andrews, J.S. (1942). J. Agric. Res., 65, 1.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.D.S. & Urquhart, G.M. (1965). Vet. Rec., 77, 1196.

Anon. (1948). E. Afr. Agric. J., 14, 1.

Armour, J. & Bruce, R.G. (1974). Int. J. Parasit. In press.

Baker, N.F., Cook, E.F., Douglas, J.R. & Cornelius, C.E. (1959). J. Parasit., 45, 643.

Baker, N.F. & Douglas, J.R. (1966). Biology of Parasites. Academic Press, London.

Berberian, J.E. & Mizelle, J.D. (1957). Amer. Midl. Nat., 57, 421.

Blood, D.C. & Henderson, J.A. (1963). Veterinary Medicine. Bailliere, Tindall & Cox, London.

Boughton, I.B. & Hardy, W.I. (1936). Rep. Tex. Agric. Exptl. St., 49, 276.

Brunsdon, R.V. (1970). N.Z. Vet. J., 18, 47.

Chan, G. (1968). Rev. Vet. Inst. Trop. High Alt. Res., Lima (July), 7.

Clark, C.H., Kiesel, G.K. & Goby, C.H. (1962). Amer. J. Vet. Res., 23, 977.

Crofton, H.D. (1958). Parasitology, 48, 243.

Cvetkovic, L., Golosin, R. & Kosanovic, M. (1971). Acta Vet., 21, 77.

Dacie, J.V. & Lewis, S.M. (1963). Practical Haematology. 3rd Edit. J. & A. Churchill, London.

Dagg, M. (1965). E. Afr. Agric. & For. J., 30, 296.

Das, K.M. & Whitlock, J.H. (1960). Cornell Vet., 50, 182.

References (Cont'd.)

Dassat, P. & Sartore, G. (1963). Zootec. Vet., 18, 17.

Dassat, P. (1964). Alti. Ass. genet. Italy, 9, 146.

Dinaburg, A.G. (1944). J. Agric. Res., 69, 421.

Dineen, J.K. & Wagland, B.M. (1966). Parasitology, 56, 639.

Dinnik, J.A. & Dinnik, N.N. (1958). Bull. Epiz. Dis. Afr., 6, 11.

Donald, A.D. (1968). Austral. Vet. J., 44, 139.

Donald, A.D. & Waller, P.J. (1973). Int. J. Parasit., 3, 219.

Ellenby, C. (1968). J. Expt. Biol., 49, 469.

Evans, J.V. & Blunt, M.H. (1961). Austral. J. Biol., 14, 100.

Evans, J.V., Blunt, M.H. & Southcott, W.H. (1963). Austral. J. Agric. Res., 14, 549.

Evans, J.V., Harris, H. & Warren, F.L. (1958a). Proc. Roy. Soc., London. B.149, 249.

Evans, J.V., Harris, H. & Warren, F.L. (1958b). Nature, Lond., 182, 320.

Evans, J.V., King, J.W.B., Cohen, B.L., Harris, H. & Warren, F.L. (1956). Nature, Lond., 178, 849.

Evans, J.V. & Whitlock, J.H. (1964). Science, 145, 1318.

Field, A.C., Brambell, M.R. & Campbell, J.A. (1960). Parasitology, 50, 387.

Fisher, E.W. (1962). Brit. Vet. J., 118, 513.

Fourie, P.J.J. (1931). Rept. Dir. Vet. Res. S. Africa, 17, 495.

Gabuzda, T.G., Schuman, M.A.A., Silver, R.K. & Lewis, H.B. (1968). J. Clin. Invest., 47, 1895.

Gethin-Jones, G.H. & Scott, R.M. (1970). Atlas of Kenya. 3rd Edition, Nairobi.

Gibbs, H.C. (1967). The Reaction of the Host to Parasitism. Vet. Med. Rev., 160.

/-

References (Cont'd.)

Gordon, H.McL. (1948). Austral. Vet. J., 24, 17.

Gordon, H.McL. (1949). Rept. Austral. & N.Z. Ass. Adv. Sci., 27, 131.

Gordon, H.McL (1950). Austral. Vet. J., 26, 14, 46, 65, 93.

Gordon, H.McL. (1958). Austral. Vet. J., 34, 5.

Gordon, H.McL. (1967). The Reaction of the Host to Parasitism. Vet. Med. Rev. pp. 174-190.

Gordon, H.McL. & Whitlock, H.V. (1939). J. Counc. Sci. Indust. Res. Austral., 12, 50.

Harris, H. & Warren, F.L. (1955). Biochem. J., 60, XX1X.

Helm, H.J., van Vliet, G. & Huisman, T.H.J.(1957). Arch. Biochem. & Physiol., 72, 331.

Hudson, J.P. (1965). Exptl. Agric., 1, 23.

Hughes, K.L. (1960). M.V.Sc. Thesis. Univ. Queensland, Australia.

Huisman, T.H.J. (1963). Adv. Cli . Chem., 6, 231. Academ.Press, N.Y.

Huisman, T.H.J., van Vliet, G. & Sebens, T. (1958a). Nature, Lond., 182, 171.

Huisman, T.H.J., van Vliet, G. & Sebens, T. (1958b). Nature, Lond., 182, 172.

Jilek, A.F. & Bradley, R.E. (1969). Amer. J. Vet. Res., 30, 1773.

Kates, K.C. (1950). Proc. Helm. Soc. Wash., 17, 39.

Levine, N.D. (1963). Advances in Veterinary Science. Academic Press, New York. Vol. 8, 215.

Lewis, K.H.C. & Stauber, V.V. (1969). Proc. N.Z. Soc. An. Prod., 29, 177.

Loggins, P.E., Radhakrishnan, C.V., Bradley, R.E. & Franke, D.E. (1973). Proc. Wld. Cong. An. Prod. Melbourne. 1973.

Lopez, V. & Urquhart, G.M. (1967). The Reaction of the Host to Parasitism. Vet. Med. Rev. pp. 153-159.

Marsh, H. (1958). Newsom's Sheep Diseases. Bailliere, Tindall & Cox, London.

Meyer, H. (1963). Zach. Tier. Zucht. Biol., 79, 162.

/-

References (Cont'd.)

Monning, G. (1962). Veterinary Helminthology & Entomology. 5th Edition. Bailliere, Tindall & Cox, London.

Nadi, A.H.E.L. & Hudson, J.P. (1965). Exptl. Agric., 1, 289.

Ollerenshaw, C.B. & Rowlands, W.T. (1959). Vet. Rec., 71, 591.

Ollerenshaw, C.B. & Smith, L.P. (1966). Vet. Rec., 79, 536.

Ollerenshaw, C.B. & Smith, L.P. (1969). Advances in Parasitology. 7, 283. Academic Press Inc., New York.

Penman, H.L. (1948). Proc. Roy. Soc. Lond. (A), 193, 120.

Pratt, D.J. Greenway, P.J. & Gwynne, M.D. (1966). J. App. Ecol., 3, 369.

Pradhan, S.L. & Johnston, I.L. (1972). Parasitol., 64, 153.

Radhakrishnan, C.V., Bradley, R.E. & Loggins, P.E. (1972). Amer. J. Vet. Res., 33, 817.

Rees, F.G. (1950). Parasitology, 40, 127.

Roberts, F.H.S. & O'Sullivan, P.J. (1950). Austral. J. Agric. Res., 1, 99.

Rose, J.H. (1963). Parasitology, 53, 469.

Round, M.C. (1962). J. Helminth., 36, 375.

Salter, P.J. (1967). Exptl. Agric., 3, 163.

Shorb, D.A. (1943). Jnl. of Parasitology, 29, 284-289.

Shorb, D.A. (1944). Jnl. Agric. Research, 69, 279.

Silangwa, S.M. & Todd, A.C. (1964). J. Parasit., 50, 278.

Silverman, P.H. & Campbell, J.A. (1959). Parasitology, 49, 23.

Smith, L.P. (1969). Weather and Animal Diseases. Tech. Note No. 113. W.M.O., Geneva.

Smithies, O. (1955). Biochem. J., 61, 629.

Soulsby, E.J.L. (1965). Textbook of Veterinary Clinical Parasitology. Blackwell Scientific Publications, Oxford.

Spedding, C.R.W. & Brown, T.H. (1956). J. Helminth., 29, 171.

/-

References (Cont'd.)

Spindler, L.A. (1936). Agric. Notes No. 74. Puerto Rico Agric. Exptl. St.

Stewart, D.F. (1950a). Austral. J. Agric. Res., 1, 301.

Stewart, D.F. (1950b). Austral. J. Agric. Res., 1, 427.

Stewart, D.F. (1953). Austral. J. Agric. Res., 4, 100.

Stoll, N.R. (1929). Amer. J. Hyg., 10, 384.

Stoll, N.R. (1943). J. Parasit., 29, 407.

Swan, R.A. (1970). Austral. Vet. J., 46, 485.

Taylor, E.L. (1935). J. Parasit., 21, 175.

Todd, A.C., Levine, N.D. & Whiteside, L. (1970). J. Nematol., 2, 330.

Veglia, F. (1915). Rept. Dir. Vet. Res. S. Africa, 3, & 4, 347.

Waller, P.J. & Donald, A.D. (1970). Parasitology, 61, 195.

Weide, K.D., Trapp, A.L., Weaver, C.R. & Lagace, A. (1962). Amer. J. Vet. Res., 23, 632.

Whitlock, H.V. (1948). J. Counc. Sci. Indust. Res. Austral., 21, 177.

Whitlock, J.H. (1960). The Diagnosis of Veterinary Parasitisms. Kempton, London.

Whitlock, J.H. & Georgi, J.R. (1968). Cronell Vet., 58, 90.

Woodhead, T. (1970). J. App. Ecol., 7, 647.