STUDIES ON HELMINTHIASIS IN THE PIG

BY

DENNIS ERIC JACOBS

A THESIS SUBMITTED FOR
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SUMMARY

When this project was initiated in 1964, there was no comprehensive information available on the helminth fauna of British pigs. In order to establish a base for epidemiological studies, post-mortem material from 3,800 pigs was examined.

Twelve helminth species were identified, including five not previously recorded from pigs in the United Kingdom. The incidence of Ascaris suum and Trichuris suis was found to decline with the increasing age of the host, whilst Oesophagostomum spp. and Hyostrongylus rubidus were encountered most frequently in the adult stock. The latter genera occurred in the greatest numbers in the spring and early summer. The significance of these observations, including the prominent role of the sow in the epidemiology of oesophagostomiasis, is discussed.

Data collected from field studies showed that the number of Oesophagostomum ova shed in the faeces of sows is greatest when the piglets are suckling. After weaning, there is a sudden drop in values caused by the expulsion of adult worms. The periparturient rise in egg-counts does not take place if the progeny are taken away at birth. Smaller, regular peaks in the Oesophagostomum egg output of one non-pregnant sow coincided with oestrus. The administration of certain hormones (FSH and LH; progesterone; ACTH) modified this pattern.

The periparturient egg-rise appears to enhance the opportunities for the transmission of infection to successive

generations of pigs. In addition, biological pathways exist for the dispersion of larvae from the dung-pat. Third stage Oesophagostomum juveniles will, for example, coil around the abdomen of certain manure breeding flies (Psychoda spp.), which then function as transport hosts. Under laboratory conditions, porcine oesophagostome larvae can invade the intestinal mucosa of rats where they become encapsulated. If the rat tissues are fed to pigs they are able to resume their development and grow to maturity.

A single dose of a broad spectrum anthelmintic given to gilts or sows one to two weeks before the expected date of farrowing was shown to eliminate the periparturient egg-rise. A new anthelmintic, 0,0-dimethyl-2,2-dichlorovinyl phosphate, was evaluated for the first time in adult pigs. It proved to be highly effective and well tolerated. Litters from sows treated with this compound were approximately 4% heavier at weaning than those from control dams.

Other workers have demonstrated that one sequel of oesophagostomiasis in ruminants is an accelerated rate of plasma protein catabolism. It seemed possible therefore that the reduced growth-rate of infected piglets may be associated with a similar dysfunction. Serum samples from experimentally infected pigs did not, however, reveal any marked hypoalbuminaemia. When albumin catabolism was measured by studying the fate of injected ^{125}I-albumin in parasitized and worm-free animals, no difference could be demonstrated. This result was confirmed by observing the rate of passage of ^{131}I-polyvinylpyrrolidone into the gastro-intestinal tract following intravenous injection. Again, identical values were obtained for test and control animals.

Finally, the use of the guinea-pig as a possible substitute for the pig in experimental Oesophagostomum infections was examined. It was discovered that larvae would invade the intestinal mucosa and that those remaining at this site formed the nucleus for nodule formation.

Others migrated further to the mesenteric lymph nodes, peritoneum and liver. The worms neither grew nor moulted even though many remained alive for 48 days. The behaviour of Oesophagostomum in the guinea-pig thus differs from that normally displayed in the natural host.

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(A) HISTORY

0.1. Recent History

After thirty years of neglect, a renewed interest in the subject of porcine helminthiasis was generated in the early part of the nineteen-sixties. This revival was not spontaneous but was dictated by economic and commercial pressures. The financial structure of the pig industry was changing. More capital investment was needed yet profit margins were becoming ever smaller. Successful business depended more and more on the extraction of the maximum profit from each pig. In order to attain the highest productivity, every factor that might influence the viability, growth-rate or food conversion ratio of growers and fatteners had to be taken into consideration. The Pig Industry Development Authority undertook to stimulate and co-ordinate research in the fields of pig husbandry, genetics, economics and veterinary science. This organisation was able to take advantage of the fact that more expertise could be devoted to topics formally of lesser importance now that many of the major pig diseases such as foot-and-mouth, swine fever, erysipelas etc. had been eradicated or controlled. A

number of projects including that reported within these covers were thereby promoted with the object of defining the significance of nematode infestations to the pig industry.

In addition, the advent of the broad-spectrum anthelmintics created a situation whereby highly active products were made available before sufficient knowledge had accumulated to enable them to be used in the most effective manner. It was, therefore, in the interest of a sector of the pharmaceutical industry to encourage academic and applied studies into the biology and pathogenesis of each parasite in order to elucidate the benefits of treatment and to provide a rational basis for the planning of therapeutic programmes.

In this way, a number of independent investigations were initiated simultaneously in different parts of the United Kingdom. The major deficiencies in the British literature were obvious and as a result a certain amount of duplication of work took place,

there being regrettably little communication or co-operation between interested parties. In general, however, the separate projects were complimentary and experiences gained from each could be used in the design of subsequent experiments. A consequence of this mode of development is an occasional diversion from the apparently logical progression of argument and experiment in this thesis.

0.2. Early History

It is strange to think that these studies are the most recent of a succession of clinical and philosophical treatises that started three thousand years ago in twentieth dynasty Egypt (Cheng, 1964). The observations of the ancients were largely centred around *Ascaris* because of its large size. Taffs (1961, 1966) has contributed a comprehensive review of the development and present state of our knowledge of ascariasis. Though it is natural that this aesthetically repugnant parasite should have dominated the attention of farmers and veterinarians over the ages, this thesis is primarily concerned with the gastro-intestinal

strongylate genera Oesophagostomum and Hyostrongylus.

(B) OESOPHAGOSTOMUM SPP.

0.3. The Adult

Oesophagostomum dentatum from the pig was discovered by Rudolphi (1803). Krabbe, in an undated manuscript from the last century, states his belief that the oesophagostomes of Danish pigs are the larval stages of Stephanurus dentatus and expresses his surprise at not being able to find the adult kidney worm in Denmark. Ostertag and Olt, in Germany, realized that the rod-like nematodes in the porcine caecum and colon were the final stage of a parasite whose larvae lived in the mucosa provoking nodular swellings (Olt, 1898). Stiles and Hassall (1920) record this parasite as the type species of the genus. The first detailed descriptions of its morphology were made by Goodey (1924, 1926).

Oe. dentatum is a bursate nematode belonging to the superfamily, Strongyloidea. The females measure 11 - 14 mm. in length by 0.4-0.5 mm. in breadth, whilst the males are smaller: 8-10 mm. x 0.2-0.3 mm. The cuticle

is transversely striated and inflated anteriorly to form a mouth collar with six circumoral papillae and a cervical vesicle which is terminated at a point one-third of the way along the oesophagus by the cervical groove. This latter structure is well developed ventrally but becomes more shallow laterally and disappears on the dorsal surface. Cervical papillae are present being found almost level with the posterior end of the oesophagus. The mouth is centrally placed and leads into the mouth capsule which consists of a ring of cuticular material. To this is attached the external leaf crown composed of nine large triangular units and the internal leaf crown made up of 18 smaller structures. Two large cervical glands are very conspicuous extending back as far as the gonads.

Two features of the male are particularly useful for specific identification: the spicules and the gubernaculum. The anterior ends of the long featureless and pliable spicules are expanded at right angles to the longitudonal axis. The posterior extremities terminate in a fine rounded point, but just anterior to this is a small

membranous wing. The total length is 1.15-1.3 mm. The gubernaculum is shaped like a garden trowel with the handle and blade each of the same length. The female tail is straight and tapers to a point. The anus is situated 0.35 mm. from the tip of the tail and the vulva 0.36-0.38 mm. in front of this. Goodey's description was so detailed and accurate that few additions have since been made. Tarzcinski (1955) comments on small details concerning the genital cone and Zakhryalov (1956) describes variations in the shape and position of the female sex organs, the shape of the dorsal ray of the bursa and the size of the spicules and gubernaculum. An extensive account of the nervous system has been contributed by Ramisz (1966).

Four other species are known to occur in the domestic pig. Oe. quadrispinulatum, originally discovered by Marcone (1901), was described fully by Goodey (1925) under the name Oe. longicaudum. That these two designations referred to the same parasite was demonstrated by Alicata (1935a). The males of

this species have shorter spicules (0.91-0.95 mm.) and the handle of the gubernaculum is only one half as long as the blade. The female tail is longer (0.38-0.46 mm.) and the anterior part of the oesophagus of both sexes is expanded. Other morphological descriptions of this species have been given by Ozerkaja (1930), Linzcano Herrera (1958), Schmeer (1958), Popova (1958), Diaouré (1964) and Costa (1965). Additional details for identification have been given by Schwartz and Alicata (1930) and Haupt (1966). Aberrant forms are described by Spindler (1932a).

Oe. brevicaudum and Oe. georgianum were discovered by Schwartz and Alicata (1930). The former has 14-16 and 28-32 elements in the external and internal leaf crowns, other mouth structures being like Oe. quadrispinulatum; spicules and gubernaculum like Oe. dentatum and female tail measuring no more than 81-120 μ long. This species was described by Maplestone (1930) under the name

of Oe. suis (Schwartz, 1931). Oe. georgianum differs from Oe. dentatum in the shape of the gubernaculum (the handle is short and twisted) and the female tail is shorter (217 μ) possessing a distinct convexity on the terminal part of the ventral surface. Finally, Oe. conicum, renamed Oe. maplestoni by Schwartz (1931), lacks a cephalic vesicle (Maplestone, 1930).

Three other new species have been claimed but their validity has not yet been confirmed. Oe. granatensis has a large cephalic vesicle with large cervical papillae more anteriorly placed than in Oe. dentatum. The spicules and female tail are a little shorter (1,010-1,100 μ and 250-350 μ respectively) than in the latter species (Linzcano Herrera, 1958). The body lengths of the specimens that he examined were, however, rather shorter than normal and it is possible that these may have been immature Oe. dentatum or aberrant forms that had developed in an unfavourable environment. Linzcano Herrera does not state how many examples of the new species he found or how many pigs were infected. No

more recent reports of its discovery have been made from Spain or elsewhere.

Oe. hsiungi is a new species that resembles Oe. maplestoni in that it has no cephalic vesicle. Since the characteristics of this parasite have only been published in Chinese (Ling, 1959), it is difficult to decide whether or not these two species are synonymous. Oe. rousseloti has 30-32 elements in the external leaf crown and 60-64 in the internal. It also has extremely long spicules (2.3 mm.) and a heart-shaped gubernaculum (Diaoure, 1964).

Numerous other Oesophagostomum species have been recovered from other members of the genus Sus, but these are not relevant to the present discussion.

0.4. Geographical Distribution

Oe. dentatum is ubiquitous being recorded from all parts of the world where records are available. It has been recovered from the peccary but is otherwise confined to domestic and wild pigs. Oe. quadrispinulatum is

distributed almost as widely although its occurrence tends to be more sporadic. It is found in North and South America, the Far East, the U.S.S.R., the Pacific Islands, Africa and Europe (Goodey, 1925; Schwartz, 1925; Kotlan, 1948; Popova, 1958; Costa, 1965a; Diaouré, 1964; et multi alia). In the United States, it is more common in the southern than in the more northern states (Schwartz, 1931), as are Oe. brevicaudum and Oe. georgianum. The latter species appears to be restricted to that one locality, but Oe. brevicaudum is known to occur on the Indian sub-continent (Maplestone, 1930; Alwar, 1958) so that its distribution may be greater than at present suspected. Kotlan (1956) makes casual reference to the contamination of his Oe. dentatum cultures with Oe. brevicaudum larvae. As far as is known, Oe. maplestoni is confined to India, Oe. granatensis to Spain, Oe. hsiungi to China and Oe. rousseloti to the Congo.

In Britain, Morgan (1924) found two of 17 pigs to be infected with Oe. dentatum; Jones (1926) two of 19; Lewis

(1930) one of 62; White (1955) 47 of 53 and Jenkins and Erasmus (1963) seven of 27.

0.5. Larval Stages

The larvae hatch from typical 'strongyle' eggs passed in the faeces of the host. These contain 2-16 cells and measure approximately 68 x 36 μ (Alicata, 1935b; Supperer, 1955; White, 1955). The inner wall of the ovum has several discrete thickenings (Honer, 1967). It is impossible to differentiate the eggs of Oe. dentatum and Oe. quadrispinulatum (Haupt, 1966).

The first larvae will be found 23 hours after deposition into an environmental temperature of 22-24°C. This rhabditiform juvenile has a long pointed tail that accounts for one-quarter of its total body length which is 304-433 μ. The buccal cavity is long with parallel rod-shaped walls (Goodey, 1924; Alicata, 1935b).

In a further 27 hours, the first ecdysis takes place. The second stage larva is very much like its predecessor but measures up to 655 μ. The next moult occurs after the

123rd hour. The cuticle of the second stage is retained and forms numerous well defined folds when the body is bent during locomotion. The origin of the sheath-tail has a small solid enlargement (Honer, 1967). The larva is 660-670 μ long including the sheath, or 500-532 μ without. The head has small lateral depressions posterior to which are six protruberences. The tail has a conical end bearing a characteristically pointed tip (Goodey, 1924; Alicata, 1935b; Kotlan and Vajda, 1939; White, 1955; Pogrebnyak, 1962). The larval tail measures 43-46 μ in Oe. dentatum and 50 μ in Oe. quadrispinulatum (Haupt, 1966).

Goodey (1924) and Alicata (1935b) also observed the behaviour of the ensheathed third stage larvae. They move comparatively sluggishly with a sinuous motion and do not attempt to swim or to climb. They will remain alive for ten months if kept moist but desiccation will kill some in as little as 22 hours. Freezing at -15°C for one month is lethal. The larvae will not penetrate unbroken skin.

The parasitic phase of the life-cycle commences when the third stage larva is swallowed. Exsheathment is

followed by penetration of the wall of the caecum or colon. Here, the first parasitic moult takes place four to five days after infestation and the emergent larvae begin their return to the lumen of the intestine on the sixth day (Kotlan, 1948; Shorb, 1948), although a proportion are inhibited in their development (Shorb and Shalkop, 1959). The structural changes that take place follow the sequence usual for the Strongyloidea. The fourth stage larva is recognisable because of the large rectangular provisional buccal capsule. The female tail lengthens whilst that of the male, still without a bursa, shortens. The genital primordium slowly develops into the gonads and associated organs. Detailed accounts of the transitional process culminating in the formation of the features characteristic of the adult worm after the second parasitic moult on the 20th-30th day have been provided by Goodey (1926) and Kotlan (1948).

The prepatent period of the pig oesophagostomes is generally acknowledged to be five to seven weeks, but recently Nickel and Haupt (1964) and Taffs (1966, 1968a)

have noticed a small transitory shedding of ova at about 21 days after experimental infection. Supperer (1955) has observed positive egg-counts in piglets 26 days old.

Third stage larvae can be induced to exsheath and moult twice *in vitro* to attain the fifth stage of their development (*vide* review by Taylor and Baker, 1968).

0.6. Ecology of Free-Living Forms

No publications exist describing the ecology of the free-living stages of the oesophagostomes of pigs under British conditions. Spindler (1933a) has, however, conducted extensive studies in the state of Georgia, U.S.A. He found that the larvae were killed in all areas exposed to direct sunlight and desiccation, but that numerous infective forms could be recovered from any cool, damp situation including the ground beneath trees or huts. The worms could retain their viability under faecal piles even in the hottest, most exposed places. In cooler areas, larvae could be recovered up to 30 cms. from the dung-pat. Wind, rain and moving creatures were responsible for this dispersion, whilst

only the pigs themselves could have carried infective material into the pig-huts as faeces were not deposited in these. The degree of infestation was related to the feeding regime. The more the pigs were encouraged to root about for food, the heavier the worm burdens. Ploughing and re-cropping were completely effective for ridding the ground of infection. Morgan and Hawkins (1949) state that the larvae can survive in the field for at least 14 months, although the source of this information is obscure. The free-living larvae on soil may be killed by the application of sodium borate at a rate of 5 lb. per 100 square feet (Alicata, 1955).

On the basis of his experiences, Spindler (1933a) believed that an Oesophagostomum-free herd could be established by practicing a modified form of the M'Clean County swine sanitation system. This method was intended for the control of Ascaris and Stephanurus and involved the treatment of the sow with an anthelmintic before parturition. She was then washed and scrubbed and

placed in a disinfected farrowing pen. Ten days after the pigs were born, the dam and litter were transported to a non-infected field surrounded by an unplanted border. The feeding troughs, watering tanks and huts were so placed that most of the dung and urine would be deposited on bare ground where the hot sun would soon destroy helminth eggs and larvae.

El Rafaii (1962) presents experimental evidence to show that the earthworm and cockroach may act as "intermediary vectors" for Oesophagostomum spp. of the pig. These investigations were conducted without control animals and the possibility that the experimental pigs had acquired natural infections cannot, therefore, be excluded. This work must be confirmed before the results can be fully accepted.

0.7. Population Dynamics

Aspects of this subject have been studied in depth by Tarczinski (1961). He compared the structure of Oe. dentatum populations from domestic pigs and wild swine. Only small numbers of this nematode species were

found in the 11.7% of the feral pigs that carried infection. On the other hand, large infestations were frequently encountered in the domesticated animals, 75% of which were parasitized. Such infections could reduce the growth-rate of the host by 10-60%. Calculations based on the length and breadth of the worms showed that a population size of 51-100 Oe. dentatum seemed to be optimal for the growth of the parasite. More ova were produced by the members of small than large populations and the highest egg-counts were recorded in the warmer months of the year (April-October). The domestication of the pig, by altering the physiology of the host and the nature of the external environment, has created conditions more favourable to the parasite i.e. the host-parasite balance has been disturbed to the detriment of the host.

The same author (Tarczinski, 1955) noted that Oe. dentatum is less prevalent in pigs suffering from swine fever than in healthy stock, presumably because the intestinal environment is altered. This explanation

may also account for an observation made by Spindler and Zimmerman, (1944). They were able to eliminate worm parasites, including Oesophagostomum spp. from the pig by withholding solid food for three to five days and feeding skim-milk ad libitum. Growing pigs remained relatively free of parasites if skim-milk was fed once a day. It should be noted, however, that skim-milk has been the most important item of the ration given to Scandinavian pigs for many years, but oesophagostomes are still frequently encountered.

0.8. Pathology

Entry of the larvae into the intestinal mucosa occurs only in the caecum and colon, the site of entry being marked by petechiation. Later, small nodular lesions develop on the luminal surface of the intestinal wall. In the case of Oe. dentatum, these take the form of a flat elevation which does not become conspicuous until after the sixth day of its development. After larval emergence, the lesion is "lentil-sized" and umbilicated, sometimes with a plug of caseous material

(Kotlan, 1948). The lesion takes six to seven weeks to regress (Kotlan, 1956). The nodules formed by Oe. brevicaudum are a little larger and are accompanied by a slight inflammation of the tissues at the edge of the lesion (Stewart and Becklund, 1957). Ten-day old Oe. quadrispinulatum lesions are 2 mm. high by 5 mm. broad with a central depressed area and a marked inflammatory zone around each (Spindler, 1933). Histologically, the latter author observed the deposition of a homogenous eosinophilic substance around the larvae within 48 hours of infection with associated "intense inflammation" and "extensive liquifaction of the tissues". At ten days, the nodules contained "a mass of fibrin, coagulated serum and connective tissue cells" and by the 17th day the cysts left by emergent worms had become filled with leucocytes and connective tissue. Only scar tissue remained on Day 35. Taffs (1968a) does not share the view that the nodules disappear so quickly as he frequently found nodules 55 days after experimental infections. Shorb (1948) gave pigs pure cultures of Oe. dentatum or of Oe. quadrispinulatum but does not record any differences in the pathological effects of the two species. The gross

pathology in Shorb's series varied with the number of larvae administered. Thus, with less than 1,000 the mucosa became slightly hyperaemic; with 10,000-130,000 there was hyperplasia of the local lymph nodes, oedema of the meso-colon, thickening of the intestinal wall and widespread haemorrhages. In the larger infections, the mucosa was sometimes covered by a diphtheritic membrane, a feature also noted by other workers (Schwartz, 1931; Kotlan, 1956; Nickel and Haupt, 1964). Schwartz (1931) considered that nodules may become ulcerated and invaded by micro-organisms, whilst Kotlan (1956) and Nickel and Haupt (1964) describe the onset of bacterial disease exacerbated by Oesophagostomum invasion.

Goodey (1926) was unable to find any nodular lesions in experimentally or naturally infected pigs. In view of the other studies reported above, it would seem that this author was mistaken even though his conclusion is supported by Kouno and Niimi (1953). Kotlan (1948) charitably suggests that the lesions had regressed in the

animals examined by Goodey. The latter author, however, states that he was unable to see "any sign of intestinal nodules such as are produced by O. columbianum in sheep and goats and O. radiatum in cattle". This implies that he was searching for the protruding subserosal lesions and did not survey the luminal surface of the intestine. Grossly, Oe. dentatum nodules resemble to some extent the lymphoid aggregations that can be found in non-parasitized pigs and this may provide an alternative explanation for the anomalous observations, as well as for Ahluwalia's opinion (1960) that the third stage larvae of the genus in question select lymphatic tissue for their site of penetration.

Little is known of the feeding habits of Oesophagostomum. Wetzel (1934) states that the adult worms cause a chronic catarrh of the mucosa and apparently use the products of inflammation to satisfy their dietary needs. Taffs and Davidson (1967), however, claim to have shown the presence of blood in the intestine of the nematodes by means of the benzidine test.

Alicata (1934) infected four guinea-pigs with 3,000 larvae. He observed nodules scattered throughout the walls of the caecum, colon and rarely the small intestine. Two fourth stage larvae were recovered from the mucosal lymph follicles. In a further experiment during which a rabbit was infected with Oe. dentatum, one larva was recovered from the liver.

0.9. Clinical Aspects

The early American authors were primarily concerned about the economic damage caused by the presence of lesions in the intestinal wall. The tissues of the digestive tract were needed for human consumption, but if badly affected by Oesophagostomum nodules, were often condemned on aesthetic grounds (Schwartz, 1931).

In Britain, Blisset and Little (1930) attributed two deaths in pigs to heavy Oe. dentatum infections, but Cameron (1933) does not consider this parasite to be of any practical significance. The modern opinion is that Oesophagostomum does not often cause overt disease in the

United Kingdom but that it is probably associated with reduced productivity measured in terms of live-weight gains or, perhaps, sow fertility (Taffs, 1969; et multi alia). Some European authors believe that Oesophagostomum spp. may provoke outbreaks of disease caused by Salmonella cholerae-suis (Kotlan, 1956; Nickel and Haupt, 1964) or pantothenic acid deficiency (Oakley, 1965a, 1965b).

Artificial infection may or may not produce clinical disease. Nickel and Haupt (1964), for example, found that a challenge with up to 11,000 larvae caused no noticeable effect, but if 20,000 or more were used there was a marked depression of appetite and weight-gain. Taffs (1966), reporting the effects of administration of 30,000 larvae, describes anorexia, retardation of the growth-rate, loosening of the faeces or diarrhoea with associated flecks of blood and mucus. Shorb (1948) administered between 1,000 and 130,000 larvae and noted approximately the same symptoms although some of his animals were constipated. The worst affected became emaciated and died.

0.10. Epidemiology and Immunity

The results of a large number of German surveys (reviewed in Chapter 1 of this thesis) have shown that breeding pigs are often more heavily infested with Oesophagostomum than immature pigs. In the United Kingdom, Blisset and Little (1930) noted that, on the one farm they investigated, the sows and suckling pigs shed large numbers of 'strongyle' eggs in the faeces, whilst egg-counts of seven to ten-week old fatteners were very low. This picture would suggest that any acquired immunity that may be built up by the young pig is of little functional significance later in life.

Nickel and Haupt (1964) gave multiple doses of Oesophagostomum larvae and concluded that the last challenge had taken the same course as a primary infestation. How they reached this opinion is not clear from the text of their paper as they gave no details of the criteria they used for distinguishing the sequelae of the final and earlier infections. The first infections caused retardation of the growth-rate, whereas subsequent challenge did not. There is, therefore, some evidence in their data to

suggest that a partial immune response did, in fact, take place.

Taffs (1966) believes that infection does provoke an immunity although this may be small and wane quickly. In his experience reinfection is possible but it does not result in such large egg-counts or worm burdens as primary infections. He also found that experimental reinfection caused an expulsion of the existing Oesophagostomum population. Using the agar gel diffusion test with adult worm antigen, antibodies could be demonstrated from the 36th day of infection. Soulsby (1958) claims to have demonstrated the presence of heterophil antibodies in the serum and faeces of experimentally infected pigs. Jaggers (1965) developed a skin sensitivity test for the diagnosis of Metastrongylus infections but discovered that the presence of Oe. dentatum caused false positives to occur.

(C) HYOSTRONGYLUS RUBIDUS

0.11. The Adult

Clinical parasitologists regard H. rubidus and

Oesophagostomum spp. collectively as the 'strongyle worms' of the pig. This has come about because of the apparent similarity in the age incidences of these genera in the host species and because the ova of the two genera are almost identical. In fact, the two nematodes have little in common. H. rubidus is a member of the Trichostrongyloidea and bears the characteristics typical of this sub-order. It is the only species within the genus and has previously been ascribed to the genera Strongylus, Haemonchus, Trichostrongylus and Ostertagia (Alicata, 1935b). The genus Hyostrongylus was erected by Hall (1921). The great similarity between Hyostrongylus and Ostertagia will become apparent during the course of this thesis.

The morphology of the adult worm has been documented by Hassall and Stiles (1892), Hall (1921) and Goodey (1924). It is small and thread-like measuring 5-7 x 0.09-0.12 mm. (male) or 8-9 x 0.09-0.1 mm. (female). The head has a small but distinct cephalic button and four circumoral papillae. The cervical papillae point backwards and are situated 0.4-0.6 mm. from the anterior extremity. The vulva, which may or may not

be associated with a flap-like structure, is found 1.5-1.7 mm. in front of the anus, which in turn is 0.2-0.68 mm. from the tip of the tail. The posterior extremity of the body is rounded with three circular cuticular indentations.

The bursal structures of the male are characteristic. The dorsal ray is long and bifid with a pair of accessory branches along its length. The externo-dorsal and postero-lateral rays are divergent whilst the medio- and externo-lateral rays are fleshy and parallel as are the latero- and ventro-ventral rays. The tip of the latero-ventral ray, however, turns through ninety degrees to point towards the ventro-ventral. Each spicule is 120 μ long and is stout with a membranous wing extending along the posterior two-thirds of its length ending in a second point. The gubernaculum takes the form of a thin featureless rod, but the telamon is very well developed with a bar running along each lateral aspect of the cloaca and a more delicate curved ventral joining piece.

The adult worms are found lying on the surface of the

cardiac or fundic areas of the pig's stomach, usually under a layer of mucus. They are often clustered together in "nests", sometimes in ulcerated portions of the mucosa (Porter, 1940; Dodd, 1960; Kendall, Thurley and Pierce, 1969).

Hyostrongylosis is of world-wide occurrence and has been known in the United Kingdom since 1923 (Blackwell, 1923; Pillers, 1923). The only quantitative estimate of the frequency of occurrence of this parasite in Britain prior to 1964 was made by White (1955) who found that 30 of 53 faeces samples he examined yielded H. rubidus larvae.

Jansen (1965) draws attention to the complete absence of H. rubidus in European wild boar. He believes that this supports the view put forward by Oppermann (1905) and Tarczinski (1956) that this parasite was imported from the Americas with shipments of domestic pigs or contaminated feeding stuffs.

H. rubidus has been recovered on one occasion from

the abomasum of sheep (Becklund and Walker, 1967). Experimentally, it can grow to maturity in calves (Douvres and Tromba, 1958), goats (Tromba and Douvres, 1958a; Fitzsimmons and Harness, 1966), rabbits (Kotlan, 1949) and guinea-pigs (Alicata, 1934). As Oesophagostomum will not complete its life-cycle in the latter species, these laboratory animals may be used as biological 'filters' for obtaining pure H. rubidus cultures from the mixed larval samples usually obtained from field cases. Partial success has been achieved with the axenic culture of this genus (Diamond and Douvres, 1962).

The adult worms are red in colour and are therefore assumed to be blood suckers. Larsen (1967) has observed erythrocytes in the intestines of a proportion of the adults he observed in microscopic sections.

0.12. The Egg and Larval Stages

The egg of H. rubidus differs from that of Oesophagostomum in that it lacks the internal sculpturing seen in the latter, is a little narrower on average (31-42 μ v. 35-45 μ) and

contains 16 or 32 cells when voided from the host. Generic identification cannot be made with confidence on morphological grounds alone (Alicata, 1935b; White, 1955; Honer, 1967). White (1955) believes that the figure given for the length of the ova by Hassall and Stiles (1892) is a misprint. The correct figure is in the order of 60-80 μ, not 45 μ.

If kept at 22-24°C the eggs start to hatch around the 39th hour. The first moult begins at the 113th hour and the second at the 168th (Alicata, 1935b). The third stage larva remains within the cuticle shed during the previous ecdysis. It is very easily differentiated from the corresponding Oesophagostomum larvae even under the low power microscope (x 23 magnification). It is slimmer and moves characteristically by violently thrashing from side to side and actively swimming. Diagnosis can be confirmed in dead specimens by examination of the tail of the L3 form under the high power of the microscope. If viewed dorso-ventrally, the tip has a digitiform process giving the appearance of "a round ended peg" (Goodey, 1924b; Alicata, 1935b; Kotlan and Vajda, 1939; White, 1955;

Honer, 1967). Comparative measurements are given in Appendix 2, Table 1.

Goodey (1924) investigated the properties of ensheathed juveniles but used only twenty specimens in all. He found that they climbed the walls of the containing vessels and could not tolerate desiccation for even one hour. Alicata (1935b) confirmed this susceptibility to drying by showing that a 240 minute exposure was fatal. In addition, the latter author observed that larvae could remain alive for 144 hours in a temperature of 3-5°C, but not at -5-1°C. In water, viability was maintained at room temperature for $2\frac{1}{2}$ but not $4\frac{1}{2}$ months. Both Goodey and Alicata agree that this genus cannot penetrate intact skin, infection being, therefore, by ingestion only.

The anatomical changes that occur during the parasitic phase of the life-cycle resemble those typical of all the trichostrongylids except that the

sexes can be differentiated at a very early stage by the position of the giant genital cell in relation to the genital primordium (Alicata, 1934c, 1935b). Whilst that author could sex larvae at the time of the third moult, Kendall, Thurley and Pierce (1969) were not able to do this until the fourth day after infection. Other differences are seen when one compares the findings of these workers. In Kendall's series, fourth stage larvae were first seen on the fourth day after infection and the fifth stage on the 12th, whereas the corresponding figures in Alicata's work are five and 13 days. As Kendall and his co-workers point out, however, their observations were made on the natural host whilst those of Alicata were made on guinea-pigs.

The exsheathed infective larva takes up a position within the lumen of a gastric gland without penetrating the mucosal epithelium. Both parasitic moults take place at this site. The worms start to leave the tissues on the 13th or 14th day (Kotlan, 1949) although many do not do so until they become adult on the 20th day

(Kendall, Thurley and Pierce, 1969). Other worms become inhibited in their development and remain in the histotropic phase for prolonged periods (Kotlan, 1949).

0.13. Clinical Aspects and Pathology

The disease hyostrongylosis is most prevalent in adult pigs (Oppermann, 1905; Castle, 1932; Nicolson and Gordon, 1959) although it does appear on rare occasions in growing swine (Crocker and Biester, 1920; Skrjabin and Bekensky, 1925; Clay, 1938). It is often precipitated by stress conditions which disturb the host-parasite balance such as bad husbandry and the abnormally high level of milk production achieved in modern stock. Castle (1932) was the first to remark on the association between outbreaks of porcine parasitic gastritis and lactation. It is possible that a co-ordinated release of inhibited larvae in relation to the peri-parturient egg-rise (vide Chapter 2 of this thesis) may create a situation analogous to that seen in winter (type II) ostertagiasis in cattle as described by Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965).

Typically, a succession of sows grazing a paddock that has been in continual use for several years become overthin during lactation and fail to recover after weaning. This happens despite the receipt of extra rations given to satisfy a ravenous appetite (Oppermann, 1905; Castle, 1932; Nicolson and Gordon, 1959; Shanks, 1965; Cairns and Hargreaves, 1966; Davenport and Stockdale, 1967; Larsen, 1967; Davidson, Murray and Sutherland, 1968; Mouwen, Jansen, van Jaarsveld, Dorrestijn and Baars, 1968). Infrequently, death may occur as a result of severe emaciation or massive haemorrhages from associated gastric ulcers (Witte, 1938; Dodd, 1960; Karlovic, Vrazic and Drezancic, 1964). Diarrhoea was noted in at least a proportion of the cases recorded by Castle (1932) and Dodd (1960) but most authors report normal faecal evacuation or even constipation. Vomiting may be seen on occasion (Larsen, 1967).

The underlying pathological lesions are very similar to those described for ostertagiasis in ruminants, involving nodule ("morocco leather") formation and the transformation of zymogen and parietal cells to non-functional

units or to mucus secreting cells (Davidson, Murray and Sutherland, 1968; Kendall, Thurley and Pierce, 1969). These changes result in elevated plasma pepsinogen levels and an increased pH value for the gastric secretions. In apparent contradiction to these observations are the findings of Neilsen (1966a) who failed to demonstrate an increased rate of plasma protein catabolism in severely affected sows.

Often the pathological picture described above is complicated by diphtheresis (Oppermann, 1905; Crocker and Beister, 1920; Skrjabin and Bekensky, 1925; Castle, 1932; Nicolson and Gordon, 1959; Larsen, 1967) and less frequently with deep or shallow circumscribed gastric ulcers (Porter, 1940; Dodd, 1960; Kendall, Thurley and Pierce, 1969). In severe cases the lesions produced by the presence of H. rubidus larvae and adults cause a marked digestive dysfunction (Davidson, Murray and Sutherland, 1968). Secondary invasion by bacteria (Hoogland and Seyffers, 1928) or protozoa (Larsen, 1967) may occur. Affected animals may be severely anaemic (Larsen, 1967).

0.14. Immunity

The only data at present available on the immunological responses of the pig to invasion with H. rubidus are those of Porter (1940) whose experimental infections became patent at around the 21st day. Thereafter, the egg-counts quickly rose to a maximum and fell to very low values. Post mortem worm counts suggested that the number of H. rubidus decreased as the duration of the parasitism increased. Reinfection 115 days after the primary infection resulted in a rise in the egg-count three weeks later, but this was minute in comparison with the original response. There is, therefore, reason to suspect that an acquired immunity can occur, but, as in the case of Oesophagostomum infections in pigs, any protection afforded is of little functional value in later life.

(D) THIS THESIS

0.15. Aims and Objectives .

An attempt has been made in the previous paragraphs to list those publications that have contributed

significantly to our understanding of the biology of H. rubidus and the Oesophagostomum species of the pig. Many papers that merely consolidate existing knowledge or describe local anomalies have been omitted. References that appeared after the work reported in this thesis had started (1964) have only been included if necessary for the continuity of the narrative. More detailed discussions of many of the papers will be found in the subsequent chapters.

Survey:

Between the years 1932 and 1963, only three investigations making a positive addition to the study of the gastro-intestinal strongylates of the pig were performed in Great Britain (White, 1955; Soulsby, 1958; Nicolson and Gordon, 1959). The obvious absence of any reliable figures on the prevalence of the various pig parasites in the United Kingdom stimulated the survey that forms the first chapter of this thesis.

Epidemiology:

The results of the first phase of the project suggested a potentially fruitful means of studying one facet of the epidemiology of porcine oesophagostomiasis and the next chapter shows that this approach yielded useful data. It became apparent, however, that other aspects of the epizootiology could not be explained in these terms. Further observations enabled two hypotheses to be forwarded to account for these anomalies and these form the basis for the third chapter.

Control:

Experiences gained in the early part of the project supported the view that the therapeutic procedures then recommended for the control of these parasites were irrational, ineffective and wasteful. The newly acquired knowledge of the prevalence and epidemiology of the nematode genera of the pig was therefore applied to the design of trials intended to demonstrate methods by which the new broad-spectrum anthelmintics could be used to greater advantage

(Chapter 4).

Patho-physiology:

The work performed in the fourth chapter also gave an indication of the extent of the economic losses caused by gastro-intestinal helminthiasis in young pigs. Published data concerning analogous host-parasite systems enabled an hypothesis to be postulated for the mechanism of the physiological dysfunction that results in the sub-optimal growth-rate of heavily parasitized pigs. Chapter 5 describes how this theoretical concept was tested experimentally.

Laboratory Model:

Finally, the large expense of keeping experimental pigs, together with difficulties in finding suitable accommodation, led to an investigation into the feasibility of using the guinea-pig as a laboratory model for studies with Oesophagostomum.

0.16. Acknowledgements

All the original work reported within these covers was performed by the author alone except in the few instances specifically mentioned in the text.

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MRS. B. WHITEHEAD typed the manuscript and MR. S.R. CLAUDOT was responsible for the final presentation.

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Chapter 1

A SURVEY OF THE HELMINTHS OCCURRING IN THE SCOTTISH AND DANISH PIG POPULATIONS

INTRODUCTION

When this project was started in 1964, very little information was available on the composition of the helminth fauna of the domestic pig (*Sus scrofa domestica*) in Britain. The prevalence of the lungworms, *Metastrongylus* spp., has been well documented (*vide infra*), but the only comprehensive *post mortem* studies have been those of Morgan (1924) and Jenkins and Erasmus (1963) who examined 17 and 27 animals respectively. The purpose of this first chapter is to present the results of an investigation during which organs were examined from some 3,800 pigs of specified age groups, slaughtered at different seasons of the year.

Studies by two other groups of workers were also initiated in 1964 but differed from the present investigation in that each depended upon the detection of nematode ova in faeces for the diagnosis of helminthiasis. Preliminary results from all three surveys were published in the following spring (Davidson and Taffs, 1965; Gitter, Kidd and Davies, 1965; Jacobs, 1965).

A sojourn in Denmark provided the opportunity for a small parallel study to be conducted in that country. The Danes have developed a highly sophisticated pig industry, yet the parasitic status of the national herd was found to be grossly underestimated.

MATERIALS AND METHODS

1.1.1. Experimental Designs

Investigation 1.1.

Post mortem material from Scottish abattoirs was collected and examined with the following main objectives:-

(1) to discover what helminth parasites occur in Scottish pigs.

(2) to study aspects of the distribution of each species within the Scottish pig population.

(3) to detect any seasonal fluctuations that may occur.

Investigation 1.2.

A similar abattoir survey was performed in Denmark but with the object only of obtaining an indication of the type and size of the nematode burdens carried by adult pigs on the island of Zealand.

Investigation 1.3.

In order to establish whether the results obtained in Investigation 1.2. were typical for Denmark as a whole, faecal samples were obtained from other regions of the

country for laboratory examination to determine the presence or absence of parasitic nematode larvae. This project was nearing completion when the author had to depart from Denmark and was therefore finished by hr. mag. scien. J. Andreassen of the Zoological Institute, Copenhagen.

Investigation 1.4.

Another study (Nickel and Haupt, 1964) had shown that the Oesophagostomum population of the caecum of the host is dominated numerically by Oe. quadrispinulatum. As Investigations 1.1. and 1.2. had demonstrated the presence of this species in both British and Danish pigs, the present study was designed to supply quantitative data in support of these observations. Examinations were made of the large intestine of ten sows, three Scottish and seven Danish. Nine were chosen at random from the available material. The tenth, a sow from which the Oesophagostomum individuals were being expelled, was selected specially so that a comparison could be made between the nematode populations in equilibrium and one undergoing spontaneous displacement from the normal habitat. In each case, the caecum was separated from the

colon and the latter divided into ten to twelve sections each measuring about half a metre in length. A comparison was made of the numbers and types of worm recovered from each portion of the intestine.

1.1.2. Sources of Material

Scottish:

It was not possible to examine every organ of each individual pig. The survey was therefore divided into a series of separate investigations, each concerned with one particular anatomical system. The origins and numbers of pigs in each series are listed in Table 1.1. The pigs could be placed into three groups on the basis of their weight, giving an approximate indication of their age: porkers (90 lbs. dead weight, four to five months old); baconers (160 lbs. dead weight, six to eight months old); and adults (gilts, sows and boars). All the material was chosen randomly from abattoir-slaughtered pigs and represented an area extending from Aberdeenshire to Ayrshire.

Danish:

Sixty-five stomachs and 29 intestines were collected from sows slaughtered at a bacon factory at Roskilde and a meat and bone-meal factory at Ortved, near Ringsted.

In addition, practising veterinarians and other interested workers from all regions of metropolitan Denmark (i.e. excluding Greenland and the Faroe Isles) were asked to submit 20 gramme faecal samples to the laboratory for examination. A request was made that each batch of material should be representative of one herd, being collected from several breeding animals that had spent part of their lives at grass. One hundred and five responses were obtained from 12 locations.

1.1.3. Methods of Examination

Gastro-intestinal tract:

This was divided into four portions: stomach, small intestine, caecum and colon. In ten instances, the colon was subdivided (§ 1.1.1. Investigation 1.4.). Each section of the gastro-intestinal tract was treated

separately. Each was opened longitudinally and its contents, together with any worms attached to the surface of the mucosa, washed into a bucket. No attempt was made to recover larvae in the histotropic phase of their development. After thorough mixing, a measured random sample was taken, amounting to between one third and one twentieth of the total contents.

Material from the stomach and small intestine was cleared by repeated sedimentation and resuspension in water. The residue was scanned under the stereoscopic microscope (X23 magnification) and the worms removed. The contents of the caecum and colon were washed over a sieve (22 mesh) and examined macroscopically against a black background for the presence of worms.

Lungs:

The predilection site of Metastrongylus is the smaller bronchioles at the most distal part of the diaphragmatic lobe of the lung, across which an incision was made about one inch from its distal tip. The tissues

on either side of the cut were squeezed and if worms were observed protruding from the cut surface, the whole of the bronchial tree was opened and searched.

Body Cavities:

These were examined visually at the abattoir.

Livers:

The hepatic tissue was cut into strips about one centimetre thick and the bile ducts examined for trematodes. In Scotland, adult pigs, especially in-pig sows and gilts, are often put out to pasture where they may be exposed to infection with Fasciola hepatica, but younger pigs are habitually housed. For this reason the collection of livers was limited to the older age groups.

Muscle:

The acquisition of Trichinella spiralis larvae is cumulative and sows and boars are more likely to be fed kitchen waste than are fattening pigs. Five gramme samples were therefore taken from the diaphragm muscle of adult swine and fed to Wistar rats, each receiving meat from 25 pigs. One month later

the rats were killed and their diaphragms removed, compressed between heavy glass plates and examined under the stereoscopic microscope for the presence of T. spiralis larvae.

Faeces:

The faecal samples often took several days to reach the laboratory. Since it was impossible to refrigerate them whilst in transit, the 'strongyle' eggs had in many cases hatched before arrival, and therefore no egg-counts were attempted. Diagnosis was based on the identification of third stage infective larvae harvested by means of a modified Baermann technique. The samples were placed in a jam-jar and mixed with an equal amount of granulated charcoal. The mixture was kept slightly damp and maintained at a temperature of 17-20^{o}C. The lid was pierced so that air could circulate whilst evaporation was kept low. After 10 days the larvae were fully developed and could be harvested in the following way:- the jar was filled with lukewarm water and a petri-dish placed over the mouth in place of the lid.

The bottle was turned so that the base was uppermost. The faeces floated to the top and within a few hours larvae could be found in the water beneath. This was poured off by inclining the jar at an angle to the petri-dish. With practice, a larval suspension could be obtained without any contamination with solid faecal particles. The nematodes were concentrated by sedimentation and the ratio of Hyostrongylus rubidus to Oesophagostomum spp. found by microscopical inspection. Identification is so easy (Appendix 2, Table 1) that X23 magnification was found to be adequate.

As the faecal samples were of approximately equal size, it was possible to make a subjective estimate of whether there were 'few' or 'many' larvae present. The material was not known with certainty to be representative of the respective farms and so no more accurate quantitative studies were attempted. The designation 'many' is indicative of a faecal egg-count of several hundred per gramme in respect of H. rubidus and several thousand in the case of Oesophagostomum spp.

1.1.4. Preservation of Recovered Helminths

All helminths were fixed in 5% formalin. Ascarids and cestodes were kept in this solution, but the others were put into 30% alcohol. After successive periods of 48 hours, more concentrated alcohols were substituted. Eventually, the worms were placed in a liquid containing 70% alcohol, 5% glycerin and 25% water. The preparation was placed in an incubator at 30°C so that the water and alcohol slowly evaporated leaving the dehydrated worms in almost pure glycerin. Lungworms and Trichuris were left in this, but the smaller nematodes were mounted on microscope slides in glycerin jelly after being stored in a desiccator for several days.

Mounted specimens were identified by microscopic examination at the appropriate magnifications. For closer inspection of spicules, the specimens were sometimes remounted in Berlese's fluid. Worms were measured with a tape-measure (ascarids) or by projecting their image onto a screen and using an opisometer to establish their lengths in relation to a calibrated graticule.

1.1.5. A Method of Representing the Distribution and Abundance of Helminths

Usually, the results of a survey such as the one recorded in this chapter are tabulated as a series of arithmetic means (e.g. Tables 1.2. to 1.6.), perhaps qualified with the range of values in absolute terms or as the standard error of the mean. This mode of expression conveys little information on the distribution of infection within the host population, though this defect can be overcome by using the data to build up a histogram (e.g. Fig. 1.17.). This method is rather clumsy, especially if the comparison of several sets of figures is involved. Bradley (1965) has described the application of logarithmic-probability curves to the field of helminthology, a form of presentation which has so many advantages and is so simple to use that it was adopted in the present study (Figs. 1.8. to 1.16).

The divisions on log-probability paper are such that a normal frequency distribution curve would be depicted as a straight line. The vertical scale shows the percentage of the examined host population that harboured as many as

or fewer than the corresponding number of worms on the horizontal axis. Theoretical considerations demand that a factor of 0.5 be added to the latter figure.

The following account is modified from the example given by Bradley to explain this point. If 19% of a population have a worm-count of 14 or less, then the remaining 81% must harbour more than 14 worms i.e. they possess a worm-count of 15 or more. For epidemiological studies it is useful to be able to read off both the proportion of the population that carry less than a specified parasite burden and the percentage that house more than this number. This would involve plotting two points on the abscissor for each value on the ordinate. To avoid having two separate curves joining the duplicated points, one half is added to each worm-count so that both sets of information can be portrayed by a single line. Thus, 19% have less than $14\frac{1}{2}$ worms whilst 81% have more than $14\frac{1}{2}$.

If two populations are parasitized to a different degree, the curve representing the less heavily infested group will lie closer to the top lefthand corner of the diagram.

RESULTS

1.2.1. Occurrence

The following parasites were found in the Scottish series:

Nematoda: Trichostrongyloidea:

Hyostrongylus rubidus (Hassall and Stiles, 1892).

Trichostrongylus axei (Cobbold, 1897) - a new record for the United Kingdom.

Trichostrongylus colubriformis (Giles, 1892) - a new record for the United Kingdom.

Trichostrongylus vitrinus (Looss, 1905) - a new host record.

Nematoda: Strongyloidea:

Oesophagostomum dentatum (Rudolphi, 1803) - Fig. 1.1.

Oesophagostomum quadrispinulatum (Marcone, 1901) - a new record for the United Kingdom, Fig. 1.2.

Globocephalus urosubulatus (Alessandrini, 1909) - a new record for the United Kingdom, Fig. 1.3.

Nematoda: Metastrongyloidea:

Metastrongylus apri (Gmelin, 1790).

Metastrongylus pudendotectus (Wostokow, 1905).

Nematoda: Trichuroidea:

Trichuris suis (Schrank, 1788).

Cestoda: Taeniidae:

Cysticercus tenuicollis.

There was no evidence of *T. spiralis* in the musculature and no sign of *F. hepatica* in the bile ducts. *C. tenuicollis* was seen on one occasion only, on the peritoneum covering the liver of a baconer. One old sow - the largest of the series - harboured a remarkable fauna which included *G. urosubulatus* and the three *Trichostrongylus* species. None of these parasites was encountered again during the course of the survey.

The following parasites were found in the Danish series:

Nematoda: Trichostrongyloidea:

Hyostrongylus rubidus (Hassall and Stiles, 1892) - a new record for Scandinavia.

Nematoda: Strongyloidea:

Oesophagostomum dentatum (Rudolphi, 1803).

Oesophagostomum quadrispinulatum (Marcone, 1901) - a new record for Scandinavia.

Nematoda: Trichuroidea:

Trichuris suis (Schrank, 1788).

1.2.2: The Linear Distribution of the Two Oesophagostomum Species

Oe. quadrispinulatum clearly favours a site closer to the ileocaecal valve than does Oe. dentatum (Figs. 1.4. to 1.6.). The territories occupied by each species were observed, however, to overlap to varying degrees, Oe. quadrispinulatum sometimes extending into a more distal predilection site and Oe. dentatum sometimes taking a more proximal position. Attempts were made to correlate these variations with (1) the absolute magnitude of the Oesophagostomum populations (2) the relative numbers of each Oesophagostomum species (3) the presence or absence of Trichuris suis (4) the reproductive status of the sow.

No constant pattern could be determined.

The results in Fig. 1.4. are expressed in terms of the percentage of each Oesophagostomum population in each segment of the intestinal canal. Similar curves are produced by plotting the density of each Oesophagostomum species per unit area of mucosal surface against the distance from the ileocaecal valve, so that the distribution of Oesophagostomum species along the large intestine appears to be independent of the surface area or volume of luminal contents available in each section (Fig. 1.5.).

Predictably, the males and females of each species occupied similar positions in the intestine (Fig. 1.6.). Pairs of Oesophagostomum were often found in coitu and in each case mating involved males and females of the same species.

Measurement of over 2,000 Oesophagostomum spp. individuals did not reveal any significant difference in the lengths of the worms from each section of the large intestine (Appendix 1, Table 18), nor did microscopical

examination show any morphological differences which might indicate a progressively older population in the more distal segments (as is seen, for example, in Trichonema infections in horses - Mathieson, 1964).

When spontaneous expulsion of a population occurs, it naturally disrupts the customary distribution beyond recognition (Fig. 1.7.).

1.2.3. The Incidence (Extensity) and Size (Intensity) of Infections

The information collected during the Scottish survey is too voluminous to be recorded in narrative form. The data for each parasite are therefore summarized in Tables 1.2. to 1.7. and Figs. 1.8. to 1.16. In the Tables, the degree of infestation is expressed as the percentage of the host population harbouring each helminth, or as the arithmetic mean of the worm burden of each group. A more comprehensive picture of the parasitic status of each population is given by means of the probability curves in Figs. 1.8. to 1.16. which are drawn according to the method of Bradley (§ 1.1.5.).

Figs. 1.8. to 1.11. compare age incidences and Figs. 1.13. to 1.16. the seasonal fluctuations in the population structure. The worm burdens of individual pigs are supplied in Appendix 1, Tables 1 to 13.

The extensity, intensity and geographical distribution of the infections studied in the Danish survey are shown in Tables 1.7. and 1.8. and in Figs. 1.17. and 1.18.

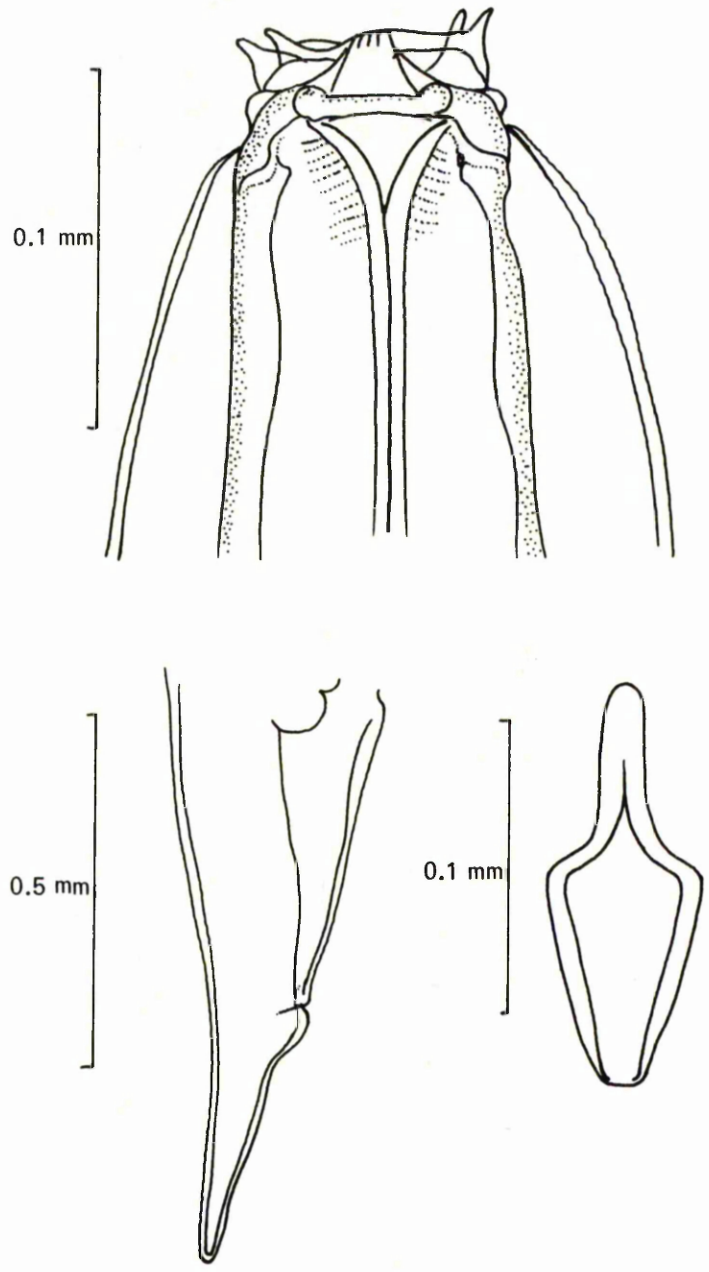


Fig 1.1 ***Oesophagostomum dentatum*** **from Scotland: head, female tail and gubernaculum of male. Drawn by Dr. A.M. Dunn**

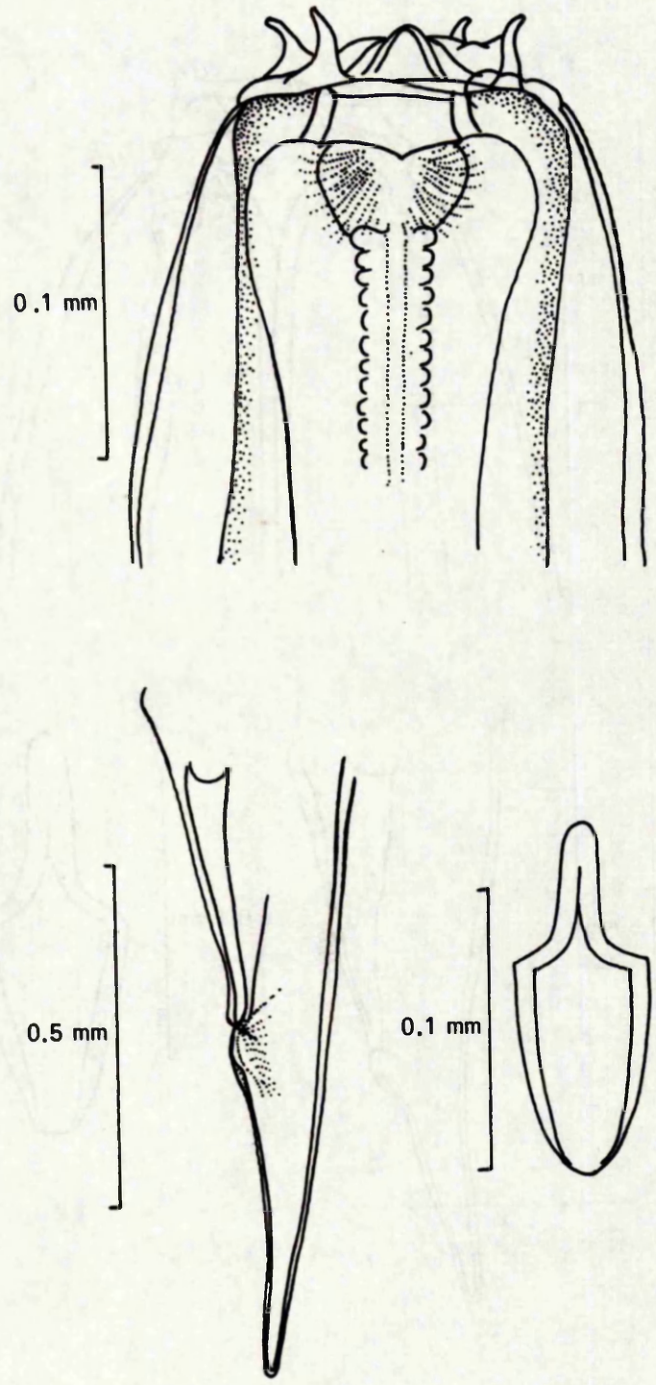


Fig 1.2 ***Oesophagostomum quadrispinulatum*** **from Scotland: head, female tail and gubernaculum of male. Drawn by Dr. A.M. Dunn**

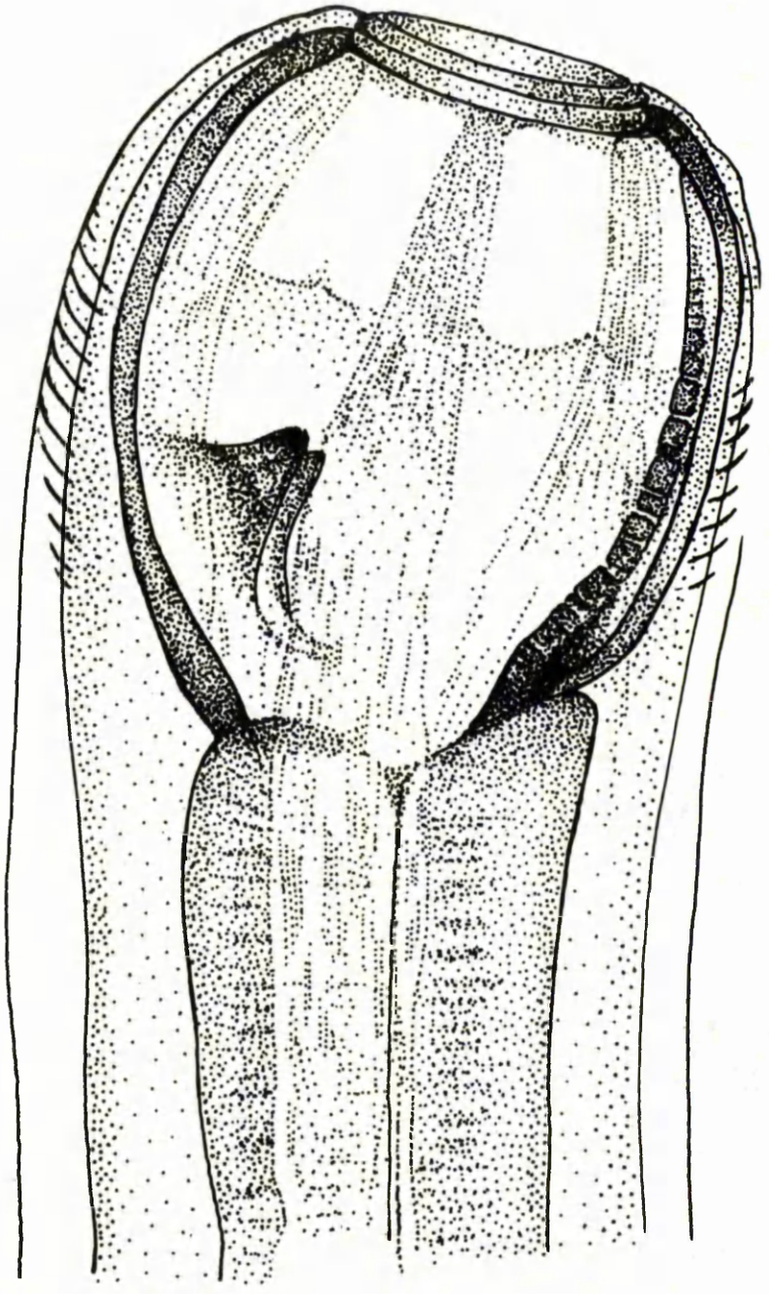


Fig 1.3 *Globocephalus urosubulatus* from Scotland : head
Drawn by Dr. A.M. Dunn

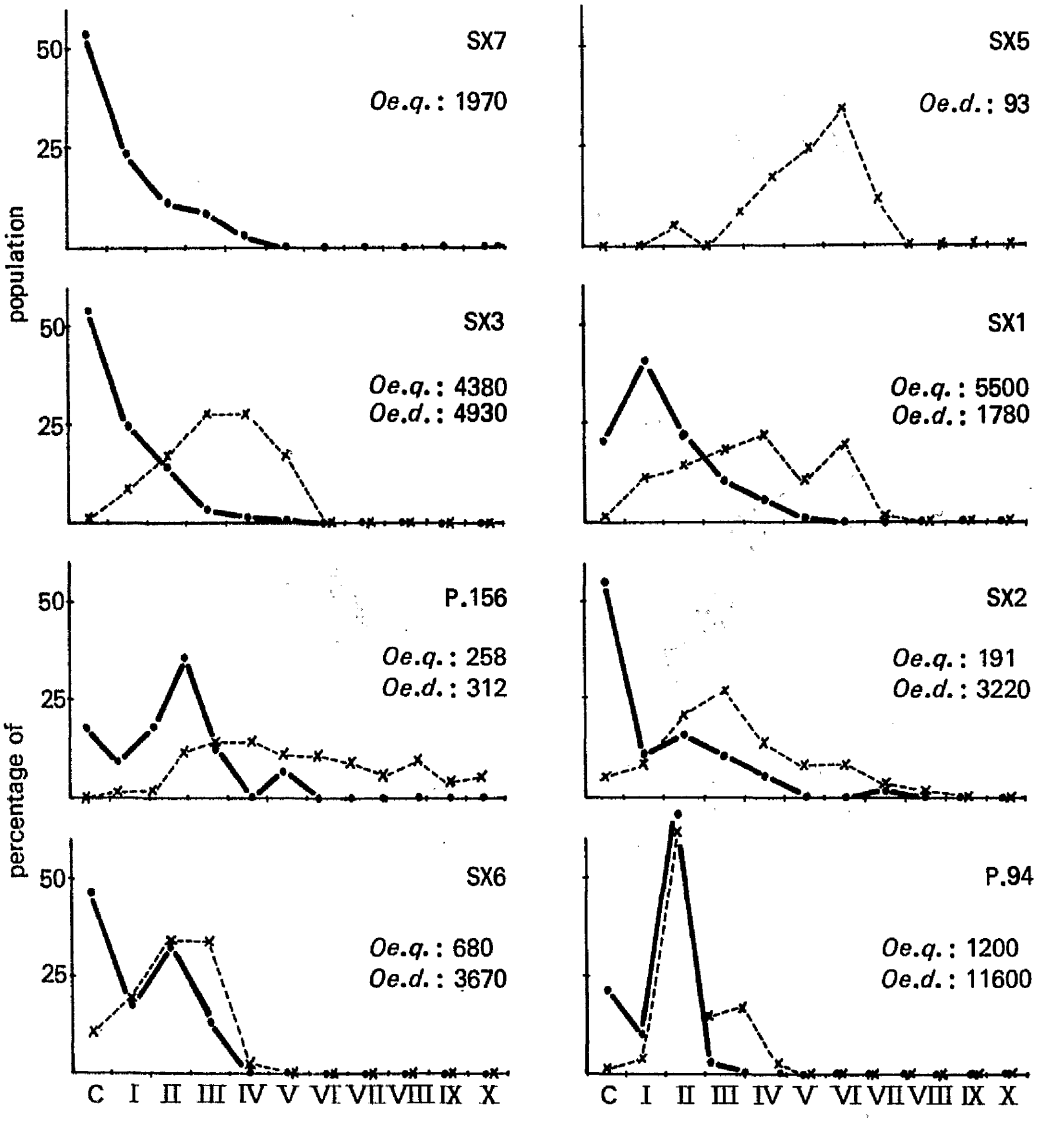


Fig 1.4 Linear distribution of *Oesophagostomum* spp. in the large intestine of the pig. C = caecum; I, II, III etc. = sections of colon and rectum. Oe.q. = *Oe. quadrispinulatum;* Oe.d. = *Oe. dentatum* and adjacent numbers refer to the population size

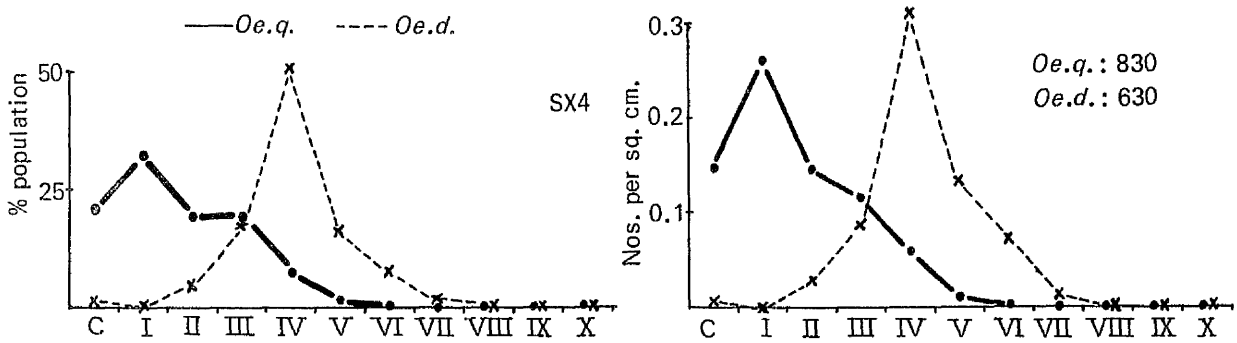


Fig 1.5 Linear distribution of *Oesophagostomum* spp. in the large intestine of the pig expressed as the proportion of the two populations in each section and the numbers of worms per unit area of mucosal surface

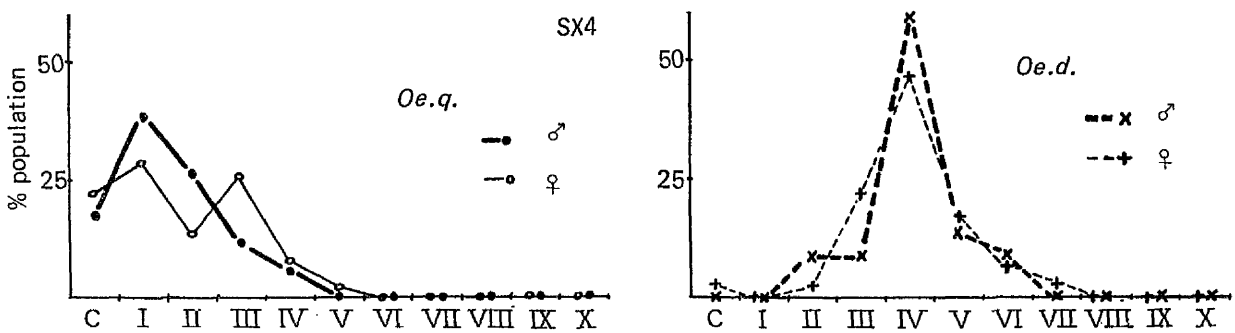


Fig 1.6 A comparison of the linear distribution of male and female *Oesophagostomum* spp. in the large intestine of the pig

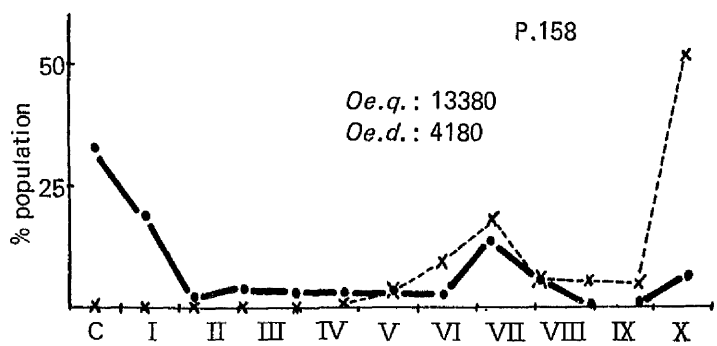


Fig 1.7 Linear distribution in the large intestine of the pig of *Oesophagostomum* spp. undergoing spontaneous population expulsion

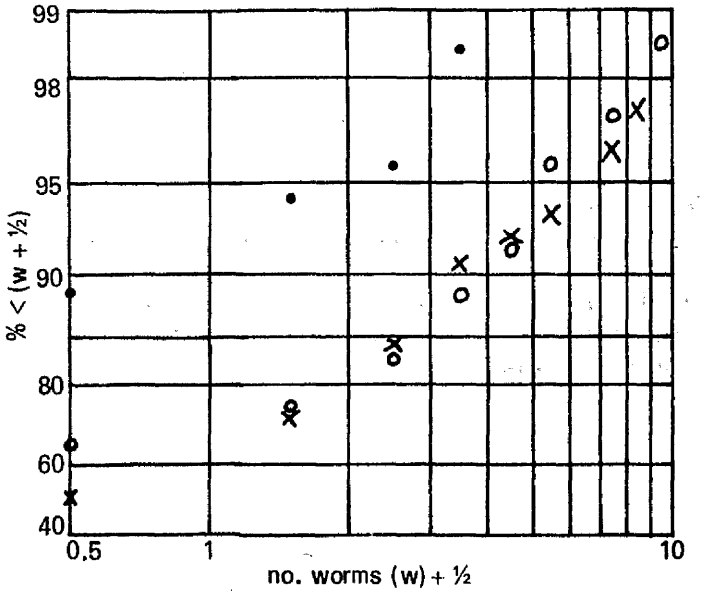


Fig 1.8 ***Ascaris suum*** **- age incidence: for explanation of log-probability curves see Section 1.1.5.. × = porker; o = baconer; . = adult**

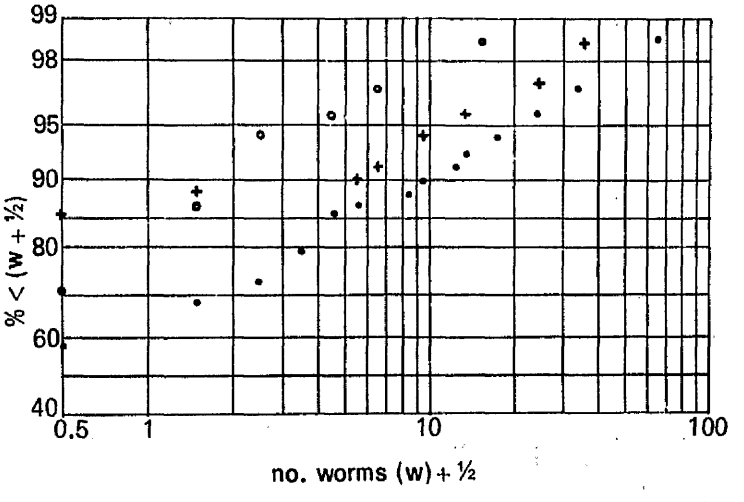


Fig 1.9 ***Trichuris suis*** **- age incidence: for explanation of log-probability curves see Section 1.1.5.. . = porker; o = baconer; + = adult**

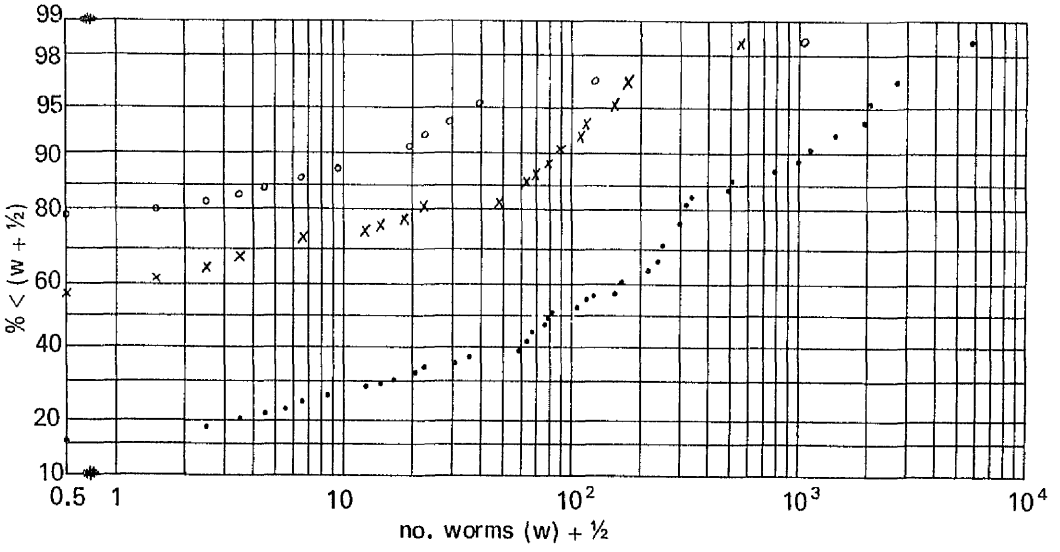


Fig 1.10 ***Oesophagostomum quadrispinulatum*** **- age incidence : for explanation of log-probability curves see Section 1.1.5.. × = porker; o = baconer; . = adult**

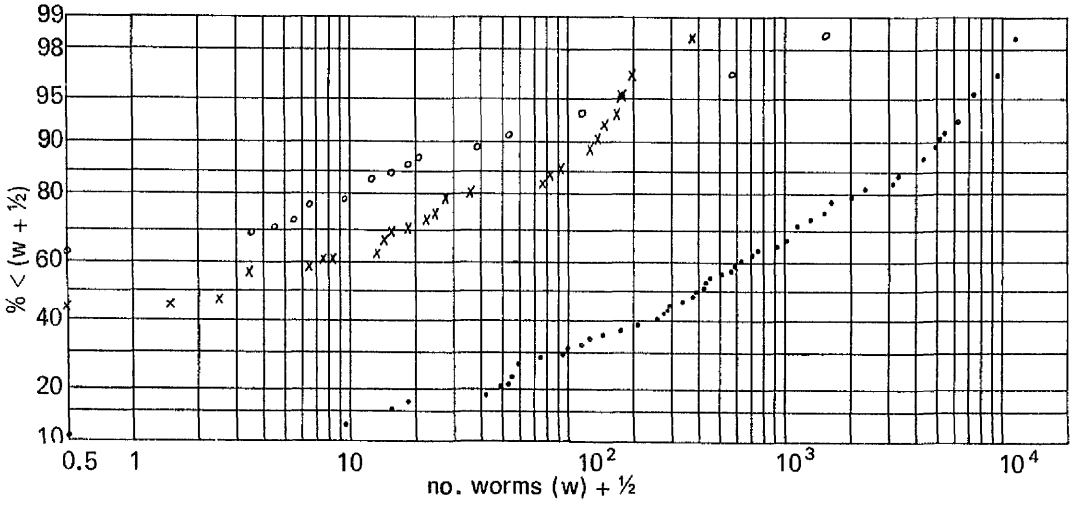


Fig 1.11 ***Oesophagostomum dentatum*** **- age incidence; for explanation of log-probability curves see Section 1.1.5..**
× = porker; o = baconer; . = adult

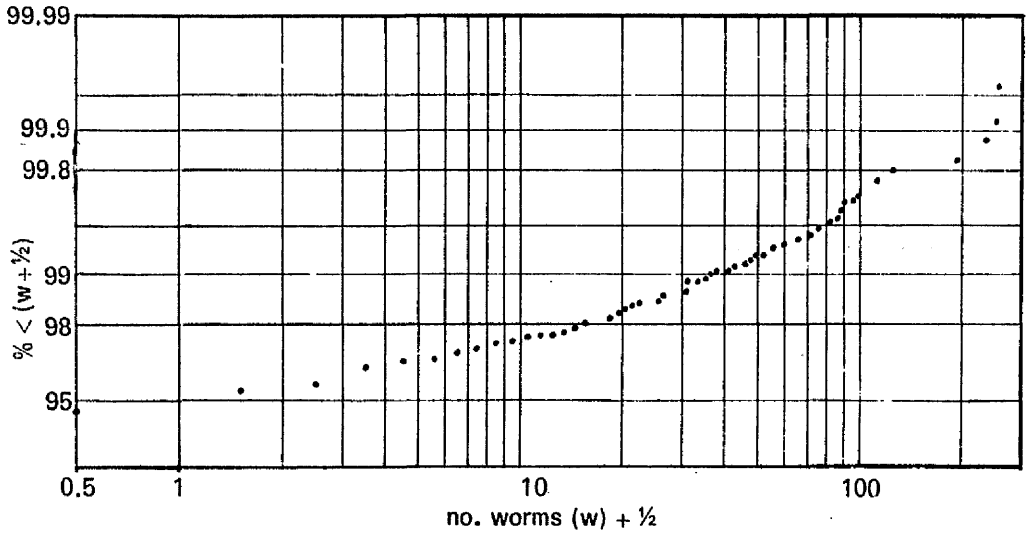


Fig 1.12 The incidence of *Metastrongylus* spp. in baconers: for explanation of log-probability curves see Section 1.1.5..

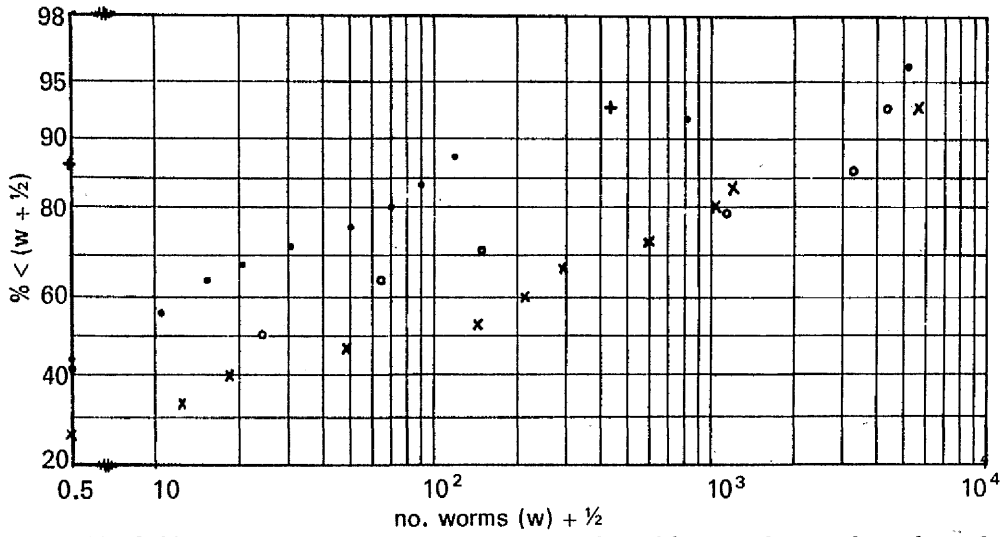


Fig 1.13 *Hyostrongylus rubidus* - seasonal incidence: for explanation of log-probability curves see Section 1.1.5..
. = Nov. - Jan.; x = Feb. - Apr.; o = May - Jul.; + = Aug. - Oct.

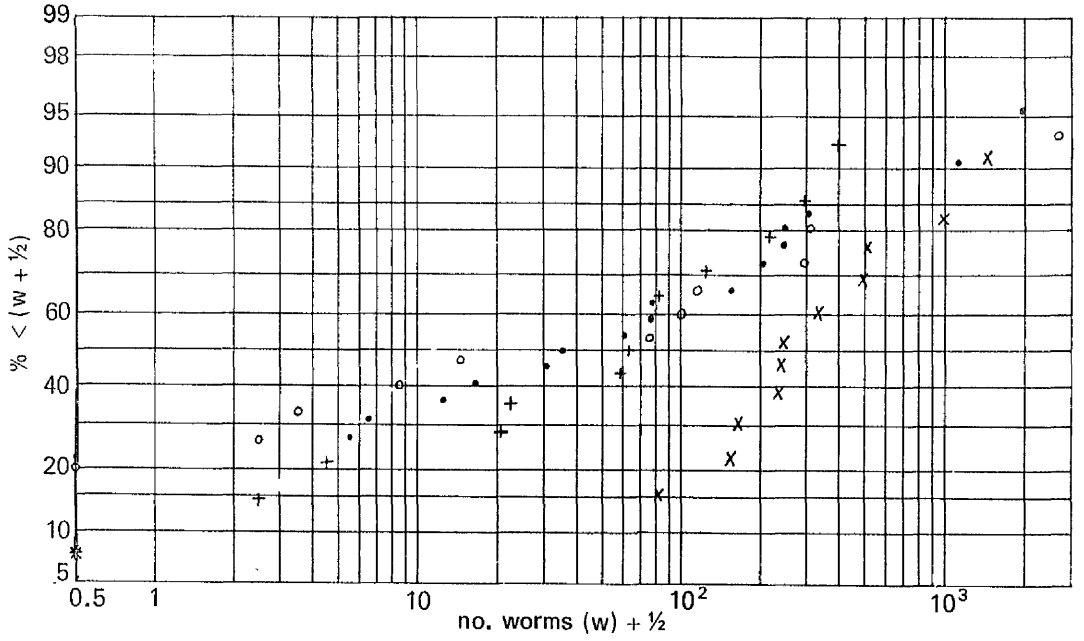


Fig 1.14 *Oesophagostomum quadrispinulatum* **- seasonal incidence in adult pigs: for explanation of log-probability curves see Section 1.1.5.. . = Nov. - Jan.; × = Feb. - Apr.; o = May - Jul.; + = Aug. - Oct..**

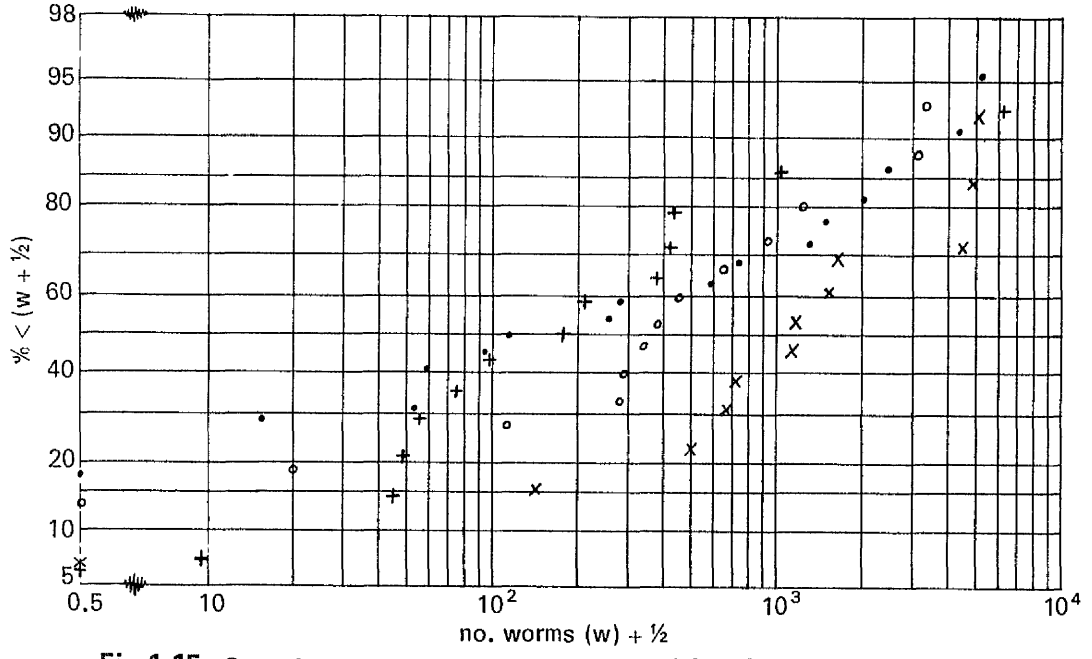


Fig 1.15 *Oesophagostomum dentatum* **- seasonal incidence in adult pigs: for explanation of log-probability curves see Section 1.1.5.. . = Nov. - Jan.; × = Feb. - Apr.; o = May - Jul.; + = Aug. - Oct.**

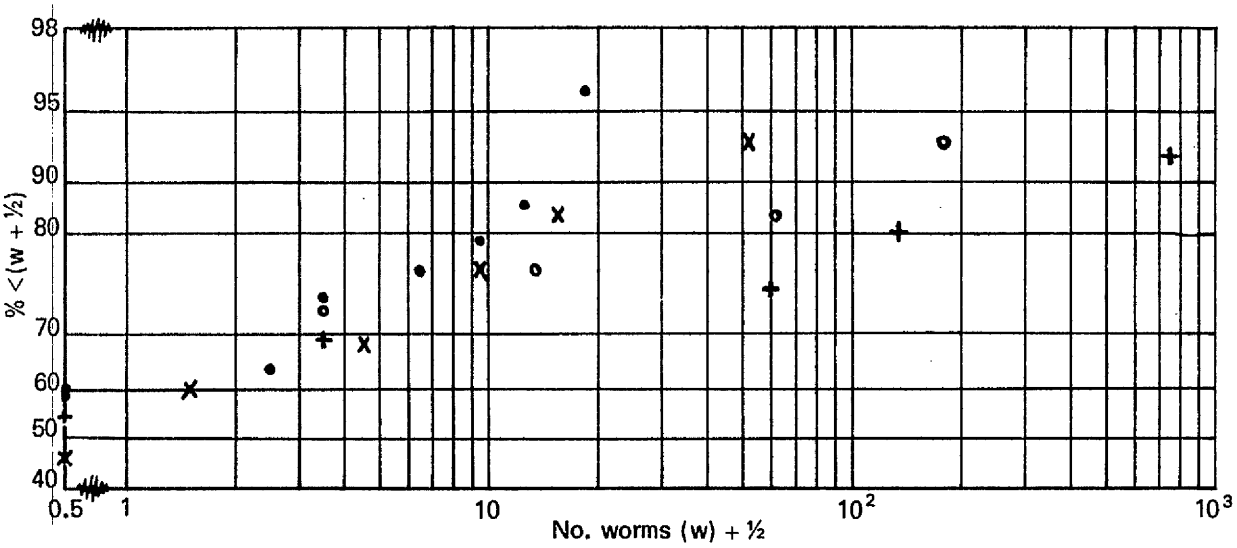


Fig 1.16 ***Oesophagostomum*** **spp. - seasonal incidence in growing pigs (combined results for porkers and baconers); for explanation of log-probability curves see Section 1.1.5..**
. = Nov. - Jan.; x = Feb. - Apr.; o = May - Jul.; + = Aug. - Oct.

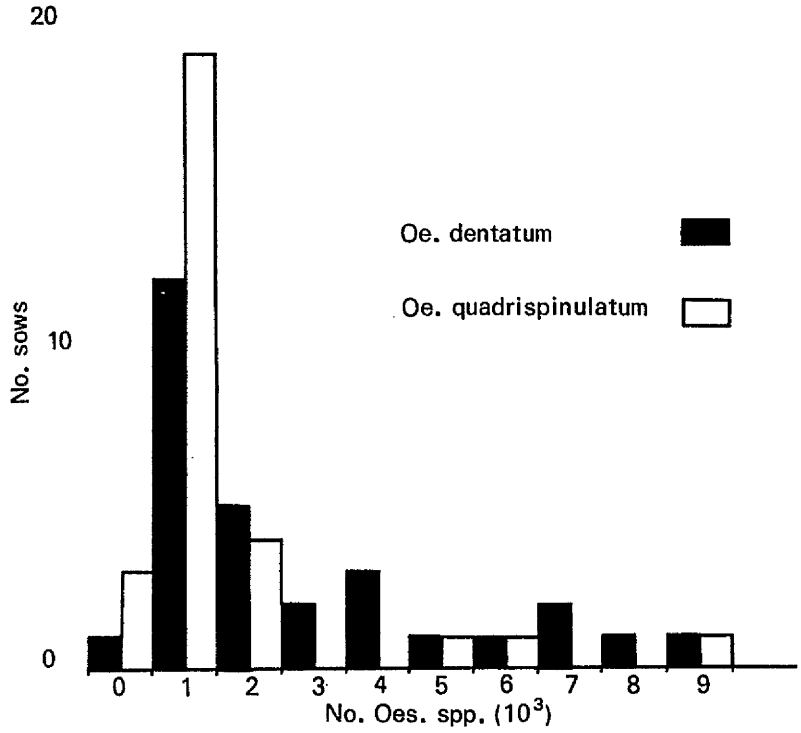
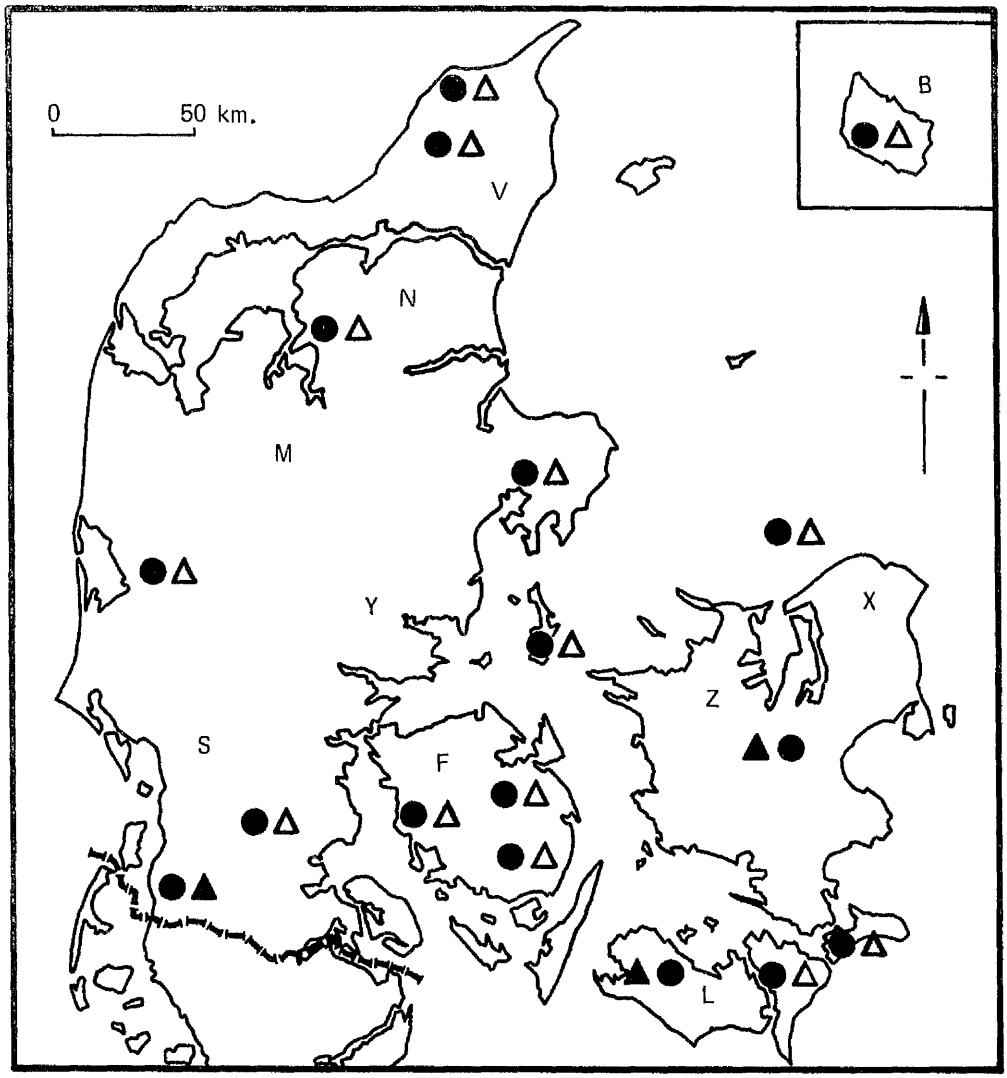


Fig 1.17 Frequency distribution of the intensity of ***Oesophagostomum*** **spp. infections in Danish adult pigs**

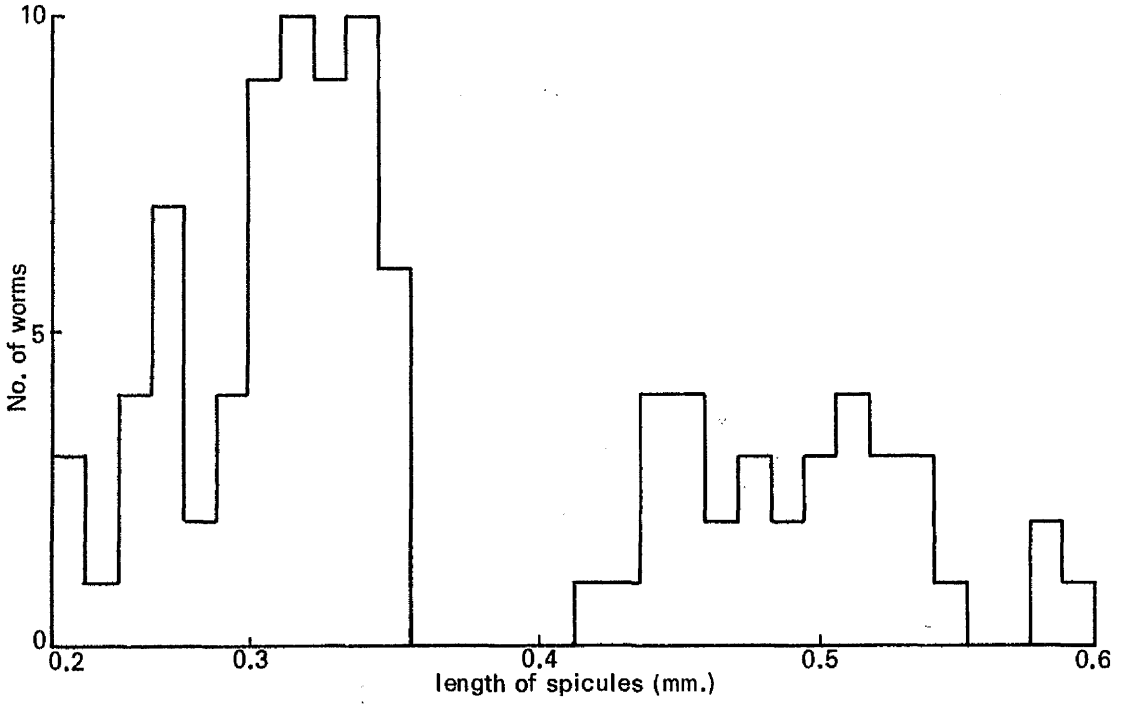


▲ *Hyostrongylus rubidus* positive

● *Oesophagostomum* spp. positive

△ *Hyostrongylus rubidus* negative

Fig 1.18 The geographical distribution in Denmark of *Hyostrongylus rubidus* and *Oesophagostomum* spp.. The letters indicate the regions listed in Table 1.10.. X shows the position of the clinical outbreak (Nielsen, 1966; Larsen, 1967) and Y the location of Ludvigsen and van Abrichem's investigation



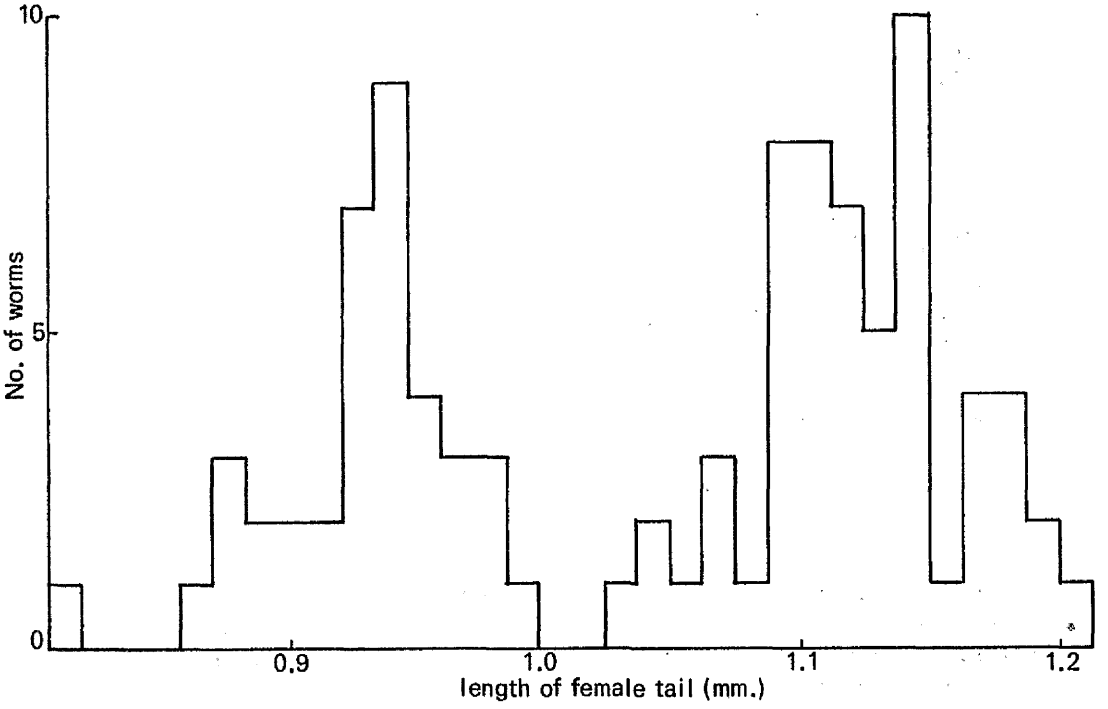


Fig 1.19 Frequency distribution curves for the length of the female tail and length of the spicules of the male in two series of 100 *Oesophagostomum* chosen at random from the Scottish collection

N.B The horizontal scales have become interchanged. Thus, 0.2 etc. should appear on the lower diagram and vice versa

DEJ

Table 1.1. - Quantity and sources of material examined during the Scottish survey

Organ	Age-group	Number examined	When examined	Abattoir
Gastro-intestinal tract	porker	76	Oct. 1964 - Sept. 1965	Glasgow Paisley Barrhead Stirling
	baconer	70		
	adult	70		
Lungs	baconer	2,513	Jan. 1965 - Dec. 1965	Stirling
	adult	33		
Body cavities	baconer	2,513	Jan. 1965 - Dec. 1965	Stirling
	adult	33		
Liver	adult	65	May 1967 - Aug. 1967	Glasgow
Muscle	adult	1,000	July 1967 - Aug. 1967	Glasgow

Table 1.2. - Percentage incidence of helminths in Scottish pigs

	Porker	Baconer	Adult
STOMACH			
Hyostrongylus rubidus	0	0	50.7
SMALL INTESTINE			
Ascaris suum	34.2	28.6	11.4
Globocephalus urosubulatus	0	0	1.4
Trichostrongylus axei	0	0	1.4
Trichostrongylus colubriformis	0	0	1.4
Trichostrongylus vitrinus	0	0	1.4
LARGE INTESTINE			
Trichuris suis	42.0	29.4	14.3
Oesophagostomum quadrispinulatum	46.4	23.5	84.4
Oesophagostomum dentatum	58.0	39.7	89.1
LUNG			
Metastrongylus apri	-	5.6	15.0
Metastrongylus pudendotectus	-	0.04	0
BODY CAVITIES			
Cysticercus tenuicollis	-	0.04	0

Table 1.3. - Average number of helminths per infected pig found in Scottish survey

	Porker	Baconer	Adult
STOMACH			
Hyostrongylus rubidus	-	-	4,255
SMALL INTESTINE			
Ascaris suum	$3\frac{1}{2}$	$4\frac{1}{2}$	$2\frac{1}{2}$
Globocephalus urosubulatus	-	-	447
Trichostrongylus axei	-	-	31
Trichostrongylus colubriformis	-	-	158
Trichostrongylus vitrinus	-	-	31
LARGE INTESTINE			
Trichuris suis	10	6	19
Oesophagostomum quadrispinulatum	77	156	547
Oesophagostomum dentatum	72	168	1,773
LUNG			
Metastrongylus apri	-	28	7
Metastrongylus pudendotectus	-	2	-
BODY CAVITIES			
Cysticercus tenuicollis	-	1	-

Table 1.4. - Seasonal incidence of Ascaris suum and Trichuris suis in growing pigs (combined results for porkers and baconers) during Scottish survey

Nov./Jan.	Feb./Apr.	May/July	Aug./Oct.	
A.suum				
26	32	29	43	percentage incidence
4	2	3	4½	av.no./infected pig
T.suis				
48	16	32	39	percentage incidence
10	3½	11	5	av.no./infected pig

Table 1.5. - Seasonal variation in the intensity and extensity of strongylate infections, based on post-mortem examinations of 70 Scottish sows during 1964/65

Nov./Jan.	Feb./Apr.	May/July	Aug./Oct.	
Oesophagostomum spp.				
96	93	87	100	% incidence
1371	3204	2519	1548	Mean worm count/infected pig
Hyostrongylus rubidus				
56	73	57	13	% incidence
1521	9536	1830	4056	Mean worm count/infected pig

Table 1.6. - Male:female ratios found during Scottish survey

			Porker		Baconer		Adult
Hyostrongylus rubidus	Nov.-Jan. Feb.-Apr. May -Jul. Aug.-Oct.		-		-	1:1.96) 1:1.24) 1:1.53) 1:2.01)	1:1.13
Ascaris suum			1:1.05		1:1.35		1:1.50
Globocephalus urosubulatus			-		-		1:1.29
Trichostrongylus spp.			-		-		1:2.14
Trichuris suis			1:1.43		1:1.22		1:1.92
Oesophagostomum spp.	Nov.-Jan. Feb.-Apr. May -Jul. Aug.-Oct.	1:1.04) 1:0.77) 1:0.99) 1:1.36)	1:1.18	1:0.75) 1:0.86) 1:1.45) 1:1.09)	1:1.22	1:1.13) 1:0.97) 1:1.03) 1:1.54)	1:1.12
Metastrongylus spp.			-		1:1.13		1:3.81

Table 1.7. - Seasonal fluctuations in Metastrongylus spp. populations in Scotland

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Percentage incidence		3.54	5.29	8.04	3.85	5.02	3.40	1.23	13.02	5.24	5.04	5.39	10.99
Av.no.worms per infected pig		48	16	47	16	64	32	121	8	18	7	40	11
Male:female ratio	1:	0.61	0.92	1.69	1.30	1.13	1.33	1.15	3.02	1.51	1.00	1.61	1.75

Table 1.8. - The gastro-intestinal helminth fauna of the Danish sows

	Hyostrongylus rubidus	Oesophagostomum dentatum	Oesophagostomum quadrispinulatum	Trichuris suis	Ascaris suum
Percentage of sows infested	11	97	90	21	21
Average number of helminths per infested sow	<100	2286	1143	15	3

Table 1.9. - The distribution of helminths throughout the Danish pig population

	Percentage Incidence			Av. no. helminths per infected pig		
	Porker	Baconer	Adult	Porker	Baconer	Adult
Ascaris suum	-	53	21	-	12	3
Trichuris suis	-	0.4	21	-	-	15
Oesophagostomum spp.	-	73	100	-	-	3,227
Hyostrongylus rubidus	-	-	11	-	-	<100

These figures, representing the examination of 469 pigs, are compiled from the present surveys, and from Mogensen (1962), and Avlund and Mandrup (1965 - unpublished data)

Table 1.10. - The geographical distribution of gastro-intestinal strongylate parasites throughout the Danish pig population

	District	No. of herds investigated	Hyostrongylus rubidus			Oesophagostomum spp		
			negative	"few"	"many"	negative	"few"	"many"
B.	Bornholm	6	6	0	0	0	6	0
F.	Funen	11	11	0	0	0	1	10
L.	Lolland	10	7	2	1	0	5	5
M.	Mid-Jutland	10	10	0	0	0	4	6
N.	North Jutland	5	5	0	0	0	1	4
S.	South Jutland	12	11	1	0	3	3	6
V.	Vendsyssel	9	9	0	0	1	6	2
Z.	Zealand	30	28	2	0	5	13	12
	Other Islands (Falster, Møn, Samsø and Hesselø)	12	12	0	0	0	2	10
	Totals	105	99	5	1	9	41	55
			6 positive = 5.7%			96 positive = 91.4%		

Table 1.11. - Some measurements made on Oe. dentatum and Oe. quadrispinulatum: a comparison between those obtained during the present study and those of other authors. This table is adapted from Haupt (1966). The two original columns represent measurements made on Roth's collection (62 female: 13 male) and on a random sample from the Scottish collection (100 female: 100 male)

				Oesophagostomum quadrispinulatum				
Body length male mm.	-	ca 6	7.8-9.6	6.5-7.0	8.1-9.2	8.5-10	av. 8.3 max. 8.4	av. 9.1 max. 10.2
Spicule length µ	910-950	750-994	815-951	768-958	850-970	816-960	810-830	820-950
Body length female mm.	-	7-9	8.0-11.6	8-11	9.8-10.7	9-12	av. 10.3 max.11.4	av. 11.4 max. 13.4
Distance vulva - anus µ	380-460	324-432	453-528	343-458	370-465	368-490	380-480	400-550
Distance anus - tip of tail µ	400-460	405-580	453-543	415-586	500-640	400-560	460-530	430-610
Author	Goodey (1925)	Mikacic (1937)	Bobkova (1956)	Schmeer (1958)	Linzcano Herrera (1958)	Haupt (1966)	Original (Roth)	Original (Scottish)
				Oesophagostomum dentatum				
Body length male mm.	8-10	8-10	8.8	7-10	11-11.5	8.5-11	av. 9.0 max. 9.9	av. 9.8 max. 11.4
Spicule length µ	1150-1300	1000-1220	896-937	1001-1187	1250-1300	1000-1184	1040-1130	1030-1220
Body length female mm.	11-14	10-14	7.5-13.4	9-12	13.4-15.0	11-13	av. 11.2 max.12.9	av. 12.0 max. 13.2
Distance vulva - anus µ	360-380	270-324	315-366	272-400	320-375	248-350	250-480	290-440
Distance anus - tip of tail µ	ca 350	270-338	255-265	257-362	320-360	224-350	260-340	260-390
Author	Goodey (1924)	Mikacic (1937)	Ozerkaja (1930)	Schmeer (1958)	Linzcano Herrera (1958)	Haupt (1966)	Original (Roth)	Original (Scottish)

Table 1.12. - The predilection sites of the gastro-intestinal nematodes of pigs commonly found in Europe

Nematode species	Predilection site		Author
	Organ	Position	
Hyostrongylus rubidus	Stomach	cardiac and fundic regions	Kendall, Thurley and Pierce (1969)
Strongyloides ransomi	small intestine	first two metres	Suppurer and Pfeiffer (1960)
Globocephalus urosubulatus	small intestine	$5\frac{1}{2}$ - $7\frac{1}{2}$ metres from pylorus	Schultze (1956)
Ascaris suum	small intestine	8 - 9 metres from pylorus	Melchiors (1963) Jacobs (1963)
Trichuris suis	large intestine	caecum, less often proximal colon	Present studies
Oesophagostomum quadrispinulatum	large intestine	caecum, proximal colon	Present studies
Oesophagostomum dentatum	large intestine	colon, situated more distally than *Oe. quadrispinulatum*	Present studies

DISCUSSION

1.3.1. Occurrence

Five parasites are reported for the first time from British pigs: Oe. quadrispinulatum, G. urosubulatus and the three Trichostrongylus species.

Pasture grazed by ruminants is commonly contaminated by the infective stages of Trichostrongylus. Pigs may be experimentally infected with T. colubriformis (Tromba and Douvres, 1958b) and it is surprising that this genus is so rarely isolated from this host in areas where swine and sheep are kept in close proximity. Costa (1965b) records an incidence of 12.9% from the Bahia district of Brazil, but there have only been five other reports of this parasite occurring naturally in pigs (Kotlan and Mocsy, 1933; Roberts, 1940; Bobkova, 1960; Frietas and Costa, 1962; Mullee and Cox, 1967). There is only one previous record of T. axei in the domestic pig (Roberts, 1940) although it has been recovered from wild boar (Jansen, 1965). Whilst these species are known to parasitize a number of animals, the third member of this genus, T. vitrinus, has a much narrower host range and has been reported from only two non-ruminant hosts, the vulpine opposum (Trichosuros

vulpecula) and man (Gordon and Sommerville, 1958; Euzeby, 1963). The isolation of T. vitrinus from the pig on this occasion, therefore, provides a new host record. Bobkova (1960) has described a Trichostrongylus species (T. suis) which has the pig as its natural host. There cannot be any doubt, however, that the worms found during the present study were representatives of the ovine species. A careful investigation of the circumstances of this discovery eliminated the possibility that the observed nematodes were contaminants from other projects taking place in the laboratory.

G. urosubulatus has not been found in any host animal not belonging to the genus Sus. The 'pig hookworm' (a misnomer as this parasite, although having some characters in common with the Ancylostomatidae, is a member of the Strongylidae) is therefore host specific and consequently, it must be endemic in the British pig population. Figure 1.3. is a drawing made from a worm of this genus collected during the Scottish survey reported here.

Oesophagostomum is the commonest helminth parasite of Scottish pigs, being found in 67% of porkers, 43% of baconers and 94% of the breeding stock. Despite the widespread occurrence of Oe. quadrispinulatum throughout Europe: e.g. Italy (Marcone, 1901); Jugoslavia (Mickacic, 1937); Hungary (Kotlan, 1948); Austria (Supperer, 1955); Spain (Linzcano Herrera, 1958); Germany (Schmeer, 1958) and Holland (Baars, Dorrestijn, van Jaarsveld, Jansen and Mouwen, 1967), Oe. dentatum has been commonly assumed to be the only species of porcine nodular worm present in the United Kingdom. The present survey, however, demonstrated a high incidence of the second species in Scotland. Taffs (1967a) has confirmed the presence of Oe. quadrispinulatum in Britain and shown it to be equally common in East Anglia.

One of the first workers to study the porcine oesophagostomes in detail was Dr. T. Goodey. He contributed a thorough account of the morphology of Oe. dentatum recovered from natural infections in British pigs (Goodey, 1924a). In the following year, he

published a description of a new species, Oe. longicaudum (later shown by Alicata, 1935 to be synonymous with Oe. quadrispinulatum) based on specimens from a collection made in New Guinea! It seems strange that such a meticulous worker should have overlooked the presence of Oe. quadrispinulatum in the United Kingdom under these circumstances.

Other nematodes frequently encountered in Scotland are A. suum, T. suis, H. rubidus and rather less commonly, Metastrongylus apri.

Three parasites that are known to occur in pigs from time to time in the United Kingdom were not found in the present series: T. spiralis (Bruce, 1966), Strongyloides spp. (Walley, 1967; Taffs, 1968b) and F. hepatica (Ross, Dow and Todd, 1967; Ellicott and Colgreaves, 1968). The reasons for these negative findings are different in each case. Strongyloides infections, for example, are most often seen in baby pigs or weaners. All the pigs of the present series

were over four months of age, and so the results do not exclude the possibility that this worm is endemic in the Scottish pig population. Experience in Northern Europe (Madsen, 1961) and more recently in the United States of America (Zimmermann, 1966) has shown that the incidence of T. spiralis infections in pigs can be reduced dramatically by ensuring that meat containing live larvae is not fed to swine. The boiling of kitchen wastes intended for pig food (compulsory on British farms) achieves this purpose and accounts for the extreme rarity of porcine trichinosis in the U.K. The sporadic cases that do occur from time to time may originate from feral sources or from badly prepared swill. Pigs are naturally very resistant to infection by F. hepatica, so that flukes seldom become established in the bile ducts of this species (Ross, Dow and Todd, 1967).

Even though the spiruroid parasites, Ascarops strongylina and Physocephalus sexalatus are commonly found in feral swine on the continent of Europe, they were absent from the British pigs examined during the

present survey. Jansen (1965) records incidences of 93 and 75% respectively for the two nematodes in Dutch wild boar, but these species have never been found in domestic pigs in the Netherlands or neighbouring countries. It is difficult to envisage how the farm environment could give protection from the intermediate hosts (coprophagous beetles) or paratenic hosts (small mammals and birds) should the pigs be kept at grass, but nevertheless freedom from these parasites is probably related directly or indirectly to husbandry practices.

1.3.2. Age Incidences

When the parasitic fauna of different age-groups of the host species are compared, the nematodes are seen to fall into two broad categories:

(1) those whose incidence decreases with the increasing age of the host.

(2) those found more frequently in the adult animal.

Group 1:

One might have anticipated that the adult animals

of the present survey would be less heavily parasitized with A. suum or T. suis than the younger age groups (Tables 1.2. and 1.3.; Figs. 1.8. and 1.9.) since the ability of the pig to develop an active immunity to infections with these helminths has been acknowledged by several authors (Kelley and Nayak, 1964; Powers, Todd and Goldsby, 1959; Taffs, 1958, 1964a, 1964b). Table 1.2. suggests that the baconers displayed a greater degree of immunity to Ascaris than did the porkers, but Fig. 1.8. shows this to be false. The curves for the two sets of data coincide and thus the parasitic status of each is identical. The same diagram, however, clearly shows the differences between the parasitic burdens carried by the growing pigs and the mature animals.

Group 2:

The second category covers those genera that were found most frequently in the adult animal and includes the strongylate genera Hyostrongylus, Oesophagostomum and Metastrongylus. Hyostrongylus was never seen in a growing pig during the whole course of the survey, but

the two Oesophagostomum species were commonly found in fatteners as is shown in Figs. 1.10, and 1.11. In each case, the worm burden of the baconers is less than that of the porkers, which might suggest that some immunity or resistance is built up during the early life of the pig, the percentage incidences (Table 1.2.) resembling those for T. suis (Table 1.2.), in which species immunological factors are known to be involved (Powers, Todd and Goldsby, 1959). Later, however, this trend is reversed so that almost every adult pig harbours Oesophagostomum, often in very great numbers.

The statement that Metastrongylus was more common in adult than growing pigs may be misleading as it is based on a comparison of data collected from 2,315 baconers and that from only 33 sows. Such a situation might have been predicted on theoretical grounds even though pigs are able to build up an acquired immunity to these infections (Jaggers, 1965; Dixon, 1968). This nematode genus requires an intermediate host for the completion of its life-cycle (Hobmaier and Hobmaier, 1929), the ova being able to

hatch and attain the infective stage only if eaten by an earth-worm. Fattening pigs are kept indoors on concrete where contact with annelids is unlikely, although not impossible (Taffs, 1967b; Herbert, 1967). Therefore, if gilts are taken from a housed herd and put onto grass, as they often are in Scotland, they will be fully susceptible to the infection to which they become exposed.

1.3.3. Seasonal Incidence

The monthly collections of slaughterhouse material (with the exception of lungs) were too small for the detection of seasonal variations. Quarterly results were therefore pooled and the year divided into the following 'seasons': early winter (November - January); late winter/spring (February - April); early summer (May - July); late summer/autumn (August - October).

The population size of the gastro-intestinal strongylate genera showed marked seasonal fluctuations, being greatest in the spring (Oesophagostomum spp. - Table 1.5., Figs. 1.14. and 1.15.), or in the spring and

early summer (Hyostrongylus - Table 1.5., Fig. 1.13.), although these changes only occurred in adult animals. No corresponding variations were seen in growing pigs (Fig. 1.16.). This may be because younger pigs are kept indoors at a constant temperature throughout the year and are therefore shielded from external environmental influences.

There is little change in the actual percentage of sows infested with Oesophagostomum from season to season, however, whereas there is a marked decline in the numbers harbouring Hyostrongylus in the autumn (Table 1.5.).

In contrast to the other gastro-intestinal strongylates, ascarid infections tended to be more common in the late summer and autumn; and trichurid infestations in the winter (Table 1.4.).

The incidence of Metastrongylus spp. was below the annual average in January, April, June and July and above the mean value in March, August and December (Table 1.7.).

These fluctuations were, however, of a low magnitude.

1.3.4. A Comparison with Other British Surveys

Cameron (1933) published a paper entitled "The internal parasites of pigs: a survey". He records the presence, in the United Kingdom, of the following helminths: 'Ascaris lumbricoides', Hyostrongylus rubidus, Oesophagostomum dentatum, Trichuris suis, Metastrongylus spp. and more rarely, Trichinella spiralis, Fasciola hepatica and Taenia solium. It is not stated whether this list is based on the author's experiences or those of other workers. Little information is offered on the relative frequencies of the parasites or the intensities of infection seen in the field. Cameron considered Ascaris to be the nematode most often encountered, Oe. dentatum, T. suis and Metastrongylus spp. to be widespread and H. rubidus "quite common in the south of England". Nicholson and Gordon (1959) were the first to diagnose hyostrongylosis as a clinically apparent disease in Scotland.

As far as the gastro-intestinal helminths are

concerned, no further progress was made until Jenkins and Erasmus (1963) conducted their survey which encompassed the examination of 27 pigs of unspecified age. They found 40.7% to be infested with A. suum (range 0 - 28 helminths), 40% with Oe. dentatum (0 - 165) and 63.6% with T. suis (0 - 43). These results correspond very closely with the figures obtained for pigs of porker weight in the present survey.

Economic and commercial pressures later created a need for more precise information and stimulated the most recent investigations i.e. those of Davidson and Taffs (1965) and Gitter, Kidd and Davies (1965) as well as the original work reported in this chapter. At the completion of their work, the Weybridge team (Gitter, Gibson, Kidd and Davies, 1966) had examined faecal samples from 614 sows on 71 farms. Of these animals, 79.8% were infected with 'Oe. dentatum', 43.3% with H. rubidus, 0.88% with Ascaris and 0.48% with T. suis. Davidson and Taffs (1965) record results from 200 pigs of all ages, but principally sows. They recovered 'strongyle' eggs from 95% of the samples.

Mixed Oesophagostomum/Hyostrongylus infections accounted for 65% of these. Only six cases of ascariasis were detected. Davidson, Murray and Sutherland (1968) found 80% of 2,000 coprological examinations to be positive for 'strongyloid eggs'. Larval cultures performed on 1,000 of these revealed that 53% were mixed infections, 44% were pure Oesophagostomum and 3% pure Hyostrongylus. These authors remark on the fact that the number of eggs per gramme of faeces tends to increase with age and that sows harbour the largest populations of gastro-intestinal strongylates.

These most recent surveys thus support some of the conclusions of the present work i.e. the sharp contrast between the high incidence of infection with Oesophagostomum and Hyostrongylus and the infrequent occurrence of Ascaris and Trichuris in adult pigs. The amount of useful information that can be obtained from faecal examinations is limited to an inaccurate assessment of the presence or absence of parasites (false negatives often occur) and an even less reliable evaluation

of the degree of infestation (egg-counts are rarely proportional to the worm burden). In addition, the sampling techniques used in these coprological surveys were not representative of the national or regional pig populations as, in each case, many if not all of the herds under observation were selected because of the occurrence of unthriftiness or other clinical abnormality. Little comparative data was presented to illustrate the changes taking place in the composition of the helminth fauna with the increasing age of the host or in accordance with the season of the year. Davidson, Murray and Sutherland (1968) give the impression that they have sufficient results to do this but, to date, they have chosen to publish only a succinct summary.

A number of studies into the prevalence of lungworm infections have been conducted over the years (Lewis, 1926; Robertson, 1937; Dunn, Gentles and White, 1955; MacKenzie, 1958; Whittlestone, 1957; Jaggers, 1965; Pirie, 1965; Taffs, 1968b). The overall incidence of Metastrongylus spp. in the present study (5.6%) was lower

than in a previous Scottish survey conducted by Robertson (1937) who recorded 13.08% but similar to the figure obtained by Pirie (1965) who drew his material from the same abattoir as the present author. Pirie found 93 (8.4%) of 1,113 pigs to be infected with M. apri and one (0.1%) with M. pudendotectus. He noted that one half of the infested animals harboured fewer than ten worms.

The scarcity of M. pudendotectus, found in only one of 2,513 pigs in the present survey, agrees with Dunn (1954), Pirie (1965) and Taffs (1968b) but is in sharp contrast with other surveys in which an attempt has been made to differentiate the species. Jenkins and Erasmus (1963), for example, found that approximately one third of the lungworms they recovered belonged to this species.

Dunn, Gentles and White (1955), MacKenzie (1958) and Jaggers (1965) have described the seasonal fluctuation in the size of lungworm infections in Britain. In

general, the incidence is high in the autumn and winter and low in the summer. The breeding habits of some species of earthworm are dependent on the amount of summer rainfall and so this factor can influence the magnitude of the parasitic population in the following months. The present results did not show any marked seasonal variation although the highest and lowest values did correspond with the pattern outlined above (with the exception of the January reading).

1.3.5. The Danish Survey

Although the incidence of Ascaris suum in bacon pigs is well documented in the Danish literature (Jensen, 1927-28; Petersen, 1941-42; Mogensen, 1962; Melchiors, 1963; Mandrup, 1965), very few references have been made to other helminths of the porcine gastro-intestinal tract, or to other age-groups of the host species. Petersen (1950) commented on the finding of T. suis in slaughter-house material and Mogensen (1962) demonstrated the occurrence of this parasite and

Oe. dentatum in baconers by coprological examination. His figures show an overall Oesophagostomum infection rate of 73%, regional results varying from 59% in Mid-Jutland and North Jutland/Vendsyssel to 100% on Lolland and Falster.

The present survey revealed the presence of two parasites that were not thought to occur in Scandinavia. These were Hyostrongylus rubidus and Oe. quadrispinulatum. The occurrence of the latter species had been previously noticed in Denmark by the late Dr. Hans Roth in 1942, as witnessed by a bottle in the helminthological collection of the Royal Veterinary and Agricultural College, Copenhagen, which is labelled: "669 - Oesophagostomum longicaudum - stortarm svin nr. 3 - Ringsted 23/1-42". This discovery, however, remained unpublished and was forgotten. H. rubidus has since been found by Nielsen (1966a), Larsen (1967) and Ludvigsen and van Abrichem (1967).

As the Danish survey was confined to the examination of material from adult pigs, Table 1.9. was

compiled summarizing the findings of other authors and giving a more complete picture of the distribution of the various parasites throughout the Danish pig population. It can be seen that the overall pattern is much the same as that demonstrated in the United Kingdom. The ascarid population declines with the increasing age of the host whilst the oesophagostomes increase in number. The intensity and extensity of infections with H. rubidus is very much lower than in Britain. The figure given for the occurrence of T. suis in baconers is also very low in comparison. This value was taken from the paper by Mogensen (1962) and is based on the detection of helminth eggs in the host's faeces by a salt flotation technique. T. suis is not one of the most prolific egg-layers and its eggs do not float readily in this solution. It is probable that a post mortem survey would have revealed a much higher incidence.

The wide distribution of H. rubidus in Denmark is indicated by the demonstration of its presence in

Southern and Mid-Jutland as well as on the islands of Lolland and Zealand (Fig. 1.18.). Since pigs have not been legally imported into Denmark for many years (except for closely controlled breeding programmes), H. rubidus is undoubtedly enzootic in the Danish national herd. Only 5.7% of the 105 selected herds investigated in the geographical survey were infected with this parasite, confirming the results of the Zealand post mortem study. It is very exceptional for Danish pigs of any age to be put out to grass and so it is likely that the actual proportion of herds infected is even lower than this figure indicates.

The geographical distribution of Oesophagostomum in Danish sows was rather different, an equally high incidence being reported from all parts of the country (Table 1.8.).

1.3.6. A Comparison with Other European Surveys

Koffman (1940) conducted an exhaustive survey of the helminth parasites of Swedish farm animals but failed

to find H. rubidus. The discovery of this nematode in Denmark stimulated a new search which revealed two positive cases out of the eleven sow stomachs that were examined (Nilsson, 1967). Across the Baltic, Tarczynski (1956) examined the worm burdens of domestic and wild pigs in Poland. He mentions neither H. rubidus nor Oe. quadrispinulatum. In both the above-mentioned countries, however, Oe. dentatum is acknowledged as being very common.

Voluminous reports have been prepared covering almost every region of Germany. Most of these are in the form of doctoral dissertations and have been listed by Behrens (1966). Of particular value are papers by Boch and Neubrand (1962), Lamina and Bohnhardt (1964) and Weissenburg and Neubrand (1967). These contributions clearly illustrate the variations in the nematode population structure that take place in relation to the advancing age of the host. Weissenburg and Neubrand (1967), for example, investigated 1,871 faecal samples and showed that the frequency of patent ascariasis and

trichuriasis in animals under six months of age was around 20-26%. In older age groups, the numbers of infested animals fell rapidly to less than 2%. The Oesophagostomum figures rose from 58% to more than 80% between the ages of three months and nine months, with an intermediate value of 72% at six months old. H. rubidus is recognized as a parasite of sows rather than fatteners but the overall incidence is somewhat lower than in the British Isles (Boch, Gerber and Horchner, 1968; Barth, 1968). The incidence of Globocephalus urosubulatus is in the order of 16-40% (vide review by Ehlert, 1962). This parasite is also found in wild boar in Germany as are Ascarops strongylina and Physocephalus sexalatus (Boch and Horchner, 1961).

All of these parasites occur in Sus scrofa in Holland as well as T. axei (Jansen, 1964). Schrooyen (1969) investigated over 800 Dutch herds and found the rate of infection with A. suum, T. suis and the 'strongyle' parasites to be 45%, 4% and 49% respectively, in growing pigs whilst the corresponding figures for sows were 28%,

0% and 81%. Mouwen, Jansen, van Jaarsveld, Dorrestijn and Baars (1968) list values of 9.5%, 3.4%, 87.7% and 97.8% for Ascaris, Trichuris, Hyostrongylus and Oesophagostomum in mature stock. In the neighbouring country of Belgium, 97% of a series of breeding farms were infested with 'strongyles' (at least 52% harboured Hyostrongylus in addition to Oesophagostomum), but only 25% of investigated fattening units were contaminated (Deceuninck and Paredis, 1967).

Further south, in France, Euzeby and Renault (1966) also demonstrated how the frequency of ascariasis and trichuriasis declines with the increasing age of the host whilst the gastro-intestinal strongylates show the opposite trend. These authors consider H. rubidus to be very rare in their native country but provide no figures to support their argument. A sporadic distribution is reported for Austria (Supperer, 1955) whilst the age incidences for the other species correspond with those described in the proceeding paragraphs (Supperer, 1961).

The numerous studies to which brief reference has been made in this section can be summarized very simply. The pattern of distribution of the gastro-intestinal helminths throughout the British pig population is very similar to that characteristic for all parts of temperate Europe for which relevant statistics are available. G. urosubulatus, however, is particularly common in Germany and it should also be mentioned that Strongyloides is a more serious problem on the continent than it is in the United Kingdom (Supperer, 1955). The spiruroid parasites of the stomach of the pig are found in European wild boar but have not been recorded in the United Kingdom (§ 1.3.1.).

1.3.7. Epidemiological Significance

The information collected during the Scottish and Danish surveys enable certain observations and tentative suggestions to be made regarding the biology of the recorded parasites. The demonstration of the heavy gastro-intestinal strongylate burdens in adult pigs, for example, leads one to consider the possibility that the sow might play a

dominant role in the transmission of Oesophagostomum spp. from one generation of pigs to the next. Epidemiological studies reported in the next chapter of this thesis proved this to be the case.

In many host-parasite systems, older animals harbour fewer helminths than do younger age-groups of the same species. This may be due to the development of "age resistance" or an acquired immunity. A comparison of the worm burdens of porkers and baconers showed declining Oesophagostomum numbers § 1.3.2., a phenomenon which appeared to be in accordance with this general principle. On the other hand, German experience does not support this view as a regular rise in the frequency of infection occurs from three months of age onwards in that country (Weissenburg and Neubrand, 1967). Between bacon weight and the attainment of sexual maturity, however, the British and German figures are in agreement. In the present survey the incidence of Oesophagostomum increased from 43% to 94%. Any immunological response that may take place in the young pig is therefore transient and of little functional

value to the adult animal.

The reason for this apparently inverted age incidence is obscure. At first it was thought that this phenomenon resulted from environmental changes as pregnant gilts and sows in Scotland are often kept collectively in grass paddocks whereas younger stock is kept indoors on concrete. The same changes occur, however, in Danish pigs (Table 1.9.) even though these are almost invariably kept inside whether intended for fattening or breeding. This hypothesis is therefore unacceptable and another explanation must be sought.

Whilst Connan (1967a) is undoubtedly correct when he states that the nature of the floor and bedding influence the size of the Oesophagostomum population within a herd to some extent, it cannot be denied that this genus can, in general, thrive equally well on grass and concrete. Oesophagostomum is a prolific egg-layer and even under good farming conditions enough larvae can survive to maintain a high level of infestation. A numerical example to illustrate

this point is given in Chapter 2 (§ 2.3.7.).

In contrast, H. rubidus has a very limited biotic potential and regular removal of faecal material from piggeries may reduce larval numbers to very low levels. Accumulation occurs on pastures, especially those that are not rested or ploughed regularly. There is much evidence in the world literature to suggest that clinical hyostrongylosis is almost always a consequence of such conditions. Thus, in Scotland where pregnant pigs are often put out to graze, Hyostrongylus is common, whereas Danish sows reared and maintained on concrete are rarely infected.

Although there are superficial similarities, the epidemiology of oesophagostomiasis is therefore quite different from that of hyostrongylosis. This principle applies also to the seasonal variations in the parasitic population size. Whilst the number of Oesophagostomum in sows is greatest in the spring and minimal in the autumn and winter, the frequency of infection remains constant throughout the year (Table 1.5.). On the other hand, the incidence of Hyostrongylus fluctuates

quite considerably. A figure of 73% was recorded in the spring, 56-57% in the summer and winter and 13% in the period August to October. H. rubidus in the stomach of the pig can be considered to be analogous to Ostertagia spp. in ruminants since the two trichostrongylids are very closely related and provoke similar pathological lesions (Davidson, Murray and Sutherland, 1968). Anderson, Armour, Jennings, Ritchie and Urquhart (1965) have shown how infective larvae of the latter genus behave differently in the late summer and autumn than at other times of the year. Instead of completing the parasitic phase of their life-cycle in the usual three weeks, a large proportion of the worms become inhibited in the early fourth stage and only resume their development after a protracted period. Unfortunately, no mucosal digests were performed during the present study and so it is impossible to judge if this phenomenon also happens in pigs. The adult worm counts suggest, however, that this may be the case.

In contrast, it is at this time of the year (late summer and autumn) that ascarids are most frequently encountered

(Table 1.4.), a pattern that has been recorded in Denmark as well (Melchiors, 1963). It is interesting to note that the quantity of liver tissue condemned as unfit for human consumption because of 'milk-spot' is also maximal during late summer (Fagerberg and Persson, 1960; Roneus, 1966; Almlof, Bjorklund and Henricson, 1968). These patches of white fibrous tissue are often caused by migrating A. suum larvae (vide review by Bindseil, 1967), although these lesions may sometimes result from a form of visceral larval migrans caused by canine, feline or equine ascarids (Done, Richardson and Gibson, 1960; Roneus, 1966). The latter author postulates that the increased incidence may be a result of the warm summer weather stimulating faster maturation of the Ascaris ova and hence providing a greater challenge. This is, however, difficult to believe as most pig-houses maintain a winter temperature high enough to allow rapid development of the eggs. On the other hand, the suggestion made by Almlof, Bjorklund and Henricson (1968) that the liver spots reflect the general non-specific resistance of the host, which is greater in the summer, is not consistent with the findings of

the present study as there are more, not less, parasites reaching maturity at this time.

It has been stated earlier that the low incidence of A. suum in older stock almost certainly indicates the acquisition of an immunity. Yet, Gitter, Kidd and Davies (1965) have put forward the theory that this is due to the widespread use of the piperazine anthelmintics. This cannot be true in Denmark as, with the exception of two campaigns aimed at controlling this parasite in local regions (Melchiors, 1963; Mandrup, 1965), ascaricides have not been routinely administered in that country. The use of these compounds is unlikely to have influenced the results of the Danish survey, but the same trend, which is seen all over Europe, was detected. The piperazine salts are not effective against Trichuris, so that they cannot be responsible for the similar age incidence displayed by this parasite.

Since the frequencies of ascariasis and trichuriasis are much greater in fattening pigs than in adults, it is

obvious that not all of the infections in the younger pigs could have been contracted from the dam. The larvae of both genera remain within the egg until this is eaten by a potential host. Thus protected, the infective forms are extremely resistant to unfavourable environmental conditions and can remain viable for four to five years. In addition, ascarid ova have adhesive properties. It seems likely therefore that the mechanical transport of ova from the heavily infested fattening house to the maternity unit is a very important mechanism for the transfer of these infections to successive generations of the host species. Kelley, Sumption, Adams and Olsen (1959) failed to eliminate A. suum from piglets by treating the dam with an ascaricide and moving her to a clean pasture before farrowing. This biological pathway is quite different from that employed by the gastro-intestinal strongylate parasites which exploit the sow as a means of ensuring the perpetuation of their species (Chapter 2).

1.3.8. The Recognition of Oe. quadrispinulatum

Detailed descriptions of the adult forms of Oe. dentatum

and Oe. quadrispinulatum have been recorded by Goodey (1924a, 1925), Ozerkaja (1930), Mikačić (1937), Bobkova (1957), Schmeer (1958) and Linzcano Herrera (1958). These papers have been reviewed comprehensively by Haupt (1966) who also provides the results of his own morphological examinations.

The two species are easily differentiated. The tail of the female Oe. quadrispinulatum is markedly longer than that of Oe. dentatum, whilst the spicules of the male of the former species are shorter (Table 1.11.). Additional points of difference are the 'handle' of the gubernaculum (longer in Oe. dentatum) and the shape of the anterior portion of the oesophagus (both the inner and outer walls are more globular in Oe. quadrispinulatum). These features are illustrated in drawings made from oesophagostomes collected during the Scottish survey (Figs. 1.1. and 1.2.)

In the present instance, identification of the species presented no problem as there were several morphological characteristics on which a diagnosis could be made. When

classification depends on the recognition of a single feature, more care is necessary. Even within a homogenous population, individual variation in the dimensions of one particular organ can be quite considerable. One cannot, therefore, compare two worms and pronounce them to be separate species on the basis of one measurement. In order to give a confident judgement one must collect a number of specimens, estimate the size of the relevant part of the body of each and analyse these data in such a way that the presence or absence of two independent populations will become apparent. An example of this principle is shown in Fig. 1.19., which shows the tail and spicule lengths of 100 female and 100 male oesophagostomes taken at random from the collection made during the Scottish survey. This information has been displayed as a histogram comparing the frequency of occurrence of organs with specific dimensions. It is quite obvious that two separate populations are present as two normal distribution curves were formed. One female fell outwith the general pattern. Aberrant female Oe. dentatum with short tails have been noted by most of

the authors cited above.

The demonstration of two populations does not necessarily imply the presence of two species. The relative sizes of two organs (e.g. tail length and body length) may not be constant throughout the life of the nematode (Inglis, 1954). Thus, only worms of the same stage of development should be compared in this way. In addition, host factors, especially immunity, may alter the physical form of the parasite. The vulval flap of Ostertagia ostertagi, for example, is smaller in those females existing in an immune animal (Michel, 1967a).

In the present study, final confirmation of the decision that there were two species present in the material was provided by the observed breeding behaviour of the worms. Copulation was only seen to take place between members of the same morphological type, even in those parts of the intestine co-inhabited by both forms.

1.3.9. Predilection Sites

The gastro-intestinal tract of the pig may be regarded

as an ecosystem in the same way as a sand-dune or a ditch. The rules of ecology should, therefore, apply to populations of parasitic nematodes. One such concept is the principle of the ecological niche. This term may be defined as the summation of the physical and biological factors required for the maintenance of any particular species. If two animals have identical requirements they will be in direct competition. Under these conditions, one species will be better suited to its environment and will, over the course of evolutionary time, replace the other unless divergence of habits eliminates antagonism. Thus, in a stable ecosystem, each niche will be filled by only one species.

Schad (1963, 1966) has tested these hypotheses in the European tortoise which harbours eight species of the oxyuroid genus Tachygonetria in the colon. It became apparent that even in this small volume most species had their own geographical teritories. Where overlapping did occur, the cohabiting species had different feeding habits. An exactly similar situation happens in the digestive tract

of the pig.

Table 1.12. shows the predilection site of each of the gastro-intestinal nematodes commonly found in European pigs. Each worm occupies its own exclusive position with the exception of T. suis and Oe. quadrispinulatum. These are both found in the caecum and proximal part of the colon. The two genera are presumably able to coexist because their different feeding habits preclude serious competition, Trichuris being found with its anterior portion buried in the tissues of the host, whilst Oesophagostomum adults lie against the mucosal wall without penetrating the surface.

Chapter 2

OBSERVATIONS ON THE EPIDEMIOLOGY OF PORCINE OESOPHAGOSTOMIASIS: THE PERIPARTURIENT EGG-RISE

INTRODUCTION

The post mortem survey described in Chapter 1 revealed that the commonest nematode parasite of the domestic pig in Britain is Oesophagostomum. It was shown that adult pigs are in general more heavily parasitized with this genus than growing pigs. These findings are confirmed by egg-count data recorded by Davidson and Taffs (1965) and Gitter, Gibson, Kidd and Davies (1966). Original work performed in Denmark and the experiences of a number of continental workers (vide Chapter 1) suggest that the patterns of infection seen in the United Kingdom are common to the whole of Europe.

The adult pig is thus an important reservoir of infection for Oesophagostomum spp. An evaluation of the possible significance of sows and boars in the epidemiology of porcine oesophagostomiasis on the farm was therefore attempted. It soon became clear that the faecal 'strongyle' egg-counts of sows undergo cyclical fluctuations in association with the reproductive cycle, and thereafter attention was concentrated on this one facet of natural infestations.

MATERIALS AND METHODS

2.1.1. Farms Visited

Farm B:

This establishment was situated in Dumbartonshire near the village of Hardgate. It was a commercial pig breeding and fattening unit comprising 30-40 sows and gilts, three boars and 150-200 fattening pigs which were slaughtered at porker weight. The Large White breed predominated, but some animals were Landrace, Wessex Saddleback or various crosses.

After service, adult females were either kept in groups of 10-15 in pens with concrete floors and a small hut for shelter, or put onto grass in a 15 acre field. When parturition was imminent, the breeding females were brought into the farrowing house. This was a building based on the design of the Danish fattening house with a central feeding passage passing between two rows of pens, each with a dunging passage along the external wall. Each pen measured about three metres square and contained the usual facilities such as an infra-red lamp and creep feed for the piglets. Weaning took place between the sixth and eighth weeks of life.

All the pigs were fed swill prepared from hotel waste with the exception of the suckling pigs and the sows at pasture. These were provided with suitable commercial concentrate rations. The indoor accommodation was swept daily, the outdoor pens rather less frequently. The general standard of hygiene and the upkeep of the buildings was poor. Faecal material from the pigs was stacked on a spare patch of ground immediately adjacent to the farrowing house.

Anthelmintics were not used on the farm during the course of these studies.

Farm C:

A second farm in the area of Hardgate was visited. This enterprise was primarily a breeding unit, most of the progeny being sold after weaning at seven to eight weeks old, although a few were kept on to porker weight. The herd was composed of around 40 Large White sows and one boar. The pregnant animals were either kept in a concrete pen which provided sufficient accommodation for eight to ten sows and gilts, or they were put out to grass. Most often, a three acre paddock was used, but occasionally other fields were set aside for this purpose.

The pigs were brought in about 10-14 days before parturition which took place in a farrowing crate built into a former railway coach. The sow and her litter were then moved to a wooden hut which was triangular in cross-section and had a small concrete run outside.

The standard of cleanliness was much superior to Farm B, but the construction of the huts made thorough removal of faecal material impossible. Appreciable worm burdens had built up even though the herd was supposedly specific-pathogen-free. The stock was wormed only when one of the pigs was observed to pass an ascarid. This did not happen during the course of these studies.

Farm EC:

Also selected was a herd of 140 Large White breeding sows located between Glasgow and Kirkintilloch. Here the pregnant animals were kept indoors in sow stalls on dry concrete without bedding. They were cleaned out twice daily, the general standard of hygiene on the farm being much above average. Young gilts were kept in groups of 20 on deep litter. One week before the expected date of

parturition, the pigs were moved to farrowing boxes or crates. A variety of converted loose boxes etc. were in use for most of the period of observation, but latterly a farrowing house was brought into operation. This housed 20 sows in individual crates with underfloor heating. Weaning was performed between the sixth and eighth weeks after birth, when the piglets were taken to another farm for fattening.

The form of management outlined in the previous paragraph had been introduced just one year prior to the start of this study. Before, the in-pig sows and gilts had been kept at grass. A severe outbreak of the 'fading sow syndrome' had occurred and the farmer's veterinary advisers had diagnosed gastro-intestinal helminthiasis. All the pigs on the farm were immediately treated with thiabendazole (Thibenzole, Merck Sharp and Dohme, Ltd.), and thereafter each sow or gilt was dosed with a thiabendazole-picadex mixture (Thiprazole, Merck Sharp and Dohme, Ltd.) one week before farrowing. The sow stalls were erected and the stock removed from the fields. An immediate response was noted, although the herd did not regain its former first class condition until treatment had

continued for two years.

Farm MJK:

This was a smallholding situated near the village of Borup to the south-west of Copenhagen, Denmark. Fifteen sows were kept in stalls in one very old building and were moved to loose boxes for farrowing. The piglets were sold as weaners at six weeks of age. The standard of hygiene was lax, and anthelmintic therapy was never implemented.

2.1.2. Sources of Post-Mortem Material

The intestines of 15 sows were collected from the Roskilde Andelssvineslagteri (Roskilde Co-operative Bacon Factory). The animals selected were either non-pregnant or in the very early stages of pregnancy. A further 14 sets of intestines were gathered from Kødfoderfabrikken 'Sjaelland' (The 'Zealand' Meat and Bone-meal Factory). These originated from sows slaughtered as casualties at about the time of parturition. The two abattoirs served the same area of Denmark and so drew sows from the same population of pigs.

The sows were all rejects from the national herd. The first group had all produced one or more litters and had been

culled for a variety of reasons such as poor breeding characteristics, lameness, old age, bad temperament etc., whereas the casualties were mostly destroyed as a consequence of obstetrical mishaps (Appendix 2, Table 7).

2.1.3. Experimental Designs

The first phase of the project was devoted to a general survey of the distribution of Oesophagostomum infections within two herds of pigs, this knowledge being later supplemented by data gathered from a third farm. As fluctuations in the egg-counts of breeding females had been detected, additional information concerning the nature of these variations was obtained by the detailed study of two sows, one of which was subjected to a series of experimental procedures. The practical and financial difficulties of maintaining non-productive animals in a heavily contaminated environment prevented this part of the work being performed on a larger scale. Direct observation of an Oesophagostomum population is only possible after the host has been killed and necropsied. The last investigation of the series was therefore performed in an abattoir.

Investigation 2.1.

Faecal samples were taken from all groups of pigs on Farms B and C every second week from February, 1965 to February, 1966 for parasitological examination (vide infra). The results were expressed in terms of averages for the year and related to the age, sex and reproductive status of the animals.

Investigation 2.2.

All the breeding females on Farm EC were sampled every second week throughout pregnancy from November, 1966 to February, 1967. This allowed a closer study to be made of the faecal egg-count data for this period of the reproductive cycle. These sows were also used for an experiment described in Chapter 5 of this thesis.

Investigation 2.3.

One sow from Farm MJK (MJK 52) was sampled twice weekly for almost the whole period of pregnancy and throughout the following lactation, so that a detailed picture could be compiled of the fluctuations in egg-counts occurring in a single animal.

Investigation 2.4.

Another sow (MJK 53) was purchased from Farm MJK but maintained on these same premises. Faeces were taken almost every day and examined for nematode ova. The piglets produced by this sow were removed soon after birth and suckled onto a foster dam. This study and the next were run concurrently with Investigation 2.3., i.e. from November, 1965 to April, 1966. The egg-counts of a suckling dam (MJK 52) could thereby be directly contrasted with those of a female that had been prematurely deprived of her offspring.

Investigation 2.5.

This was a continuation of Investigation 2.4. Sow MJK 53 was not allowed to become pregnant again and examinations were continued over a period of five months. In this way, the egg-counts of a non-pregnant sow could be compared with those of the breeding female MJK 52. Any influence exerted by the oestrous cycle on the 'strongyle' egg-output would also be detected.

Investigation 2.6.

The sexual activity of the non-pregnant sow MJK 53 was

later modified by the administration of hormone preparations. An attempt was made to stimulate a premature period of oestrous activity by the injection of 500 i.u. serum gonadotropin and a similar quantity of chorionic gonadotropin (Gonadoplex, Leo Pharmaceuticals Ltd.). On another occasion, the onset of oestrus was delayed by giving a progesterone-like substance (norethisterone acetate, Parke-Davis & Co.) per os at a rate of 26 mg. per day for seven days. Lastly, ten daily doses of norethisterone acetate were applied during the initial stages of an oestrous egg-rise. Daily intramuscular injections of adrenocorticotropic hormone (Acton Prolongatum Vet., Frederiksberg Chemical Laboratories Ltd.) were given during the progesterone therapy to simulate a state of stress. The dosage regime was as follows: days 1 and 2, 225 i.u.; days 3 and 4, 450 i.u.; days 5 and 6, 225 i.u.

Investigation 2.7.

A comparison was made of the helminth fauna recovered from the series of 15 sows slaughtered whilst they were non-pregnant or in the very early stages of pregnancy, and a series of 14 killed at about the time of parturition. Particular emphasis was placed on the size and fecundity of

the female worms. The latter parameter was quantified by comparing the faecal egg-count with the numbers of female worms present in the large intestine and was recorded as the number of Oesophagostomum eggs per female worm per gramme of faeces. A more satisfactory unit would have been eggs per female per day, but it was impossible to obtain 24 hour faecal samples from sows slaughtered as casualties.

2.1.4. Collection and Treatment of Faecal Samples

Wherever possible, the farms were visited soon after the floors had been cleaned. The faecal samples were taken from dung pats that had been deposited only minutes previously and in this way contamination with free-living nematodes was avoided. If any tractable animals had not defaecated, material was taken directly from the rectum. The pregnant animals and fatteners on Farms B and C were kept in large pens or paddocks under conditions that made restraint and identification difficult. A composite sample for each group was therefore taken by pooling small quantities of faeces from as many fresh deposits as possible. Piglet samples were also representative of the

litter rather than the individual.

Samples were examined by a modified McMaster method (Morgan, Parnell and Rayski, 1950). This technique is suitable for counting 'strongyle' eggs but, as applied in this study, does not provide accurate results for Ascaris suum, Trichuris suis, Metastrongylus spp., Balantidium coli cysts or Eimeria spp. oocysts.

The Egg-Count:

Two grammes of faeces were weighed out and put into a sieve standing in a porcelain bowl. The faeces were mixed with 30 ml. of saturated sodium chloride solution. A wooden spoon ensured that the solid clumps of material were broken up and the whole dispersed in the liquid. The sieve was then raised and a further 30 ml. of salt solution poured over the remaining particulate matter. The liquid was thoroughly stirred and a small quantity pipetted into the chambers of the McMaster slide. After a short while, the eggs within the marked areas were counted under the low power objective of the stereomicroscope. The resulting total was multiplied by 100 to give the number of eggs per

gramme of faeces.

In Investigations 2.3., 2.4., 2.5. and 2.6., this procedure was repeated four times for every sample in order to increase the sensitivity of the technique.

Larval Culture:

In order to estimate the relative numbers of Oesophagostomum and H. rubidus ova in each sample, the eggs were hatched and cultured as described in Chapter 1. The third stage larvae were examined under the stereomicroscope, the two genera being easily identified (Appendix 2, Table 1).

2.1.5. Treatment of Post-Mortem Material

The helminths in each set of intestines were recovered by the techniques outlined in Chapter 1. The species were identified and the sexes separated. The length of each female Oesophagostomum was estimated by means of an epidiascope. A magnified image was projected onto a screen and the dimensions measured with an opisometer. The image of a calibrated graticule was used to provide the necessary conversion factor.

2.1.6. Nomenclature

It would be advantageous for the clarity of the ensuing text to define certain terms that have been given special meanings for the purposes of the present discussion:

'Strongyle':

This word refers collectively to the nematode superfamilies, Trichostrongyloidea and Strongyloidea, whilst excluding the Metastrongyloidea. In the present context, the term embraces the genera Oesophagostomum and Hyostrongylus. It is a particularly useful expression since these worms produce ova that are, for practical purposes, identical. The inverted commas are used as this term is a designation of convenience that is technically meaningless.

Parturient:

This adjective indicates a period from the second week before farrowing to weaning.

Pregnant:

This signifies the 14 weeks following conception.

RESULTS

2.2.1. Investigation 2.1.

Four hundred and eighty-nine faecal samples were examined from Farm B and 364 from Farm C over the 12 month period. Table 2.1. shows the average 'strongyle' egg-counts of each group of pigs on these farms, and Appendices 2.2. and 2.3. tabulate the raw data.

The average 'strongyle' egg-count of the parturient sows on Farm B was 2,850 eggs per gramme of faeces (e.p.g.), whereas that for the pregnant animals was 480 e.p.g. The corresponding figures for Farm C were 736 and 301 e.p.g. Thus, the 'strongyle' egg-output of the dams was 5.94 and 2.45 times greater than that of the in-pig females. The boars voided even fewer ova than the latter group.

The results for the sows on each farm are displayed in more detail in Figures 2.1. and 2.2. The parturient period forms the main body of each histogram, each vertical column representing the average figure for one week. On either side are the pooled results for the pregnant sows. The sampling methods did not allow a breakdown of these data (vide supra),

but this deficiency was rectified by Investigation 2.2.

Two weeks or so before farrowing took place, the egg-count started to rise, and it continued to do so until a maximum was reached during lactation. After the piglets were removed from the dam, there was a speedy return to the minimal values of pregnancy. Investigation 2.3. demonstrated the inadequacies of fortnightly sampling when studying the egg-output of individual sows, and provided the basis for the decision to express the present results in terms of herd averages rather than for single animals.

On Farm B, small quantities of 'strongyle' eggs were noted in the faeces of piglets after the third week of life, whereas on Farm C the appearance of ova was delayed until the fifth week (Table 2.2.). In both herds, larger numbers were voided after the sixth week. Many litters, however, showed no evidence of patent infection. The fattening pigs carried rather heavier infestations but many negative values were obtained. On Farm C, the figures for fatteners and in-pig sows were approximately equal.

Larval culture revealed that Farm C was infected with Oesophagostomum alone. The pigs on Farm B harboured Hyostrongylus as well, but this genus only accounted for approximately 0.2% of the total 'strongyle' egg-count.

2.2.2. Investigation 2.2.

Observations from Farm EC gave a detailed analysis of the period of pregnancy (Fig. 2.3.). No fluctuations of a magnitude comparable to those described in the last section were seen to occur during this phase of the cycle. After service, the egg output tended to fall, representing a tailing off of the previous periparturient rise, whilst the next was presaged by a prenatal increase in values. At about the eighth, ninth and tenth weeks of pregnancy, a small transient peak in the egg-counts was detected.

Figure 2.3. was compiled from the results of 312 faecal examinations. Both Oesophagostomum and Hyostrongylus were present in this herd, but again the latter genus accounted for less than one percent of the 'strongyle' egg output. Ascarid and trichurid ova were encountered on rare occasions.

2.2.3. Investigation 2.3.

The 'strongyle' egg-counts obtained by examining the faeces of sow MJK 52 during the first half of pregnancy were consistently low (Figure 2.4. Appendix 2, Table 5), varying from 50 to 650 e.p.g. Higher figures were recorded in the second half of pregnancy, peaks of 3,225, 2,725 and 5,750 e.p.g. occurring during this period. Immediately before parturition, the counts reached 9,075 e.p.g. and during lactation maximum values of 13,800, 15,975, 19,450 and 17,900 e.p.g. were obtained. This last result was reported four days after the piglets had been weaned. After this the output was considerably reduced remaining within the limits 225 and 2,925 e.p.g. for the next month.

The egg-counts in the latter part of pregnancy and during lactation fluctuated within wide limits over short periods of time. Often, ascending or descending curves joined several regularly spaced points.

This sow also harboured A. suum. The infection was patent during the second half of pregnancy and throughout the suckling period. Ova could not be demonstrated in early

pregnancy or after weaning. (Appendix 2, Table 5).

2.2.4. Investigation 2.4.

The egg-count pattern of sow MJK 53 before and after weaning is displayed in Fig.2.5. superimposed upon the graph of the regular periparturient rise as it was seen in Fig. 2.4. In this instance, the 'strongyle' egg output behaved in a manner similar to that of sow MJK 52 up to the time of farrowing. Thereafter, no egg-rise took place. On the contrary, the egg-count fell to comparatively low values. The highest reading obtained during the six weeks following parturition was 1,425 e.p.g., in contrast to the figure of 10,625 e.p.g. that had been recorded in late pregnancy.

2.2.5. Investigation 2.5.

Although the egg-counts of sow MJK 53 remained low throughout all of the five months of this investigation, small transient peaks were observed at intervals (Figs. 2.5. and 2.6.). The first occurred 11 days after weaning and another 35 days later. The second coincided with a period of intense oestrous activity. No further signs of sexual behaviour were noted until the third rise in values took

place after another 35 days. At this time some changes in the sow's temperament were noted (excitability, some desire to stand when 'ridden'), but a clinical diagnosis of oestrus could not be made with confidence. Thereafter, the phases of the hormonal cycle were never obvious on inspection. Yet, peaks in the faecal output of 'strongyle' ova occurred thrice at three-weekly intervals (Fig. 2.6.). During periods when the animal was thought to be in dioestrus, the egg-count remained below 500 e.p.g. whereas values of 1,000 to 2,000 e.p.g. were obtained when she was thought to be in season.

2.2.6. Investigation 2.6.

The parenteral administration of gonadotropins to sow MJK 53 lengthened the period between the successive peaks from three to six weeks (Fig. 2.7.).

Oral application of norethisterone acetate not only delayed the occurrence of an anticipated peak but also reduced the egg-count to zero, no positive samples being obtained for ten days (Fig. 2.8.). During the nine months that this sow was under surveillance (for Investigations 2.4., 2.5. and 2.6.) this was

the only occasion on which a series of negative results was obtained. The egg-output started to rise steeply six days after the withdrawal of the progesterone therapy.

In the final attempt to manipulate the hormonal balance of the host, the norethisterone preparation again caused the egg-count to fall immediately (Fig. 2.9.). A rise in values did follow when the adrenocorticotropic hormone was given, but its magnitude was small in comparison with that of the two previous oestrous rises (1,200 e.p.g. in contrast to 5,850 and 4,975 e.p.g.).

2.2.7. Investigation 2.7.

In Fig. 2.10., the fecundity of the female worms is plotted against the magnitude of the corresponding Oesophagostomum populations. It can be seen that when only comparatively small numbers of worms (i.e. less than 900 females) are present, much greater numbers of eggs are produced by the individual females than are shed by those belonging to larger populations.

Fecundity figures obtained from the non-pregnant group of

sows are represented in Fig. 2.10. by closed circles and the values for the parturient group are shown by crosses. The circles tended to be closely clustered about the mean, whilst the crosses were scattered more widely on either side of this value (Appendix 2, Table 10).

A total of 2,169 female worms were measured and the results are displayed in Table 2.3 . and Appendix 2, Table 9 . On average, the Oesophagostomum recovered from the parturient sows were appreciably longer than those taken from the non-pregnant animals. In the case of Oe. dentatum, this difference was statistically significant ($P < .01$).

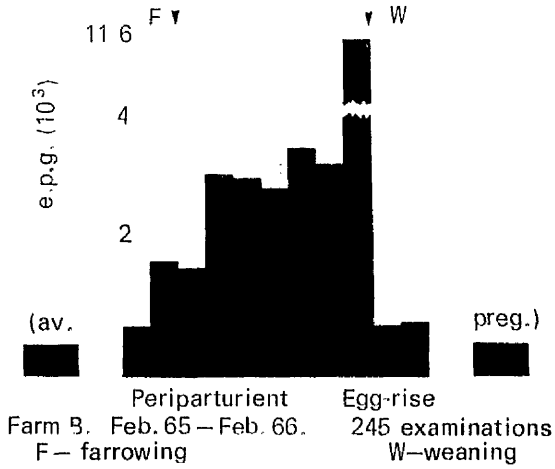


Fig 2.1 Faecal 'strongyle' egg-counts (herd averages) from sows on Farm B related to the stage of the host reproductive cycle. Each vertical column represents one week On either side of the histogram, the average value during pregnancy

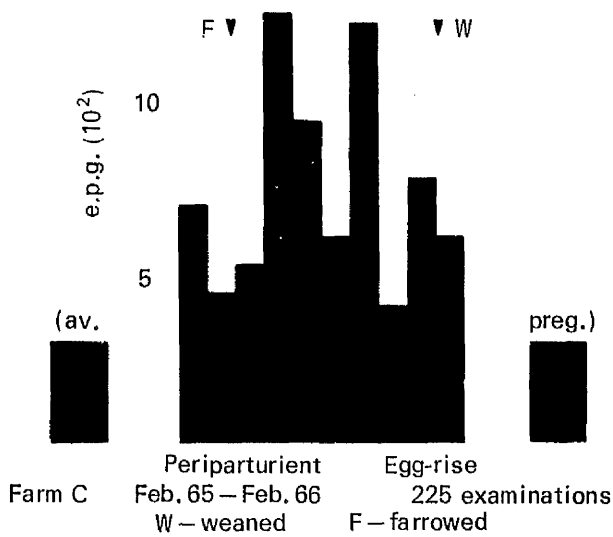


Fig 2.2 Faecal 'strongyle' egg-counts (herd averages) from sows on Farm C related to the stage of the host reproductive cycle. Each vertical column represents one week On either side of the histogram, the average value during pregnancy

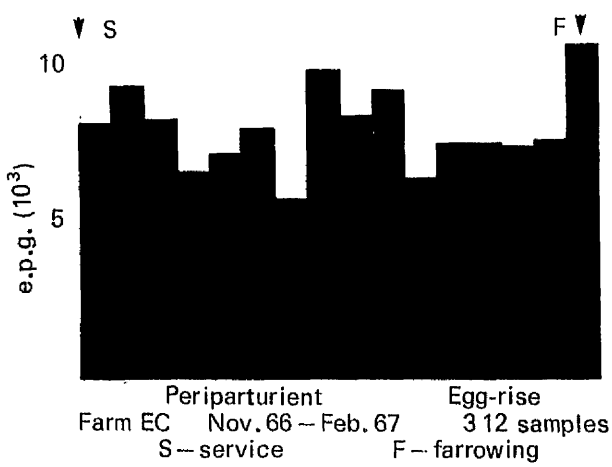


Fig 2.3 Faecal 'strongyle' egg-counts (herd averages) from sows on Farm EC during period of gestation. Each vertical column represents one week

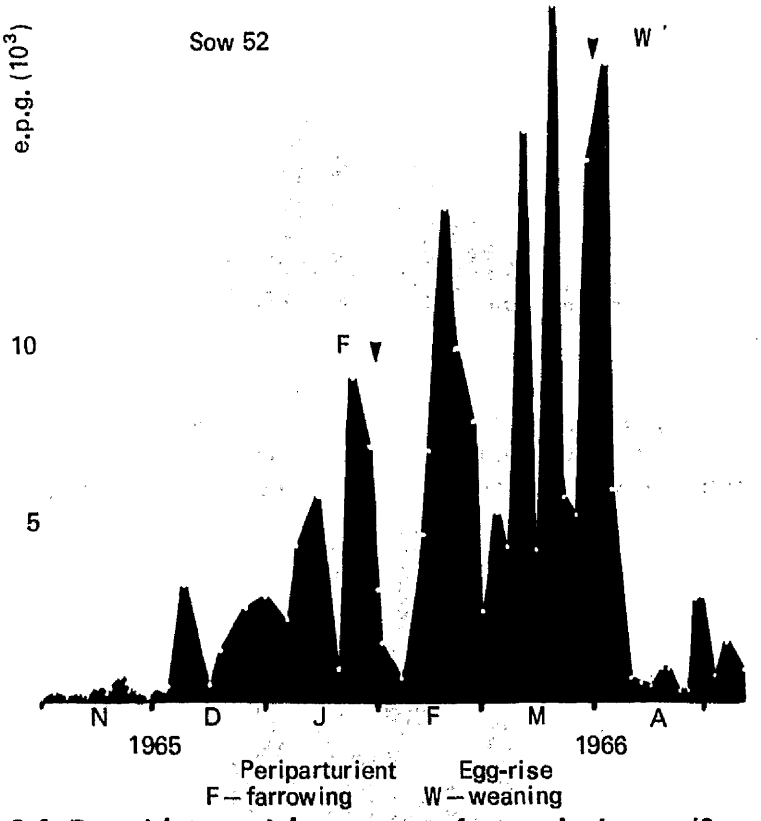


Fig 2.4 Faecal 'strongyle' egg counts from a single sow (Sow MJK52)

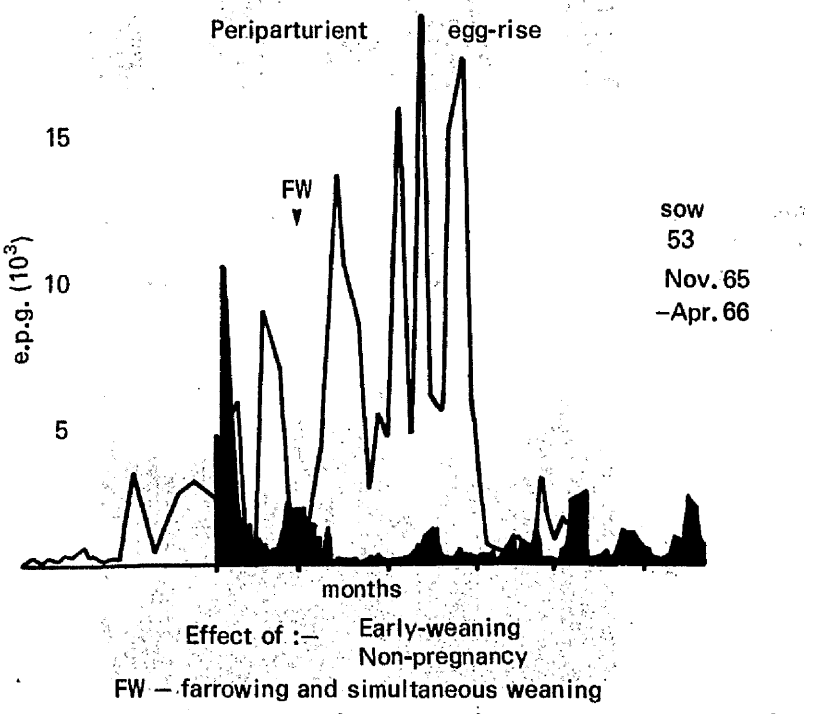


Fig 2.5 The shaded area represents the faecal 'strongyle' egg-count of a single sow (MKJ 53) whose piglets had been weaned at birth, the unshaded area those of a normal sow (MJK 52 – cf. Fig 2.4)

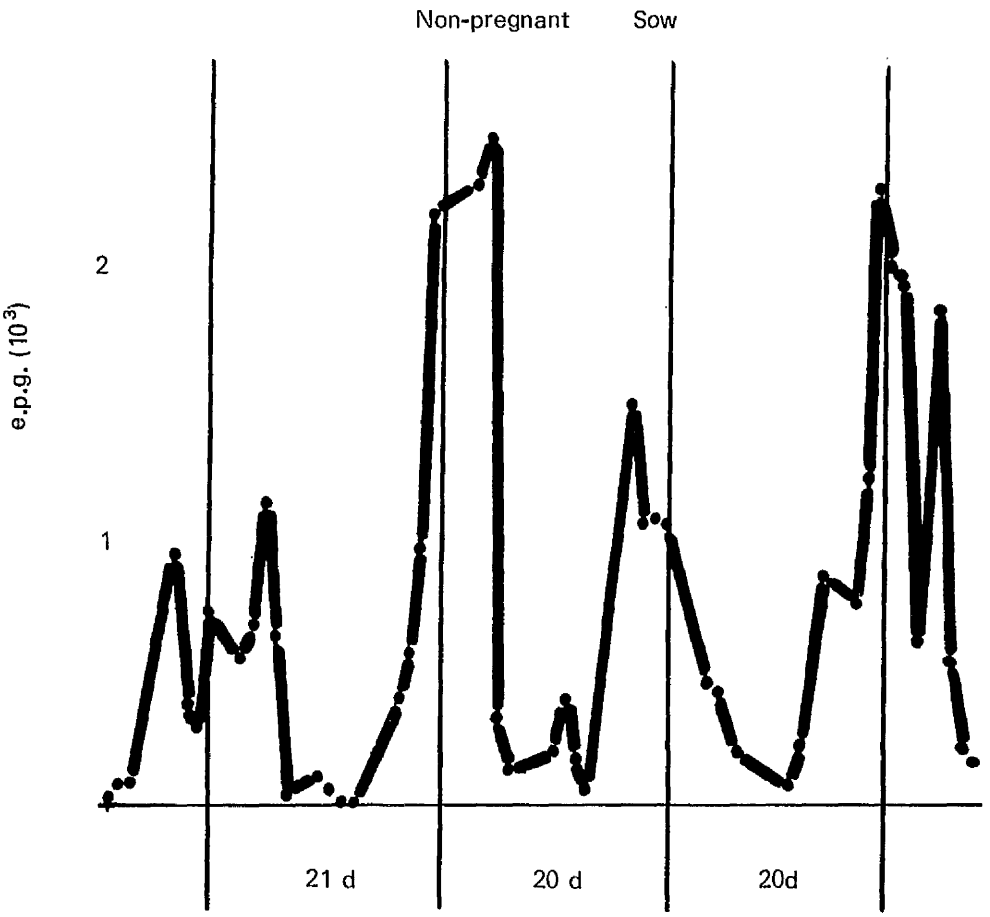


Fig 2.6 Faecal 'strongyle' egg-counts of a non-pregnant sow (MJK 53). This is an enlargement of the terminal portion of the corresponding curve on Fig 2.5

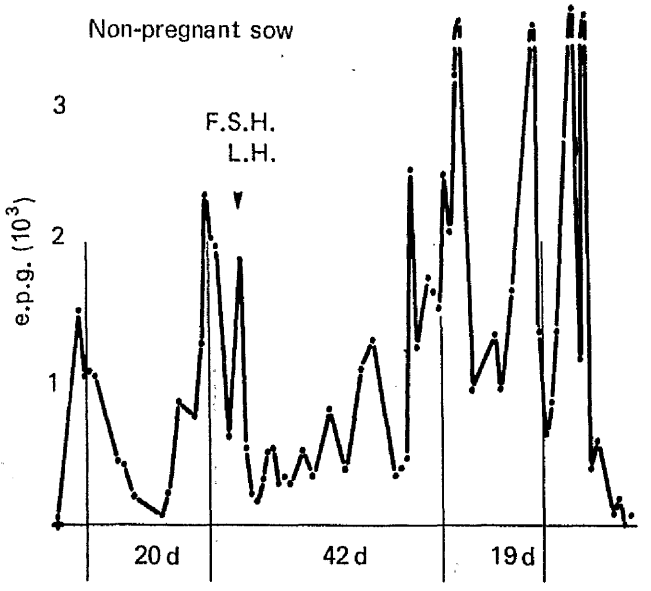


Fig 2.7 The effect of gonadotropins on the faecal 'strongyle' egg-count of a non-pregnant sow (MJK 53)

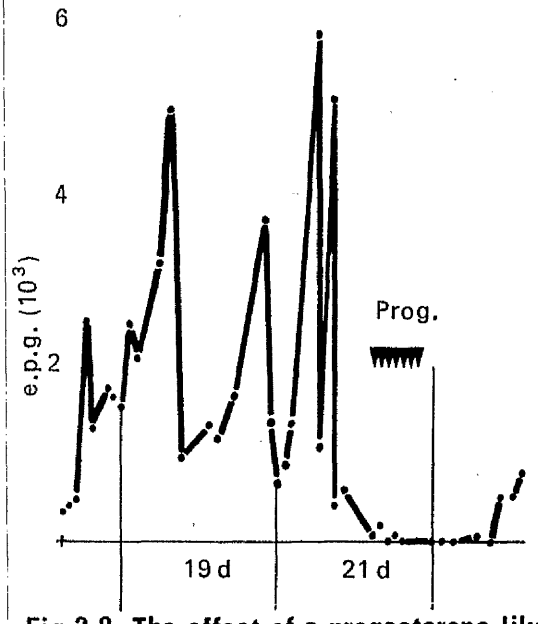


Fig 2.8 The effect of a progesterone-like compound (norethisterone acetate) on the faecal 'strongyle' egg-count of a non-pregnant sow (MJK53)

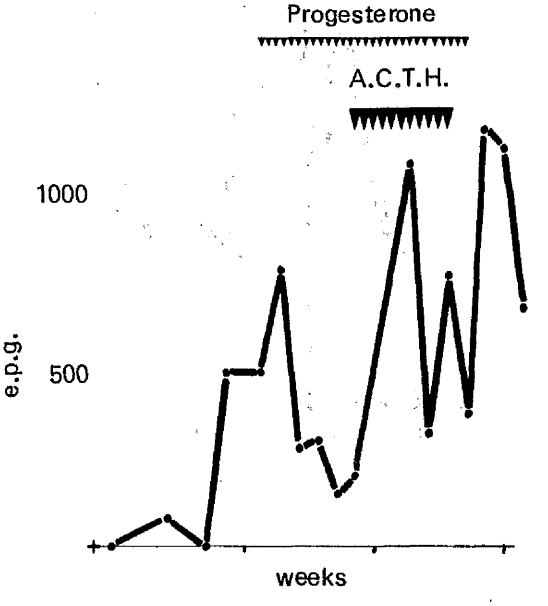


Fig 2.9 The effect of adrenocorticotropic hormone on the faecal 'strongyle' egg-count of a non-pregnant sow (MJK53) undergoing simultaneous progesterone therapy

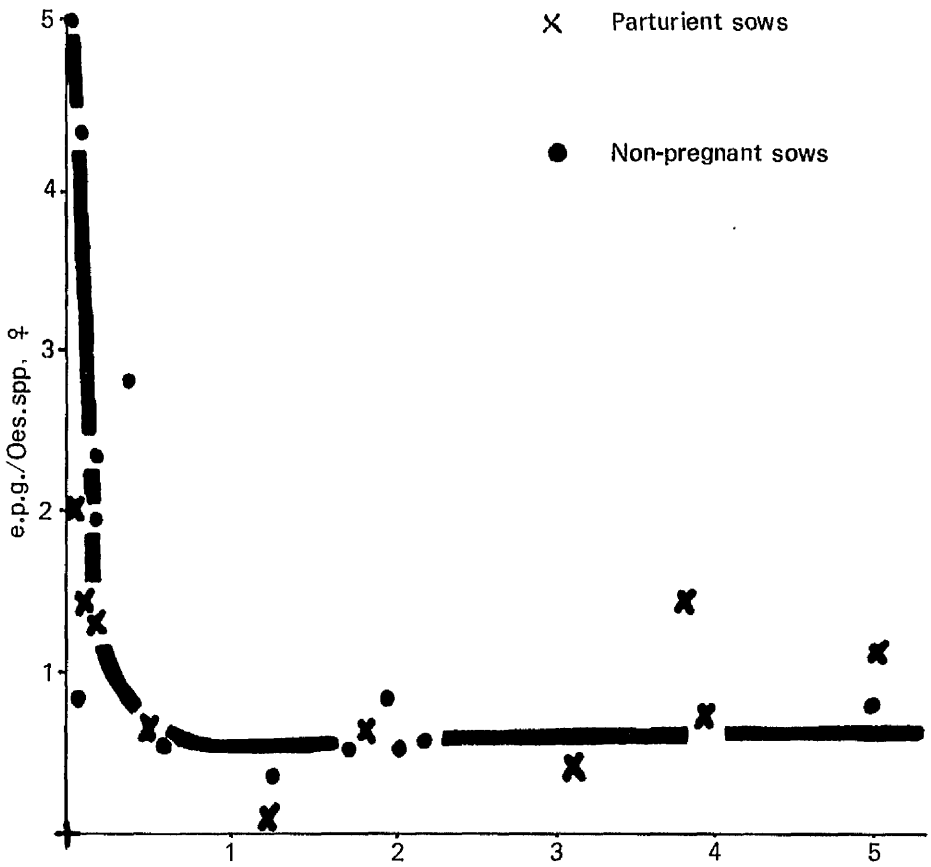


Fig 2.10 The effects of parasite population size and the reproductive status of the host on the fecundity of female *Oesophagostomum*

Table 2.1. - The average faecal 'strongyle' egg-counts of four groups of pigs within two herds (e.p.g.) during the period February, 1965 to February, 1966

	Farm B	Farm C
Parturient sows:	2,850	736
Pregnant sows:	480	301
Boars:	250	30
Fatteners:	114	341

Table 2.2. - The average faecal 'strongyle' egg-counts of suckling piglets on two farms during the period February, 1965 to February, 1966

	Farm B	Farm C
Up to 1 week old:	0	-
2 weeks old:	0	0
3 " "	0	-
4 " "	7	0
5 " "	7	17
6 " "	0	0
7 " "	36	25
8 " "	0	0
9 " "	-	40

Table 2.3. - Comparison of the lengths of 2,169 female Oesophagostomum from two groups of Danish pigs

	Parturient sows	Non-pregnant sows	Student "T" Test
Oe. dentatum females	9.85 mm.	7.96 mm.	$p<.01$
Oe. quadrispinulatum females	9.22 mm.	7.99 mm.	$p>.05$

DISCUSSION

2.3.1. General Considerations

Parasitic infections as they occur in the field represent exceedingly complex ecological systems. The helminth population is subjected to an almost infinite number of external influences. The host is not in a steady state but undergoes many physiological and pathological changes associated with season, growth and sexual activity, intercurrent disease, nutrition and husbandry and so on. Each factor may possibly alter the worms' habitat. The free-living stages of the strongylate nematodes are directly affected by climatic conditions, predators and a host of other factors. The infectivity of an area for livestock is also dependent on stocking rate, and thus there is a complicated interrelationship between host, parasite and environment.

The post mortem survey described in the previous chapter enabled several hypotheses to be advanced concerning the epidemiology of Oesophagostomum infections in pigs. It was deduced that the intensity of infection declines with age in fattening pigs and that this trend is reversed later in life so that mature animals are frequently heavily

infested. It was noted that the numbers of worms in the lumen of the intestine of sows varies according to the time of the year. It was realized, however, that to study all these facets would involve such a dilution of effort that no information of value could emerge from a system governed by so many variables. This section of the work therefore started with a general epidemiological survey, but when it became obvious that the output of 'strongyle' eggs by sows was related to the reproductive cycle, the limited resources that were available were concentrated on this one phenomenon.

2.3.2. The Periparturient Egg-Rise

The results from Farms B and C demonstrated that parturient sows shed greater numbers of 'strongyle' ova in their faeces than do pregnant sows, boars, piglets or fattening pigs. Typically, the egg-counts of breeding females are low during the greater part of pregnancy but tend to increase during the later phases. A steep rise in values takes place one or two weeks before parturition and this trend continues so that comparatively massive numbers of eggs are voided during the suckling period. Soon after weaning there is a sudden drop to

the minimal values associated with early pregnancy. These observations have subsequently been confirmed by Connan (Barnett, 1966), Davidson (1966), Connan (1967a), Ludvigsen (1967), Thomas and Smith (1968), Barth (1968) and Schrooyen (1969).

In the original announcement of this discovery (Jacobs, 1966a) the descriptive term 'preparturient egg-rise' was adopted since the increase in values was initiated before farrowing took place. Connan (Barnett, 1966), however, chose to use 'postparturient', presumably because the maximal readings occur during the time of lactation. For the sake of consistency in future publications, Jacobs (1966b) suggested that the designation 'periparturient' would be an accurate and acceptable compromise. This adjective has the additional advantage that it has not been used in descriptions of the spring rise of sheep and is therefore a useful term for comparative essays. Nevertheless, a fourth alternative has since been added to the list: 'parturient' (Thomas and Smith, 1968).

Twice weekly sampling of the individual sow MJK 52 gave results approximating to the general picture outlined

above. One additional feature was revealed by this study: the egg-output of the sow in the latter part of pregnancy and during lactation fluctuates within wide limits over short periods of time. A similar observation has been made by Connan (1967a) who attributes the irregularities to 'the inherent variability of egg-counts'. Examination of Fig. 2.4. in this thesis will show that each upsurge and decline takes several days and that each component curve is often compiled from several consecutive observations. This evidence is not consistent with Connan's explanation but suggests that real changes in the egg-output are taking place.

Faecal analysis of single samples taken from sows therefore yields little information concerning their parasitic status as they may well be shedding 600 e.p.g. at the time of examination and 13,800 e.p.g. six days later (cf. MJK 52; 15.2.66. and 21.2.66., Appendix 2, Table 5). The occurrence of this phenomenon complicates the interpretation of the results of the field trials conducted on Farms B and C, which were visited at fortnightly intervals. With such infrequent sampling it is a matter of chance whether the faeces

are taken at a time of maximal or minimal ova production in the transient cycle. By taking a sufficiently large number of samples over a long period of time, these variables average out to give a genuine impression of the true course of events as seen on a herd basis. The data compiled for individual animals or small groups of animals based on fortnightly sampling can therefore be inaccurate. Both Connan (Barnett, 1966) and Thomas and Smith (1968) relied on such infrequent collections of material. Connan was able to show the occurrence of the periparturient rise in each of the herds he examined. Thomas and Smith, however, failed in two of three attempts, the numbers of sows under observation on each occasion being 15, 10 and 11.

The hypothesis that the reproductive ability of a parasite can be intimately linked with that of its host animal is not novel as a number of examples can be quoted from the field of veterinary and zoological experience. Dunsmore (1965) has reviewed this sector of the scientific literature. The spring rise of sheep was first described by Taylor (1935) and has since been the subject of much research

and discussion. Parturient egg-rises have also been demonstrated in cattle (Parnell, Dunn and Mackintosh, 1953; Corticelli and Lai, 1960), horses (Wetzel, cit. Taylor, 1955), deer (Dunn 1965) and rabbits (Dunsmore 1966).

2.3.3. The Oestrous Egg-Rise

Egg-count data from sow MJK 53 demonstrated an association between the reproductive capacity of Oesophagostomum spp. and the oestrous cycle of the host. Larger numbers of 'strongyle' ova were shed during oestrus than during dioestrus.

Sows normally become receptive to the boar every third week (range: 18 to 24 days) starting four to nine days after the cessation of lactation (Roberts, 1956). A substantial minority, however, display irregularities in the cycle and clinical differentiation of the stages is frequently difficult or impossible.

In the present study, the first rise in the egg-counts occurred 11 days after weaning, the second 35 days later, coinciding with evident oestrus, and the

third 35 days after this. Thereafter, maxima were recorded regularly every three weeks. This circumstantial evidence strongly suggests that the periods of relatively high nematode egg production are related to oestrus.

In order to test this hypothesis, several attempts were made to alter the timing of the oestrous cycle to see whether the egg-output would be correspondingly modified. The first hormonal intervention involved the injection of gonadotropins immediately after an oestrous egg-rise. It was anticipated that this treatment would induce a premature period of sexual receptiveness, but no clinical response became obvious. The arrival of the next peak was not accelerated but markedly retarded. Regular three-weekly rises were recorded before and after this dislocation. These results are difficult to explain but it is felt that the quantities of FSH and LH that were utilized may have been inadequate for administration to an animal with new, active corpora lutea. The high levels of progesterone circulating at this time may have masked the psychological and physiological changes

usually associated with the use of gonadotropins. Within a few days, the artificially induced crop of follicles would have developed to corpora lutea which would secrete yet more progesterone, thus prolonging the period before the subsequent oestrus.

Oral administration of norethisterone acetate also delayed the occurrence of an anticipated peak. This may have been a consequence of the prolonged dioestrus caused by this drug, but the complete disappearance of 'strongyle' eggs from the faeces suggests that the compound may have had a direct action on the worms. Whatever the mechanism, the result was an inhibitory effect on ovulation in the female Oesophagostomum. The egg-count rose steeply from the sixth day after the withdrawal of the progesterone-like compound. Pigs are reported to come into season five to seven days after the therapy is terminated (Cameron, 1966).

A further possibility that had to be considered was that the oestrous rise resulted from the excitable emotional state of the in-season sow, i.e. that it is a stress phenomenon. Stimulation of the adrenal cortex did produce an increased

egg-count in the dioestrous animal, but this was small in comparison with the oestrous rise. The injection of corticosteroids is a recognized means of exaggerating the faecal egg-output of parasitized animals (Michel, 1967b).

It is not possible to extrapolate this description of an oestrous egg-rise to the pig population in general as observations on one animal may reflect an abnormal situation. Before the oestrous rise can be accepted as a genuine occurrence, its existence must be demonstrated in a series of animals.

2.3.4. The Mechanism of the Periparturient Egg-Rise: The Origin of the Ova

On a theoretical basis, three hypotheses could be advanced to explain the origin of the additional eggs shed during the periparturient rise.

(1) It could be argued that the worm population produces a constant number of ova during each 24 hour period, but that the volume of faeces voided by the host undergoes cyclical changes related to reproductive

activity. Such variations would alter the concentration of eggs in the faecal samples and directly influence the e.p.g. values. An increased egg-count would be the result of a diminished quantity of evacuated material. During lactation, however, the sow consumes far more food than at any other time in her life. Thus, at the time of the periparturient rise, food wastes are diluting the worm-egg suspension. This in turn implies that the magnitude of the rise is, in fact, greater than would appear from standard egg-count data.

(2) Alternatively, it can be suggested that the numbers of worms in the host is a constant factor, but that the reproductive capacity of the individual female nematodes is enhanced. In support of this idea, the post mortem study showed that the fecundity of Oesophagostomum is a variable function. When only small numbers of worms are present in the host, for example, much greater numbers of eggs are produced by the individual females than are shed by those belong-

ing to larger populations. This result conforms with that of Michel (1967b) who showed that Ostertagia ostertagi in the calf produces eggs at a rate considerably lower than that of which it is potentially capable, except in the case of small populations.

Of the 29 sows that were examined for this purpose, 14 were parturient at the time of death, the remainder being breeding sows that were either non-pregnant or in the very early stages of pregnancy. The groups thus represent the periods of maximal and minimal egg out-put. As indicated previously, the faecal egg-count of the parturient sow fluctuates greatly over short periods of time, and this is reflected in the fecundity values (Fig. 2.10.). The scatter of the figures for these sows about the mean was greater than that for values obtained for the non-pregnant group. This would indicate that some changes in the fecundity of the individual female worms do occur as parturition approaches and during lactation.

It may be possible to make a direct measurement of the fecundity of female Oesophagostomum recovered immediately after the death of the host. Unfortunately, it is impracticable to count the quantity of eggs in the uterus as these are so numerous, but there are indications that the degree of ovarian activity could be estimated by the application of the Feulgen method. This staining technique is specific for desoxyribonucleic acid (DNA) which is found in relatively large amounts in cells undergoing multiplication. The greater the number of cell divisions, the more DNA present in the tissue. The author has treated the long filimentous ovaries of Oesophagostomum in this way and shown that new cells are formed only at the very tip of the organ. It is anticipated that a greater length of Feulgen positive material would be found in fecund females than in relatively quiescent worms, but this has not yet been fully explored. The extent and intensity of the DNA positive area can be measured visually or by autoradiography using reagents labelled with ^{14}C.

(3) Lastly, it might be thought that the most important factor in the periparturient rise is an increase in the absolute size of the parasitic population.

Because of the high biotic potential of Oesophagostomum, pastures will be highly infective for most of the year. For the same reason, buildings will harbour large numbers of larvae unless exceptional standards of hygiene are enforced. There is, therefore, ample opportunity for the build-up of large infestations from external sources. It is also possible, however, that the population size may be determined by the rate of release of larvae inhibited in the histotropic phase of their development. It is known that a prolongation of the time spent within the host tissues is a common feature of Oesophagostomum infections in pigs (Kotlan, 1948; Shorb and Shalkop, 1959). These retarded forms become encapsulated. No information is yet available

on their subsequent ability to complete the life-cycle.

Naerland (1949) was the first to suggest the potential role of inhibited larvae in the spring rise of sheep. The original theory that the rise could be attributed wholly to an increase in the fecundity of the existing worms (Taylor, 1935) was discarded when Morgan, Parnell and Rayski (1951) demonstrated a rise in worm numbers in ewes in the spring. The increased parasitic burdens were explained in terms of the direct acquisition of new larvae from the pasture, but it has since been shown that the same egg-count patterns are obtained from ewes overwintered indoors under conditions that preclude transmission of infection (Naerland, 1949; Spedding and Brown, 1956; Field, Brambell and Campbell, 1960; Connan, 1967b; Proctor and Gibbs, 1968). Soulsby (1966) and Gibbs (1967) have performed larval counts using tissue digest techniques. The numbers of inhibited forms declined rapidly

shortly before the rise was due to take place, whilst the number of adults increased proportionately. The findings of Anderson, Armour, Jennings, Ritchie and Urquhart (1965), who studied the aetiology of winter ostertagiasis in cattle, suggest that the larval inhibition of O. ostertagi may be a seasonal rather than a host-induced function. It would therefore be interesting to study the mechanism of the parturient rise in autumn lambing Dorset Horn sheep.

2.3.5. The Mechanism of the Periparturient Egg-Rise: Its Termination

The periparturient egg-rise finishes abruptly soon after the cessation of lactation. When sow MJK 53 was weaned prematurely, the 'strongyle' egg-count failed to reach its post-parturient peak (Fig. 2.5.). Connan (1967a) has compared the faecal egg-output of breeding sows whose piglets had been removed at birth, at four weeks of age and at two months. In each case, the termination of the egg-rise coincided with weaning.

But what changes occur in the worm population to cause this decline in the numbers of nematode ova? The examination of the Oesophagostomum recovered from the two series of slaughtered sows enables an explanation to be advanced. At this point it should be explained that all the sows of the so-called 'non-pregnant' group had produced one or more litters in their life-time. No farmer will keep non-productive breeding stock longer than absolutely necessary and it may therefore be assumed that most, if not all, of the animals of this series had been lactating until a comparatively short time before their death. Culls would be sold immediately after the last litter of piglets had been removed, and few sows are allowed to return to service more than twice. The nematode populations were therefore in the 'post-weaning' stage of their cyclical development. Yet, the average length of these worms was approximately 20% less than that of the females from the parturient animals. It is unlikely that these nematodes could have shrunk to such a degree, even if the egg-laying females had become effete. The shorter population must therefore represent

a new Oesophagostomum generation. This in turn implies that the population responsible for the preceding peri-parturient rise has been expelled from the host. Connan (1967a) was able to demonstrate an exodus of adult Oesophagostomum at the fifth day after weaning in a proportion of his sows. The character of the peri-parturient egg-rise is therefore influenced by changes in the nematode population structure as well as by variations in fecundity. It must be assumed that the new population starts to become established before the former is expelled as the 'non-pregnant' group of sows were in many cases already harbouring substantial numbers of female worms containing egg-laiden uteri. The expulsion therefore appears to be a selective process, eliminating the older egg-laying nematodes and not the younger developing worms.

2.3.6. The Mechanism of the Periparturient Egg-Rise: Its Initiation

Up to this point in the dissertation, the experimental evidence that has been collected has provided

strong guidelines for the formulation of possible explanations for the observed phenomena. Further discussion must, however, be more speculative. The potential controlling factors can be discussed under eight headings:-

(1) Seasonal influences
(2) Husbandry
(3) Nutrition
(4) Stress
(5) The immunological status of the host
(6) The reproductive hormones
(7) The lactational hormones
(8) A multiple control.

(1) Seasonal Influences:

In this context, it is interesting to note the controversy that surrounded the spring rise of sheep for many years. Early authors assumed that it was a seasonal occurrence dependent upon harsh winter conditions and an inadequate plane of nutrition, although the chronological association with the date of lambing had been realized (Parnell, Dunn and Mackintosh, 1954). The discovery of a similar rise in the faecal 'strongyle' egg-counts of autumn-lambing ewes (Crofton, 1958) indicated that the primary

stimulus was associated with parturition rather than season, but this hypothesis is difficult to conciliate with the fact that egg-rises also occur in non-pregnant ewes at about the same time as the regular spring rise of breeding females (Crofton, 1954; Field, Brambell and Campbell, 1960; Proctor and Gibbs, 1968). The changes in non-pregnant sheep, however, occur less regularly and are of a smaller magnitude. Crofton (1958) also noted that non-pregnant Dorset Horns do not undergo an egg-rise in the autumn and concluded that there is, in fact, a seasonal rise in the spring but that this is overshadowed by the much larger parturient rise.

Pigs breed throughout the year and the peri-parturient egg-rise is not limited to any season. The suggestion that this phenomenon is primarily produced by a particular sequence of climatic conditions can therefore be confidently rejected. As with sheep, however, there are also seasonal fluctuations in the strongylate fauna of breeding females that are

independent of parturition (vide Chapter 1).

(2) Husbandry:

The breeding pig is subjected to great changes in its environment during the reproductive cycle. During pregnancy, she is often kept with a number of other in-pig sows, either in a field, in a large pen or in stalls. Here, the average egg-output of each animal is low but constant exposure to reinfection is likely, especially from grass. Shortly before parturition is due, the sow is placed in a farrowing pen or crate. This accommodation has usually been thoroughly cleaned and disinfected and so at first there is no further uptake of third stage larvae. Within seven to ten days, however, infective forms have developed from ova shed by the sow herself after admittance. The weight of infection builds up during lactation until the sow is removed from her off-spring to join the remainder of the herd.

Can these changes provide an explanation for the periparturient rise? Under experimental conditions a

large larval challenge can displace an existing Oesophagostomum population (Taffs, 1966). It could be postulated that the removal of the sow from the farrowing quarters to mingle with the herd might expose her to such a challenge. The termination of the periparturient rise would then be a nematode-induced immune expulsion. The primary infection having been eliminated, a small residual infection maintains a low egg-count. Newly acquired larvae start to attain maturity in mid-pregnancy and the egg-count starts to rise. Infestation continues in a cumulative fashion, so that the number of ova being shed during the suckling period is great enough to be termed an egg-rise.

This hypothesis may explain the pattern of egg-counts, albeit rather inadequately, but many objections can be raised. Why should larval challenge after weaning displace the existing population when the massive numbers ingested during suckling do not? If, on the other hand, there is

no massive challenge during lactation, why is there such a large increase in egg-count values at this time? Whilst the environmental conditions must influence the host-parasite balance to some degree, this explanation is clearly a gross over-simplification.

(3) Nutrition:

In general, the sow is kept on a low nutritional plane throughout most of pregnancy. Previously, she will have been 'steamed up' before meeting the boar as she will have lost weight during lactation and must regain good bodily condition in order to achieve her maximal breeding potential. She is also given extra rations shortly before parturition, and afterwards her food intake is adjusted according to the amount of milk she is producing.

The 'strongyle' egg-output is therefore approximately proportional to the amount of food being given to the host. This is not a physical relationship involving the volume of faeces (vide supra). It is

difficult to believe that the worms benefit from the extra food as they are so small in relation to the amount of nutrient that surrounds them. The apparent effect of the food must either be incidental or mediated via the host. In either case, the true stimulus to the periparturient rise will be found under the other headings.

(4) Stress:

The administration of adrenocorticotropic hormone to the non-pregnant sow caused a small rise in the egg-count value, and other authors have recorded similar results in other animals (Michel, 1967b). Nematode infections can, therefore, respond to adrenocortical activity by increasing their reproductive capacity. On the other hand, attempts to alter the egg-count pattern of barren ewes by the injection of corticosteroids at the time of the spring rise have failed (Dunsmore, 1965; Soulsby, 1966; and Gibbs, 1967).

Is the sow in a state of stress during the period of the periparturient rise? This may be the case as the modern pig has to perform functions quite unknown to its wild counterpart. The quantities of milk demanded from the sow are so great that she loses up to 20% of her body weight during lactation. The oestrous egg-rise might also be explained in these terms. Oestrus is accompanied by behavioural changes and excitability which may constitute psychological stress. However, it might be argued that oestrus, parturition and lactation are normal physiological functions and would not be stressful. It is of relevance to note that abortion in ewes is not associated with a rise in egg-counts (Dunsmore, 1965).

It is considered unlikely, therefore, that stress <u>per se</u> plays a major role in the initiation of the periparturient egg-rise.

(5) <u>Immunity</u>:

Connan (1967a) has advanced the hypothesis that

the periparturient egg-rise is initiated by 'a non-specific change in the immune status of the host', and that 'its termination is due to the return of this host resistance'. Soulsby (1957) suggested that the spring rise in sheep was a consequence of waning immunity following lack of antigenic stimulation during the winter period. But pigs farrow all the year and many are exposed to infection throughout the period of gestation. Why then should their immunity fade? Why, too, should it return when the sow is in the worst physical condition?

Hjelle (1967) found that the plasma immunoglobulin levels of pregnant ewes fell during the winter and Soulsby (1957) noted an inverse relationship between antibody levels and egg-counts, but there is no way of telling whether these observations are related, directly or indirectly, to seasonal factors, pregnancy or nutrition. No parallel studies have been performed in the pig.

It is doubtful if a decline in the immune status per se would produce a rise in the egg-counts. Soulsby and Owen (1965) demonstrated an egg-rise in one of five sheep given chlorambucil, an immunosuppressant drug, but Brunsdon (1966a) and Gibbs (1967) were unable to reproduce this effect under field conditions.

(6) Reproductive Hormones:

The early gestational period is characterized by high progesterone levels and low egg-counts. The terminal phases of pregnancy are associated with increasing oestrogenic activity and a rising e.p.g. value. Oxytocin and relaxin play important roles about the time of parturition. (The lactational hormone, prolactin, will be considered separately in the next section). After weaning, the endocrinological rhythms of the oestrous cycle come into play. The Graafian follicle grows under the influence of follicle stimulating hormone (FSH) and starts to produce oestrogen. Ovulation occurs 30-36 hours after the onset of oestrus,

and luteinizing hormone (LH) aids the formation of the corpus luteum which in turn generates progesterone during dioestrus.

It can be inferred from this brief summary that the periparturient and oestrous egg-rises have some characteristics in common. In both, the periods of minimal 'strongyle' egg production coincide with high progesterone activity, whilst rising egg-counts occur simultaneously with oestrogenic activity. The negative faecal egg output of sow MJK 53 during the administration of norethisterone acetate suggests that progesterone can suppress the reproductive capacity of Oesophagostomum populations. Connan (1968) treated four ewes with progesterone immediately after lambing, but attained inconclusive results. The parturient egg-rise was apparently suppressed in two animals and remained unaltered in the remainder.

Oestrogen may be parasitologically inert, the egg-rise being the consequence of the cessation of progesterone inhibition. On the other hand, it may

actively enhance the biotic potential of the Oesophagostomum population. Gibbs (1967) described experiments in which unbred ewes were treated with diethyl stilboestrol. The mean egg-count rose from 520 e.p.g. to 3,980 e.p.g. within six weeks, whilst that for the untreated controls remained below 620 e.p.g. Interpretation of this experiment is complicated by the fact that such treatments stimulate mammary development (vide infra). In contrast, however, Dunsmore (1965) has remarked upon the absence of a parturient rise following abortion, an event which is also accompanied by increases in oestrogen and oxytocin levels. Dobson (1964) lists a number of studies on a variety of host-parasite systems each of which showed that oestrogens increase the resistance of the host to nematode infections.

(7) Lactational Hormones:

The main part of the periparturient rise coincides with the period that the host is producing

milk. The cessation of lactation terminates the egg-rise and removal of the piglets at birth prevents its occurrence. A similar relationship applies to the spring rise of the ewe (Gibbs, 1967; Connan, 1968). Oshima (1961) has drawn attention to the possible influence of prolactin on the migratory patterns of Toxocara canis and this has led Dunsmore (1965) to speculate on the role of this hormone in the present context. This hypothesis will be very difficult to prove or disprove experimentally owing to the complex inter-relationships between various reproductive hormones. Injections of diethyl stilboestrol will stimulate prolactin generation (Gibbs, 1967), and prolactin itself is associated with the maintenance of the corpus luteum and progesterone secretion (Roberts, 1956).

(8) A Multiple Control:

The factors that could possibly determine the characteristics of the periparturient egg-rise have been discussed under seven separate headings. It

is unlikely that the controlling force will belong to any one of the above groupings. All the listed possibilities will influence the parasites' environment to a greater or lesser degree as each affects the physiological equilibrium of the host. Some will undoubtedly play a more important role than others. The complexity of the system accounts for the confusion and contradiction that is seen when the available experimental evidence is reviewed. Future work should not be directed towards finding the single cause of the periparturient or spring rise, but rather towards an evaluation of the contribution made by each variable. It is possible that such a study will reveal a balanced interplay between the components, the biotic potential of the parasite changing in response to small changes in the relative influences of the opposing host forces. Bawden (1969), for example, has illustrated how Nematospiroides dubius infections of mice are dependent upon the protein intake and hormonal status of the host as

well as the structure and age of the nematode population itself.

Two examples can be given of the dangers of searching for a single explanation for the experimental observations. Conflicting evidence was forwarded supporting the views that the spring rise of the ewe was on the one hand seasonally induced, and on the other related to the sexual activities of the host. It is now known that both parties were partially correct (vide supra). Similarly, there was much discussion concerning the origin of the increased worm burdens. One school of thought maintained that inhibited larvae were responsible, the other that pasture contamination was the contributing factor. The graphs produced by many authors have shown the spring rise to be a biphasic phenomenon, but only Soulsby (1957) and Dunsmore (1965) have commented on this. By combining egg-count data with herbal larvae counts Dunn (1969) has shown that the renewed activity of retarded forms is responsible for the primary rise

in the numbers of voided eggs. These ova hatch and soon reinfect the ewes resulting in the bigger, secondary phase. Again, the supporters of each cause were equally right (or wrong).

2.3.7. The Periparturient Egg-Rise: Its Significance

The practical significance of the periparturient rise is best described in numerical terms. A limited number of observations made on the farms where these studies were conducted indicated that an adult pig evacuates three kilogrammes of faecal material per day (range: $1\frac{1}{2}$-$5\frac{1}{2}$ kg./day). Over the year, the lactating sows on Farm B shed an average of 2,850 'strongyle' eggs per gramme of faeces. If this figure is related to the daily faecal output, it can be seen that, on average, each dam suckling her piglets voids 7,550,000 Oesophagostomum ova per day. If the diligent farmer removes 95% of the waste from the pen, one third of a million eggs will remain. Limited laboratory experience (Chapter 5) indicates that on many farms one could expect around 40% of these to hatch and produce

ensheathed third stage larvae in seven to ten days, i.e. over 100,000 potentially infective organisms are being made available each day.

The egg-rise occurs at precisely that point in time when two generations of the host species are in intimate contact. It would almost appear that a mechanism has evolved whereby the physiological processes of the host are utilized to ensure that successive generations of pigs are born into highly infective surroundings.

On the farm, the expectant sow is usually put into disinfected farrowing quarters a week to ten days before the anticipated date of parturition. Applying the reasoning outlined above, it is obvious that the newly-born piglets will be exposed to infection almost as soon as they are born. Contamination of the dam's teats with faeces, and the piglet's compulsion to root about in the bedding materials facilitate the transfer of the larvae.

Do field observations support this alarming

theoretical picture? This particular question was not studied in detail but some judgements can be made from the data collected. That some harm is done is witnessed by the suboptimal growth-rates of piglets born to infested dams. This topic is discussed in detail in Chapter 5 of this thesis. Yet the litters on Farms B and C did not seem to accrue massive worm burdens (Table 2.2.). Some infection did take place immediately after birth as small numbers of 'strongyle' eggs appeared in the faeces between the third and fifth weeks of life. (The prepatent period of H. rubidus is 21 days, and that of Oesophagostomum 21-42 days (Taffs, 1966)). The egg-counts were, however, very low and many litters did not display patent infections at all. Thomas and Smith (1968) were unable to show any transmission from mother to litter in the herd they kept under surveillance, whilst Connan (1967a) found that the offspring remained worm-free in two of his series of five herds.

What happens, then, to the infective larvae when transmission is not occurring? Either the majority perish before they are ingested or they fail to establish themselves in the host. Connan (1967a) suggests that regular cleaning eliminates opportunity for transmission, but the enormous numbers involved makes this theory unlikely if interpreted in the literal sense. Heavy infestations were present in Farm EC despite impeccable cleanliness. It seems more probable that there is some natural lethal influence in operation and that its relative significance varies from farm to farm. If the hostile factor is present on a farm, hygiene may upset the ecological balance to the detriment of the nematode species. The limiting force could be a biological competitor or predator. The dung-pat has a rich flora and fauna including nematode trapping fungi, for example. Alternatively, the piglet could be endowed with a defence mechanism. The immature intestinal tract or its contents could provide an unfavourable environment for the parasite, or there may even be a passive immunity passed from the dam.

It is a general feature of helminthology that the reproductive capacity of adult populations is far in excess of the value theoretically adequate for the maintenance of the species. Parasite and host are, in general, in equilibrium and so a study of the fate of juvenile forms would be of considerable interest. An exaggeration of these natural limitations might provide a means for the prophylaxis of parasitic disease.

Chapter 3

OBSERVATIONS ON THE EPIDEMIOLOGY OF PORCINE OESOPHAGOSTOMIASIS: FARM-TO-FARM TRANSMISSION

INTRODUCTION

The investigations described in Chapter 2 of this thesis revealed a mechanism by which Oesophagostomum spp. infections are passed from one generation of the host species to the next. Other methods for the dissemination of this genus must exist as a feature of the epidemiology of porcine oesophagostomiasis is the persistent reappearance of infection in herds produced by hysterectomy, despite, in some instances, stringent precautions aimed at the exclusion of disease-causing organisms.

This chapter presents results from field and laboratory experiments that demonstrate two biological pathways by means of which infective larvae may possibly be transported over longer distances, and perhaps from farm to farm.

Another project, reported in Chapter 6, had provided the information that third stage Oesophagostomum, encapsulated in the intestinal mucosa of experimentally infected guinea-pigs, can remain alive for at least 48 days. This suggested the possibility that other rodents and particularly rats, may play a role in the transmission of oesophagostomiasis. This hypothesis was therefore

tested under laboratory conditions.

The second study was stimulated by an observation made by a colleague, Miss Margaret Tod, who was conducting a survey of the insect fauna asssociated with pigs. She noticed that one particular genus of manure-breeding fly, Psychoda, often carried nematodes clinging to the abdomen and that some of these appeared to be the third stage larvae of parasitic forms.

MATERIALS AND METHODS

3.1.1. Rat Transmission

The first series of experiments was intended to show whether Oesophagostomum larvae would encyst in the intestinal wall of artificially infected rats. If this was shown to be the case, further investigations would be planned to discover whether or not the encysted forms had retained their infectivity.

3.1.2. Animals Used

Laboratory rats of the Wistar strain were purchased from a commercial source. All were female and weighed between 60 and 100 grammes when infected.

Two types of pig were utilized: nine-week old Large Whites that had been specially bred under worm free conditions by Messrs. George Pinkerton Ltd., Houston, Renfrewshire, and three-month old pigs of the Pitman-Moore X Palouse strain (Crees, 1968) that had been reared by the author.

3.1.3. Preparation of Larval Cultures

Freshly deposited faeces was collected from sows

on Farm EC (Chapter 2) that were known to be infested with Oesophagostomum spp. and no other helminth parasite. Infective third stage juveniles were cultured and recovered from this material by the methods described in Chapter 1. The larvae were washed repeatedly by mixing with large quantities of water and allowing sedimentation to take place, after which the supernatant was poured off.

3.1.4. Administration of Larvae

The number of larvae per unit volume of the final concentrated suspension was estimated by agitating the preparation and removing five 0.1 ml. aliquots which were each diluted with water in a separate petri dish. These were scanned under the stereoscopic microscope (X23) and the larvae counted. An average of five readings was used to compute the amount of fluid needed for each experimental animal.

The calculated dose was measured in a syringe and administered by stomach tube to rats that had been lightly anaesthetized with ether vapour.

3.1.5. Experimental Designs

Experiment 3.1.

As a pilot experiment, three rats were infected, respectively, with 10,000, 20,000 and 30,000 third stage Oesophagostomum spp. larvae, and were killed on the sixth, seventh and eighth days after infection. The intestinal tract of each was washed out and the washings examined for parasites. The intestinal wall was cut into short sections which were compressed between glass slides. Larvae in the mucosa were counted under the stereoscopic microscope. The viability of the parasites was tested by warming each preparation and watching for signs of larval movement. As controls, four uninfected rats from the same cage were killed and their intestinal tracts treated in a similar manner.

Experiment 3.2.

Ten rats were each infected with 20,000 larvae, killed after seven days, and the large intestine of each examined as above.

Experiment 3.3.

A further 82 rats were infected, each animal receiving 20,000 larvae. Fifty-five of these were killed after seven days, the remainder after one month. The large intestines were removed, opened longitudinally and washed in running water to remove the contents of the lumen. The mucosae were cut into small fragments and mixed with a little pig-meal. Food samples containing tissues from a known number of rats were offered to individual worm-free pigs that had been starved for 12 hours. The experimental animals were killed three to four weeks later, and their intestinal tracts searched for worms.

Throughout the experiment, one or more littermate control animals were housed in the same pen as each of the experimental animals. One control pig was fed mucosae from uninfected rats, but the others received only the standard meal. The Large White controls were slaughtered at the termination of the experiment and autopsied, but the miniature pigs were required for a

future breeding programme. In this instance, therefore, each pig was kept for five weeks and then treated with 7.5 g. formulated dichlorvos, containing 1.3 g. active ingredient (Atgard, Shell International Chemical Company). This drug is known to be highly effective against Oesophagostomum populations at rates of 15.1 mg./kg. active ingredient (Batte, Moncol, Todd and Isenstein, 1965), so that the amount used in this study was considerably in excess of the minimum effective dose. The faecal material passed by the medicated pigs was collected for three days and screened for worms.

3.1.6. Psychodid Transmission

The possibility that psychodid flies may be able to carry the larvae of parasitic nematodes as well as those of some free-living species stimulated the experiments outlined in the next paragraphs.

3.1.7. Experimental Designs

Experiment 3.4.

This experiment and the next were performed under

the leadership of Miss Tod. Two-hundred gramme quantities of faeces were collected from sows on Farm B (described in Chapter 2). Absolutely fresh deposits were ignored for this study, those just a few hours old being preferred. This was done to ensure that the local psychodid flies had had ample opportunity to deposit their eggs in the chosen sample. The material was taken to the laboratory and spread in a thin layer on the floor of a Perspex insect cage. This was watered when necessary for the maintenance of a high humidity. Emergent flies were caught and removed daily.

Living nematode larvae could be obtained from the psychodids by lightly etherizing the flies, transferring them to a muslin bag and then using the Baermann technique. The preparation was suspended in luke-warm water in a conical measuring cylinder. The larvae then left the flies, moved into the surrounding medium and sank to the bottom of the vessel.

Experiment 3.5.

The fields used for grazing the in-pig sows on

Farm B were surveyed and an area where psychodid flies could be found in abundance was noted. This spot was visited at intervals during the summer of 1968 and flies were collected for subsequent laboratory examination.

The insects were caught with a net or by trapping them in tubes against tree-trunks or other surfaces. They were killed with ether fumes and quickly transferred to alcohol for preservation. In retrospect, this was not the ideal method for the treatment of the material as the nematodes often became unwound from the flies in the alcohol so that the estimation of the percentage carrying larvae proved impossible on those occasions that specimens were pooled.

RESULTS

3.2.1. Rat Transmission Experiments

Experiments 3.1. and 3.2.

A small proportion of the larvae (0.02-0.64%) invaded the intestinal mucosa of the rats where they became encysted (Tables 3.1. and 3.2.). Penetration was confined to the caecum and colon, no larvae being found in the wall of the small intestine. No signs of disease were seen in any of the rats during the period of observation and at post mortem examination, there were no macroscopically visible lesions. When the crushed tissues were examined microscopically the tightly coiled larvae could be seen to be encompassed by a distinct capsule in a manner similar to that illustrated in Plate 6.1. All the larvae that were tested for viability seven days after infection were active when warmed on the microscope stage. No indications of helminthiasis were found in the four control rats, whereas all 20 infected animals showed signs of parasitism.

Experiment 3.3.

The rat tissues were accepted without hesitation

by the Large White pigs which even picked out the intestinal segments preferentially. The miniature swine, however, were more discerning and only consumed the treated food after several hours delay.

Whilst none of the control pigs displayed any sign of helminth infestation, four of the five experimental animals became infected with Oesophagostomum spp. (Table 3.3.). The majority of the worms were young adults, but a few fourth stage larvae were also seen.

3.2.2. Psychodid Transmission Experiments

The greatest concentration of psychodids was found in a 15 acre field bordered on the south-west by a driveway with garden trees and bushes on either side. It was found that the flies sheltered in a cypress tree in one corner, particularly on the leafless side next to the field. Other trees and gateposts only yielded a few specimens. Near the foot of the cypress tree was a muddy area where the pigs found shade on sunny days, so that the flies were never far from fresh dung.

Many psychodid flies caught in the field or cultured in the laboratory carried nematodes twined round the intersegmental furrows of the abdomen. In one sample of 142 flies caught on the farm 11 (7.7%) were involved. Some flies bore large numbers (Plate 3.1.), and although their abdomens sometimes appeared shrivelled they were still able to fly. The majority of nematodes recovered were free-living forms, but third stage ensheathed juveniles identical in size and appearance to Oesophagostomum spp. larvae were often seen on flies from the faecal cultures and from the farm. The parasitic larvae could be readily collected using the Baermann technique, and were thus presumably fully active and infective. As worm-free pigs were not available at the time of this study, no attempts could be made to infect such animals with Oesophagostomum-bearing Psychoda.

Of the 16 species of Psychoda recorded in Great Britain (Tonnoir, 1940), Miss Tod identified nine amongst the flies caught on the farm: P. albipenis,

P. brevicornis, *P. cinerea*, *P. crassipenis*, *P. grisescens*, *P. phalaenoides*, *P. setigera*, *P. severini* and *P. trinodulosa*. All these species were raised in the laboratory except *P. cinerea* and *P. severini*. Nematode larvae were observed on *P. grisescens* and *P. phalaenoides*, and may possibly have occurred on other species.

Plate 3.1 Nematode larvae coiled around the abdomen of a psychodid fly

Table 3.1. - The numbers of Oesophagostomum larvae found in the intestinal mucosa and lumen of rats infected per os one week earlier

Rat No.	No. of larvae given	No. found in small intestine	No. found in caecum	No. found in colon	Total No. in tissues	No. found in lumen
1	10,000	0	2	3	5	0
2	20,000	0	10	128	138	0
3	30,000	0	30	54	84	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0

Table 3.2. - The numbers of Oesophagostomum found in the intestinal mucosa of rats infected with 20,000 larvae per os one week earlier

Rat No.	No. larvae in tissues
8	124
9	84
10	20
11	10
12	4
13	5
14	10
15	8
16	16
17	11
Total	292

Average per rat: 29

Average 'take': 0.15%

Range: 0.02 - 0.64%

Table 3.3. - Results of feeding pigs with tissues from rats exposed to Oesophagostomum larvae

Pig		Rats		Method of examination	No. Oesophagostomum recovered	
No.	Breed	No. given	Time infected			
Experimental Group						
9901	LW	20	1 week earlier	PM		450
73	LW	15	" " "	PM		116
368	LW	20	" " "	PM		7
M1	PMxP	10	1 month earlier	PM		3
M2	PMxP	17	" " "	PM		0
					Total	576
Control Group						
9903	LW	0	-	PM		0
74	LW	15	Not infected	PM		0
366	LW	0	-	PM		0
M3	PMxP	0	-	vermifuge		0
M4	PMxP	0	-	"		0
M5	PMxP	0	-	"		0
M6	PMxP	0	-	"		0
					Total	0

LW = Large White

PMxP = Pitman-Moore x Palouse

DISCUSSION

3.3.1. The Problem

Within the last decade, there has been a widespread movement towards the establishment of 'specific-pathogen-free' (SPF) or 'minimal disease' (MD) herds of pigs. The primary object is the creation of populations that are free from enzootic pneumonia and other non-helminth porcine diseases endemic in the United Kingdom. The usual method of achieving this is to remove all pigs from the farm, disinfect the premises and to repopulate, after a suitable resting period, with stock that were delivered by Caesarian operation. Alternatively, pigs born to dams that were themselves produced by hysterectomy may be used.

This procedure had been followed by Farm C. The observations recorded from this farm in Chapter 2 were made on the original herd, which was subsequently sold. The buildings and pens were cleaned and new MD pigs introduced about five months later. Soon, however, patent Oesophagostomum infections were again detected.

This is not an isolated occurrence. Taffs (1966) mentions two MD herds that he found to be harbouring intestinal nematodes. Coop (1968) (personal communication) has described the difficulties of preventing the introduction of infection into an experimental unit housing worm-free pigs. In contrast, however, the MD herd belonging to the Rowett Institute, Aberdeen, has remained helminth-free for over a year without any especially stringent precautions being taken (Hunter, 1968, personal communication).

The most common manner by which Oesophagostomum larvae are brought into disinfected premises is undoubtedly by the mechanical transfer of dung. This may be illustrated numerically by the following example: the lactating sows on Farm B were voiding, on average, 2,850 'strongyle' ova per gramme of faeces (Chapter 2). Anyone working with these pigs could easily pick up 5-10 gramme of faeces in the tread of their wellington boots. If the visitor was to proceed to Farm C, about one mile distant, without washing his footwear, some 15-30,000 potentially infective units would have been transported to the latter herd.

Farms may acquire infection from carrier animals. Pigs carrying adult Oesophagostomum populations are dangerous in this respect because of the high biotic potential of this genus. Fattening farms are particularly at risk since they frequently purchase weaners from a variety of sources. As far as MD or SPF herds are concerned, however, the only animals liable to be imported from outside are entire males. These are frequently neglected in worming programmes but are capable of playing a major role in the dissemination of infection within a population since each sow is taken to the boar's pen twice, if not thrice, a year for mating.

Such explanations do not provide a satisfactory explanation for the presence of helminth infections in all SPF or MD herds. One outstanding example has come to the attention of the author. This was a sophisticated model farm used by an industrial concern for research purposes. In this instance, extreme efforts were made to isolate the herd. No visitors were allowed onto the premises and goods lorries bringing feedstuffs or equipment unloaded outside the boundries of the farm. Yet this herd became infested

with Oesophagostomum.

In these special cases, the usual mechanisms for the dispersion of larvae are unlikely to have been involved. Connan (1966) has suggested that birds and rodents may carry infection but he does not state the means by which these animals might perform this function. It seems quite feasible that they may, on occasion, accidentally transport faecal material from farm to farm.

Other possibilities that have not as yet been fully investigated include transmission from dam to piglet in utero or via colostrum. There is, however, little indication that the histotropic forms of Oesophagostomum are able to pass beyond the muscularis mucosa of the wall of the intestinal tract of the natural host (Chapter 6). The experimental studies that are described in this chapter demonstrate two biological pathways by which infective Oesophagostomum larvae may possibly pass from herd to herd. These are discussed in the following paragraphs.

3.3.2. Rat Transmission

The demonstration of encysted worms in all the treated rats of Experiments 3.1. and 3.2. indicates that porcine oesophagostome larvae are able to invade the intestinal wall of this abnormal host. That the observed larvae were not naturally occurring parasites of the rat is confirmed by the consistently negative results obtained from the controls.

The discovery of young adult Oesophagostomum in four of the five pigs that received tissues from the infected rats confirms that the laboratory rat can, under the conditions ofthis experiment, act as a paratenic host for this nematode. Some of the rats retained their infective potential for as long as one month. The control pigs remained worm-free even though they had been permanently housed with the experimental animals. The possibility that the infestations resulted from environmental contamination is thereby discounted.

The results of this experiment do not necessarily implicate the wild rat as a vector of oesophagostomiasis in the field, but demonstrate that there is a likelihood that this may be the case. Rats are commonly found in close

association with pig farms, where they live and feed in an environment often grossly contaminated with faecal material. Oesophagostomum is an extremely fecund worm and it is not uncommon to find several thousand eggs being passed with every gramme of host faeces. It seems inevitable that piggery rats will ingest a number of Oesophagostomum larvae each day. If the larvae are able to encyst and survive in the intestinal mucosa as they do in the laboratory strain, then wild rats are almost certainly frequent carriers of infection. It is doubtful whether rats form a regular part of the pig's diet, but diseased or debilitated specimens are certainly eaten. Rats usually form stable colonies within defined boundaries, and normally there is little communication between neighbouring groups. If, however, a community is eradicated by man, an ecological vacuum is produced which is quickly filled by an influx of rats from surrounding areas. Under these conditions, the rat may be able to transport Oesophagostomum infections from farm to farm.

3.3.3. Psychodid Transmission

Bovien (1937) found in Denmark two types of nematode twined round the intersegmental furrows of the abdomen of psychodid flies which had developed in bovine faeces. He classified them as the free-living Rhabditis dubia and an unknown species belonging to that genus. Satchell (1947) noted the same phenomenon in England and identified a third species, Rh. curvicaudata. Neither of these authors mentions the possibility that juvenile forms of nematodes parasitic in the cattle producing the dung might also be involved.

Members of the family Psychodidae are commonly known as Owl Midges or Hairy Moth-flies. The family contains five genera of which only one, Psychoda, has been found breeding in dung in Great Britain. This genus is composed of small flies, 1 - 2 mm. long, which are covered with loosely packed greyish hairs. They carry their wings folded roof-like over their body when at rest, and can be seen moving erratically on window panes or being attracted to electric lights after dark, when they often settle on lamp-shades or nearby walls.

In the field they can occur in large numbers, moving with a short jerky flight near their breeding places in decaying organic material, including dung. In the height of summer when conditions for breeding are optimal, enormous numbers are produced. One dung sample of approximately 200 g. collected in August yielded over one thousand psychodids in 18 days. The adult fly emerges from the pupa one to three weeks after the eggs are laid, depending on the temperature and species involved. On emerging, the imago stands motionless on the surface of the dung-pat for several minutes, strengthening itself and hardening its wings.

By this time 'strongyle'eggs passed with the faeces will have hatched and the emergent larvae will have attained the infective third stage. The in-pig sows on Farm B were shedding an average of 480 e.p.g. (Chapter 2). Should the nematodes brush against the imago fly, they could either attach directly onto the abdomen, or onto the legs, from where they could move up through the hairs to coil tightly around the abdominal intersegmental grooves. No larvae were ever found clinging to other manure-breeding flies.

The flies are small enough to be transported by the wind, but to what extent this happens is uncertain. Satchell (1947) studying P. alternata, a fly breeding in sewage filters, found that it could be caught one mile from the breeding ground in the direction of the prevailing wind, but not at one and a half miles. Psychoda spp. may therefore be able to transport the larval forms of parasitic nematodes from field to field, or into farm buildings from the surrounding countryside. It seems likely that the psychodid fly may play some role in the dispersion of porcine Oesophagostomum infections.

3.3.4. Zoological Significance

A variety of means are used by the parasitic nematodes for the dispersion of infections. Some rely on the adhesive nature and resistance of their eggs (e.g. Ascaris suum), others on the mobility of transport hosts (Heterakis gallinae), paratenic hosts (Toxascaris leonina) or intermediate hosts (Trichinella spiralis).

Within the Strongylata, the Metastrongyloidea require intermediate hosts for the completion of their life-cycles.

With three exceptions, however, no biological associations have been reported for the dispersion of infective larvae belonging to the other groups of veterinary interest within this suborder. The exceptions are the genera Dictyocaulus (Trichostrongyloidea) whose larvae are scattered by the exploding sporangia of the fungus Pilobilus, Stephanurus (Strongyloidea) that uses the earthworm as a transport host and Syngamus (Strongyloidea) which uses a variety of transport hosts. The genus Oesophagostomum (Strongyloidea), whose larvae can utilize the rat as a paratenic host and the psychodid fly as a transport host, can now be added to this list.

El Rafaii (1962) claims to have shown, by a series of uncontrolled laboratory experiments, that cockroaches and earthworms can also act as paratenic hosts for Oesophagostomum. These observations need to be confirmed before they can be accepted, but it would appear that this parasite may be distributed by a wide variety of biological mechanisms.

It seems unlikely that the few 'strongyle' parasites mentioned in this section are the only ones using these

biological pathways. Their larvae are incapable of moving more than a few centimetres from the dung-pat and future investigations will doubtless reveal a widespread utilization of these routes. Indeed, there is already some evidence that the behaviour of Ostertagia spp. in cattle dung containing developing Psychoda resembles that described for Oesophagostomum in this chapter (Jacobs, Tod, Dunn and Walker, 1968).

Chapter 4

STUDIES ON THE CONTROL OF HELMINTHIASIS IN ADULT PIGS WITH MODERN ANTHELMINTICS

INTRODUCTION

The advent of the broad-spectrum anthelmintic has stimulated a renewed interest in porcine helminthiases. If control of these infestations is shown to be economically desirable, a rational approach will be dependent upon a knowledge of the prevalence and epidemiology of each parasite. Earlier chapters of this thesis have shown the importance of the adult pig as a reservoir of infection for the strongylate parasites Oesophagostomum and Hyostrongylus. Any attempt to combat these genera must be concentrated on the breeding stock. Gitter, Kidd and Davies (1965) suggested that the most convenient time for the application of chemotherapeutic agents to sows was immediately prior to parturition. At this time, the animal is kept under conditions that allow individual feeding, and potential pathogens will be eliminated before the piglets are born. The demonstration of the periparturient egg-rise (Chapter 2) confirmed the wisdom of this recommendation.

Several authors have evaluated the new generation anthelmintics in sows (Table 4.1.), but little detailed work has been performed to define the epidemiological consequences of

such treatments. One important objective of the present series of investigations was to find out if the peri-parturient rise could be eliminated by strategically applied chemotherapy. The anthelmintic chosen for this study was Thiprazole (vide infra), which, at that time, was the only anthelmintic whose efficacy and safety in the sow had been adequately tested.

Later, a second broad-spectrum anthelmintic, Atgard (vide infra), became available for experimental use. Previous studies had demonstrated its activity against Oesophagostomum and Hyostrongylus in fattening pigs (Batte, Moncol, Todd and Isenstein, 1965; Ikeme, 1966) but no information had been published to substantiate its suitability for adult pigs. Since this product was due to be marketed in the United Kingdom within a few months, and as the practice of worming sows was becoming more widespread, it was decided that an evaluation of its usefulness in the treatment of breeding stock would be of value. Investigations were therefore initiated to answer the following questions:

Does a single dose adequately suppress the output of 'strongyle' eggs in the faeces, and if so, for how long?

Are the worms expelled or is egg-laying merely inhibited?

Are both Oesophagostomum and Hyostrongylus affected by the compound?

What dose-rate is required to give the necessary degree of activity?

Will this product also kill 'strongyle' eggs in the intestinal contents?

Is the substance easy to apply and readily accepted by the pigs?

Will the sow tolerate accidental overdosage?

Will this anthelmintic provoke abortion or increase the risk of peri-natal death?

Will it kill the early foetus or induce teratogenic effects?

Finally, a trial was conducted to demonstrate that economic benefit could be gained from the application of the knowledge accrued during the course of these studies.

Since the completion of this work, two products designed for the therapy of multiple parasitisms of swine have become available to the British pig industry: I.C.I. Pig Wormer® (tetramisole, Imperial Chemical Industries Limited) and Loxon Premix® (haloxon, Cooper, McDougall and Robertson Limited). In addition, parbendazole (Smith, Kline and French Limited) has shown promise on an experimental basis.

MATERIALS AND METHODS

4.1.1. Farms Visited

Farm EC:

A description of this farm, together with its clinical history, was provided in Chapter 2 (§ 2.1.1.).

Farm P:

The second herd to be studied during the course of these investigations was situated in Renfrewshire. It is a large enterprise with several hundred Large White sows and gilts. After weaning at eight weeks of age, the progeny are kept on the premises for fattening. The standard of hygiene is exceptionally high and the worm burden very low. The 'strongyle' egg-counts of the lactating sows were never observed to rise above a few hundred eggs per gramme of faeces. This particular farm was chosen because the owner and his staff were used to keeping detailed and accurate records and could be relied upon to follow an investigation through to its completion.

Farm Z:

A herd known to be heavily infested with both

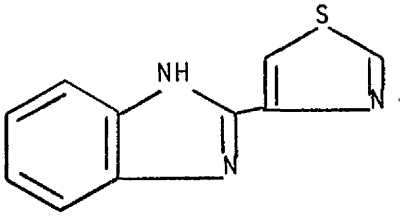
Oesophagostomum and Hyostrongylus was located in Dumfriesshire. The 60 sows and their progeny were kept in grossly overcrowded conditions. The in-pig sows wallowed in a small waterlogged paddock and were brought into dilapidated unhygienic buildings for farrowing. The piglets were weaned at eight weeks old and reared to bacon weight in a cold draughty fattening house. The younger stock harboured Ascaris and the breeding animals carried large numbers of lice (Haematopinus suis).

4.1.2. Anthelmintics Used

Thiprazole®:

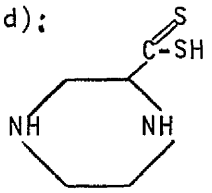
This is a product developed by Merck Sharp and Dohme Limited that, at the time of this investigation, contained 4% thiabendazole and 8% picadex.

Thiabendazole (2-(4-thiazolyl)benzimidazole) has the following structure:



This compound combines a broadspectrum of anthelmintic activity with a wide safety margin. Its use in pigs has been evaluated on several occasions (vide Tables 4.1. and 4.2.).

Picadex is a piperazine-carbon disulphide complex (piperazine-1-carbodithioic acid);

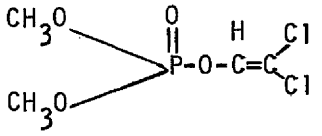


It has good ascaricidal properties (Kelly, Olsen and Garwood, 1956) and thus complements thiabendazole which is partially deficient in this respect (Leiper and Crowley, 1963). In the stomach, picadex releases carbon disulphide which has been specifically used in the past against H. rubidus (vide infra).

Atgard V®:

The active principle of this product (supplied by Shell International Chemical Company) is the organo-phosphorus compound, dichlorvos (0,0-dimethyl-2,2-

dichlorovinyl phosphate):



This substance is incorporated into polyvinyl chloride (PVC) pellets designed to give a slow and sustained release of dichlorvos during their passage through the alimentary tract. The pellets have proved to be highly effective against a wide range of gastro-intestinal nematodes including all those listed in Chapter 1 as endemic in the British pig population (Batte, Moncol, Todd and Isenstein, 1965; Ferguson, 1966; Ikeme, 1966).

4.1.3. Experimental Designs

Experiments 4.1. - 4.4. were performed on Farm EC.

Experiment 4.1.

Each sow farrowing between November, 1966 and February, 1967 was treated on a single occasion with 6 oz. Thiprazole. The drug was administered one week before the anticipated date of farrowing by mixing the medicated pellets with the normal ration. Efforts were

made to persuade the farmer to leave a proportion of his breeding stock untreated to serve as controls. This request was, however, refused. Faecal egg-counts were performed at weekly intervals. The results obtained were compared with those from nearby untreated herds (previously discussed in Chapter 2).

Experiment 4.2.

Sixty sows, all in the early stages of pregnancy, were selected during the period April-July, 1967 and randomly allotted to four experimental groups each containing 15 animals. One group was not subjected to any treatments and used as a control, whilst the sows of the other three were given single doses of dichlorvos at rates of 1.96 g., 1.44 g. and 1.15 g. (i.e. 11 g., 8.25 g. and 6.67 g. of the formulated product) per pig respectively. The animals were treated individually by incorporation of the pellets into each sow's normal ration of meal.

Twice weekly faecal samples were taken from each sow from the second week before treatment until the end of the

second week after the administration of the drug, and once weekly thereafter for a further seven weeks. Egg-counts were performed by the McMaster method (Chapter 2) and larval cultures by the Baermann technique (Chapter 1).

Experiment 4.3.

Two groups of five sows were selected randomly from the herd. The risks associated with accidental over-dosage were investigated by giving each animal a single large dose of the product one to two weeks before the expected date of farrowing. The sows of one group received 33 g. of pellets (5.76 g. dichlorvos) and those of the other 66 g. (11.52 g. active ingredient).

Experiment 4.4.

In order to estimate the influence exerted by the drug on the viability of 'strongyle' eggs lying in the intestinal contents or faeces, a further group of 20 sows was selected at random and given 1.44 g. dichlorvos (8.25 g. of pellets). Faecal samples were taken immediately before, and 24 hours after, administration

of the anthelmintic. Ten-gramme samples were weighed out and the number of larvae harvested after seven days incubation noted. Every care was taken to maintain the two sets of samples under identical conditions.

It will be shown in Chapter 6 that guinea-pigs given Oesophagostomum larvae of porcine origin by stomach tube develop easily visible lesions by the ninth day after infection. This observation was used for the development of a rapid inexpensive laboratory technique for evaluating the infectivity of third stage larvae of this genus. Four guinea-pigs were each inoculated with 10,000 third stage juveniles cultured from the post-treatment faecal samples, and a further four were given a similar number from the pre-treatment samples. The presence, after nine days, of parasitic nodules was taken as confirmation of infectivity, whereas failure to produce the characteristic lesions was assumed to indicate larval incompetence.

Experiment 4.5.

The previous experiments measured the efficacy of the

anthelmintic in terms of reduction in faecal egg-counts. This method is not entirely satisfactory as it leaves the possibility that the toxicant may only inhibit egg-laying without expelling the worm burden. Direct observations of the behaviour of the nematode populations of treated animals were therefore made using the technique described by Gibson (1965) as the critical trial. This method is extremely time consuming and finally involves the slaughter of the experimental subject. Thus, in field investigations, it can only be used to supplement information gathered by other means.

Three emaciated sows were purchased from Farm Z and placed in metabolism cages (described in Chapter 5). Each was treated with 1.44 g. dichlorvos. All faecal material produced by these animals for seven days after treatment was collected and examined for the presence of nematode parasites. The sows were slaughtered and the gastro-intestinal tract searched for helminths by the methods outlined in Chapter 1. The percentage efficacy of the anthelmintic was obtained by comparing the numbers

of worms expelled and the number retained within the host.

Experiment 4.6.

This project required the careful documentation of litter-weights and was therefore performed on Farm P. Every second sow that was due to farrow during March and April, 1967 was given a single dose of 1.44 g. dichlorvos in the PVC formulation ten days before the estimated date of farrowing. The remaining dams acted as untreated controls. The following information was collected:

Name and boar that fathered litter

Number of piglets born alive

Number born dead

Age of piglets at the '3-week' and '8-week' weighings

Number of piglets still alive at the '3-week' and '8-week' weighings.

These data were used to compute the productivity of treated and untreated sows in terms of litter size and growth-rate. The terms '3-week' and '8-week' are placed within inverted commas because the litters were often

weighed one or more days before or after the required date. Piglets grow so rapidly that account has to be taken of the exact age of the piglets in days when calculating the results of such a trial.

RESULTS

4.2.1. Suppression of the Periparturient Egg-Rise

Data obtained during Experiment 4.1. from the examination of 396 faecal samples are given in Appendix 4, Table 1, and mean values are displayed graphically in Fig. 4.1. The diagram shows the average faecal 'strongyle' egg-counts from sows during the first 14 weeks of pregnancy (to the left of the main body of the histogram) and for each week of the parturient period (i.e. two weeks before farrowing until the ninth week after). The shaded areas represent the treated herd (Farm EC). These results are superimposed upon an illustration of the regular periparturient rise as it occurred on Farm B (Chapter 2).

After treatment, the mean number of ova being shed in the host faeces fell from around 8,000 e.p.g. to a minimal value of 350 e.p.g. four weeks after parturition. The apparent delay of one week between the administration of the anthelmintic and the rapid decline in the egg-output is an artifact. The length of gestation in the pig is not a constant value and consequently the period between medication and parturition varies. Some pigs farrowed within a

week of dosing, others did not produce piglets until more than seven days had elapsed. Egg-counts remained low until after weaning, when they climbed up to the preparturient level.

Better results still might have been obtained if the sows had taken the medicated feedstuff more readily. The ration was eaten by the majority of pigs after a short period of reluctance, but it was totally rejected by a few. In order to entice these animals, sugar or molasses was added to the food. Even so, three days sometimes elapsed before all the Thiprazole had been consumed.

4.2.2. The Biological Evaluation of Dichlorvos Palatability (Experiments 4.2.-4.6.):

Each of the sows used for these trials accepted meal containing dichlorvos pellets with the eagerness usually displayed on being fed - even in those cases where considerable overdoses were offered.

Egg-Counts (Experiment 4.2.):

Administration of dichlorvos at all three dose-rates

caused an immediate decline in the faecal output of 'strongyle' eggs (Fig. 4.2.). A comparison of the average egg-counts from each group of sows for the two weeks previous to, and subsequent to treatment, showed that whereas the number of eggs per gramme of faeces shed by the control group increased by 32.9% during this period, the corresponding figures for the treated groups had fallen by up to 97.5% (Table 4.3.). A very low level of egg-production was maintained for six weeks or more after treatment.

Figure 4.3. shows the number of sows in which patent Oesophagostomum infections could be demonstrated. A very high proportion of sows of the control group shed Oesophagostomum eggs throughout the duration of the experiment. On the other hand, the treated animals yielded relatively few positive samples during the six weeks following the administration of the drug.

The numbers of sows infested with H. rubidus was much smaller (Fig. 4.4.). The two larger dose-rates

(1.44 g. and 1.96 g. dichlorvos) appeared to control this parasite, no Hyostrongylus eggs being shed for three weeks or more after treatment. A lesser degree of success was obtained with the smallest dosage rate.

Critical Trial (Experiment 4.5.):

The Oesophagostomum populations (which included fourth and fifth stage larvae as well as adults) in the lumen of the large intestines of the three experimental sows were completely, or almost completely, eliminated from the host (Table 4.5.). In addition, the few Trichuris and ascarids harboured by these animals were all expelled. Almost all of the ejected worms appeared in the faeces on the third and fourth days after treatment.

No H. rubidus were found in the faeces of these sows. This nematode is normally located under the layer of mucus covering the gastric mucosa, which may delay the passage of dead worms into the ingesta. It seems probable that the structure of the worms is altered beyond recognition by the digestive processes during the three or four days in transit

after treatment. Taffs (1968b) has reached a similar conclusion as a result of his own experiences. In addition, it may be noted that Mendes, Rocha, Rocha, Serra, de Campos and Ribeiro (1967) have observed intra-luminal disintegration of nematodes killed by dichlorvos. The copious quantities of faecal material produced by adult pigs add to the difficulties of the search, so that this negative finding does not necessarily mean that no H. rubidus had been expelled. Twelve adult members of this species were recovered from the stomach of one sow, none from the other two.

Ovicidal Effect (Experiment 4.4.):

The hatchability of the eggs shed in the faeces of the sows 24 hours after treatment was not influenced by the dichlorvos, since equal numbers of larvae were recovered from the pre- and post-treatment samples (Table 4.5.). The drug did, however, affect the development of a proportion of the hatched larvae as one-third of these were stunted, having failed to reach the second moult. Those juveniles that did become third stage ensheathed

larvae were shown by the guinea-pig test to be infective.

Toxicity (Experiments 4.2.-4.6.):

Altogether a total of 84 pregnant females were treated with between 1.15 and 1.96 g. dichlorvos. In addition, gross excesses of formulated product (up to 66 g.) were administered to ten sows one to two weeks before parturition. Without exception, farrowing proceeded normally and healthy litters were produced. No signs of acute intoxication were noted on clinical inspection of the treated animals.

4.2.3. The Effect of Dichlorvos on Sow Productivity

The productivity of the treated sows on Farm P (Experiment 4.6.) tended to be greater than that of the untreated control animals (Table 4.6., Appendix 4, Table 3). At parturition, the experiment was unintentionally biased against the treatment as larger litters (both in terms of numbers of piglets born and their combined weights) were born to the control animals. By the time of the '8-week' weighing, however, this relationship was reversed, there being fewer post-natal deaths amongst the offspring of the

treated sows. The surviving piglets of the latter group grew more rapidly so that at eight weeks of age the average weight per animal was 0.26 lbs. heavier than the corresponding figure for the progeny of the untreated dams.

These results were submitted for statistical analysis. The differences were not significant although there was an apparent tendency in favour of the treated group. In order to prove the occurrence of a 5% difference with this degree of variability, one would need to have 390 sows in each experimental group. If the real difference is in the order of 10%, 98 would be required.

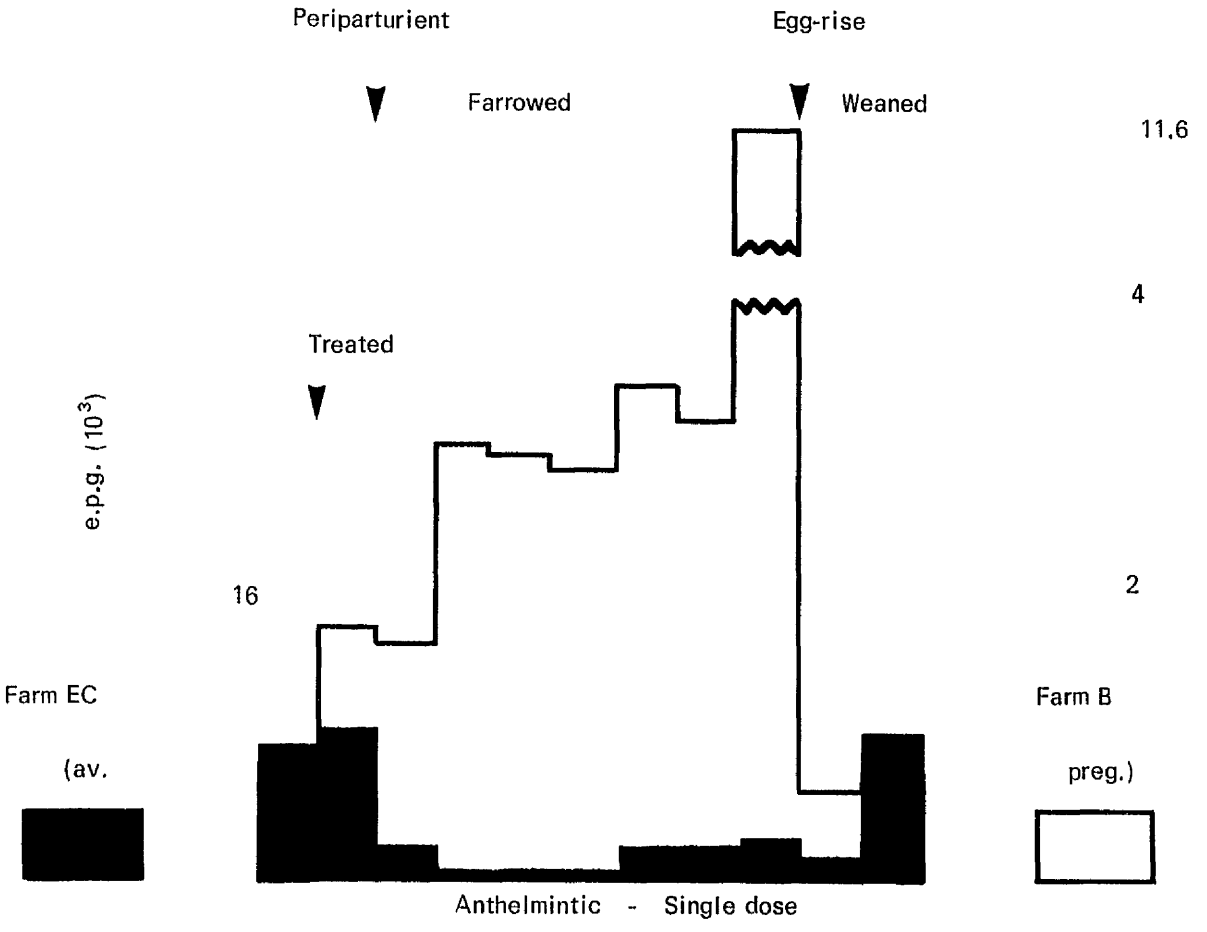


Fig 4.1 Faecal 'strongyle' egg-counts (herd averages) from sows on Farm EC (shaded), that were treated with a thiabendazole/picadex mixture one week before the expected date of farrowing, compared with those from Farm B (unshaded) which received no anthelmintic treatment (*cf* Fig 2.1.). Each vertical column represents one week. On either side of the histogram, the average value during pregnancy

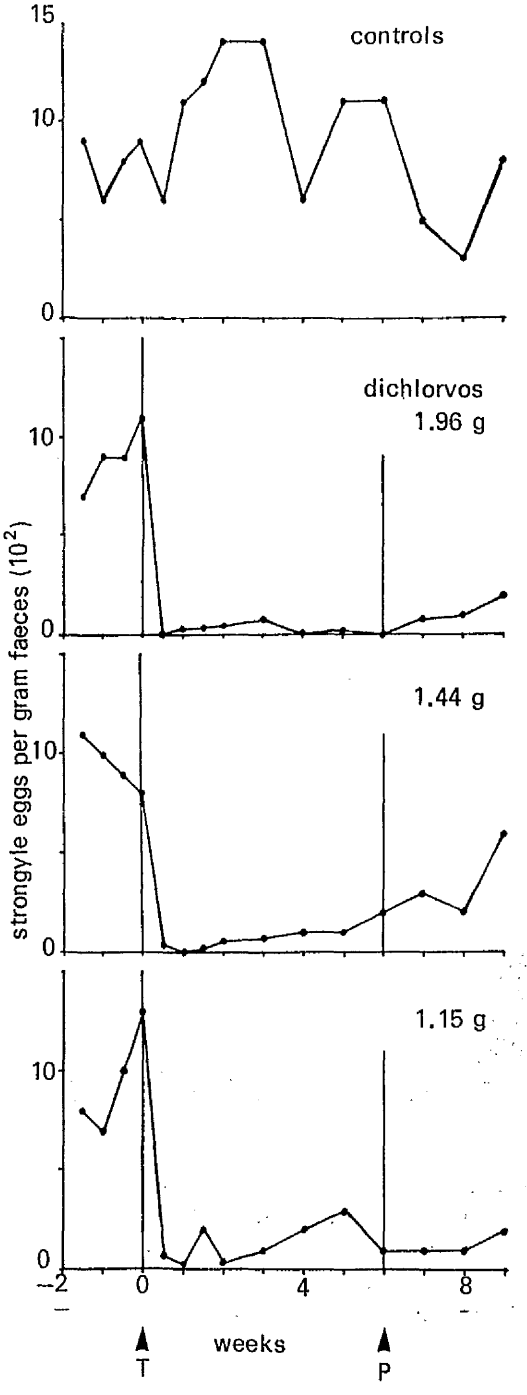


Fig 4.2 Average faecal 'strongyle' egg-counts for three groups of 15 sows treated with formulated dichlorvos (quantities refer to active principle) and one untreated control group. Treatment was given at 'T'; the interval 'T - P' represents the prepatent period of *Oesophagostomum* spp.

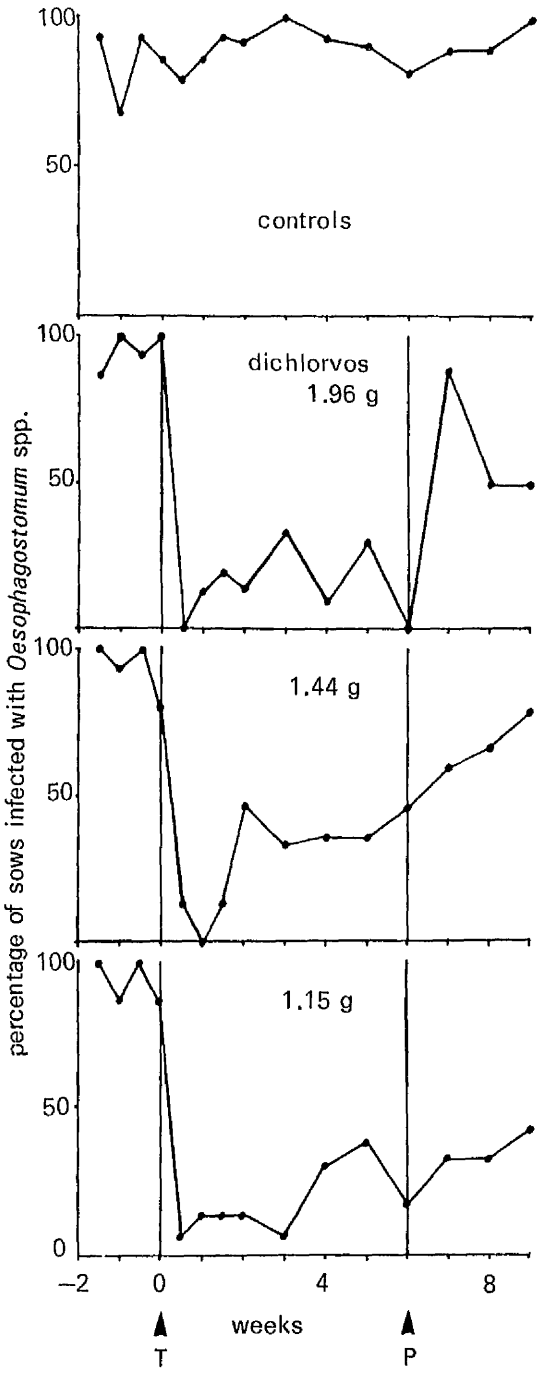


Fig 4.3 The proportion of sows from an untreated control group and three groups of 15 sows treated with formulated dichlorvos (quantities refer to active principle) in which patent *Oesophagostomum* infections could be detected. Treatment was given at 'T'; the interval 'T - P' represents the prepatent period of *Oesophagostomum*

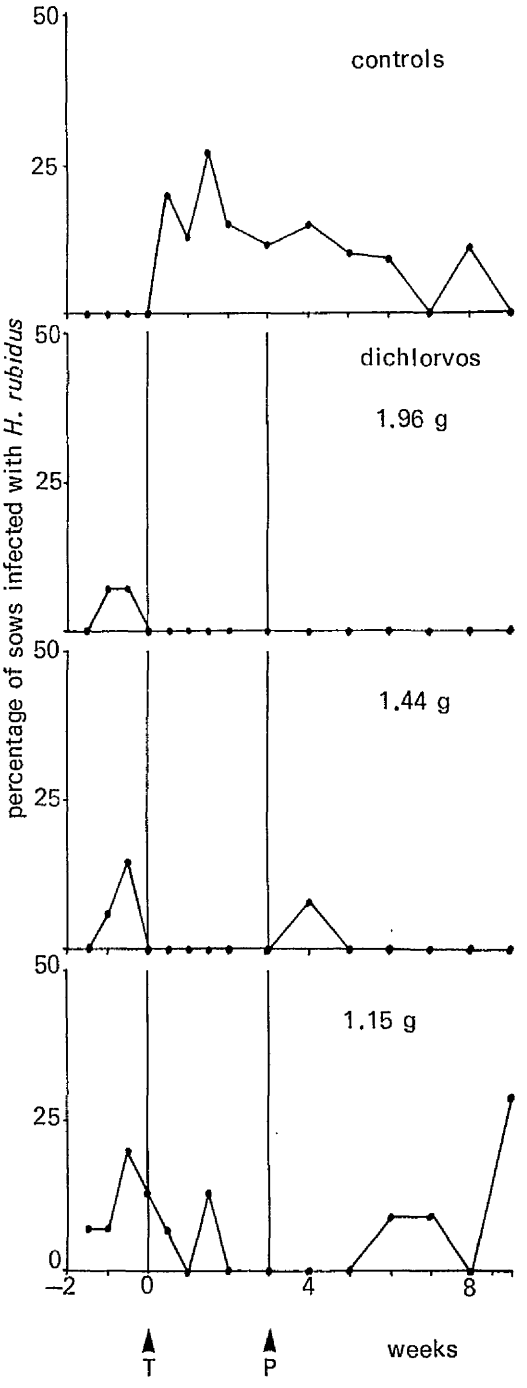


Fig 4.4 The proportion of sows in an untreated group, and three groups of 15 sows treated with formulated dichlorvos (quantities refer to active principle) in which patent *Hyostrongylus* infections could be detected. Treatment given at 'T'; the interval 'T - P' represents the prepatent period of *H. rubidus*.

Table 4.1. - The evaluation of broad-spectrum anthelmintics in sows: a synopsis of published results. The recorded experiments were performed under widely varying conditions and the results cannot be used for the direct comparison of compounds

Source	No. pigs examined	Dose given mg./kg.	% worms expelled		% reduction in 'strongyle' egg-counts	Type of infection	Notes
			Oesophagostomum	Hyostrongylus			
			THIABENDAZOLE				
Shanks (1963, 65) - U.K.	8	4.5 g./100 lbs.	-	-	100	Natural	
Cairns and Hargreaves (1966) - New Zealand	37	3 - 5 g./100 lbs.	-	-	100	Natural	
Gitter et al. (1966) - U.K.	39	100	-	-	96	Natural	
Ludvigsen (1967) - Denmark	205	50	-	-	97	Natural	
		100	-	-	97	Natural	
Taffs and Davidson (1967) - U.K.	4	44	-	-	100	Natural	+ 125 mg./kg. picadex
Davidson et al. (1968) - U.K.	33	50 - 60	-	-	99 - 100	Natural	
Thomas and Smith (1968) - U.K.	15	2 x 50	-	-	73	Natural	
Present work	-	7.5 g./sow	-	-	96	Natural	+ 15 g./sow picadex

Table 4.1. (Continued)

Source	No. pigs examined	Dose given mg./kg.	% worms expelled		% reduction in 'strongyle' egg-counts	Type of infection	Notes
			Oesophagostomum	Hyostrongylus			
			TETRAMISOLE				
Walley (1967)	10	10	96	-	-	Natural	
- U.K.	28	7 - 14	95	-	-	Natural	
Nickson et al cit. Taffs (1968a) - U.K.	-	20	-	-	42 - 89	Natural	
			DICHLORVOS				
Present work	45	1.15-1.96 g./sow	95 - 98	-	96 - 98	Natural	
			HALOXON				
Gitter et al (1966) - U.K.	16	45	-	-	91 - 96	Natural	

Table 4.2. - The evaluation of broad-spectrum anthelmintics in growing pigs: a synopsis of published results: The recorded experiments were performed under widely varying conditions and the results cannot be used for the direct comparison of compounds

Source	No. pigs examined	Dose given mg./kg.	% worms expelled		% reduction in 'strongyle' egg-counts	Type of infection	Notes
			Oesophagostomum	Hyostrongylus			
THIABENDAZOLE							
Enigk and Flucke (1962) - Germany	-	50	-	-	81	Natural	
Leiper and Crowley (1963) - U.K.	8	100	-	-	99	Natural	
Drezancic (1964) - Yugoslavia	-	100	-	-	100	Natural	
Taffs (1966) - U.K.	2	44	71 - 88	-	-	Adult	
	2	66	97 - 99	-	-	Adult	
	3	66	0	-	-	5-day old larvae	
Taffs (1968c) - U.K.	3	50	0	-	-	5-day old larvae	+ 125 mg./kg. picadex
	3	50	100	-	-	50-day old worms	+ 125 mg./kg. picadex
Taffs (1968d) - U.K.	3	66	-	57	-	5-day old larvae	
	3	66	-	81	-	16-day old larvae	
	3	66	-	96	-	30-day old worms	

Table 4.2. (Continued)

Source	No. pigs examined	Dose given mg./kg.	% worms expelled		% reduction in 'strongyle' egg-counts	Type of infection	Notes
			Oesophagostomum	Hyostrongylus			
			TETRAMISOLE				
	4	5	45	31	-	3-day old larvae	
	2	5	81	-	-	28-day old larvae	
	4	10	38	20	-	3-day old larvae	
	2	15	60	-	-	3-day old larvae	
	5	10	50 - 64	45	-	10-day old larvae	
	2	15	89	80	-	10-day old larvae	
Walley (1967) - U.K.	1	20	93	76	-	10-day old larvae	
	2	5	81	-	-	28-day old larvae	
	4	10	96 - 99	92	-	28-day old larvae	
	4	15	100	100	-	28-day old larvae	
	2	20	100	98	-	28-day old larvae	
	16	5	53 - 77	0 - 46	-	Natural	
	144	10	22 - 100	62 - 100	-	Natural	
	34	15	99 - 100	100	-	Natural	
	4	20	100	100	-	Natural	
Weissenburg and Neubrand (1967) - Germany	9	10	-	-	99+	Natural	
	4	15	0	-	-	5-day old larvae	
Taffs (1968a) - U.K.	4	15	85	-	-	16-day old larvae	
	4	15	76	-	-	48-day old worms	
			DICHLORVOS				
	27	400 ppm	98	-	-	Natural	Used as feed additive
Batte et al (1965) - U.S.A.	109	<20 - >100	91	-	-	Natural	

Table 4.2. (Continued)

Source	No. pigs examined	Dose given mg./kg.	% worms expelled		% reduction in 'strongyle' egg-counts	Type of infection	Notes
			Oesophagostomum	Hyostrongylus			
			DICHLORVOS				
Ikeme (1966) - Nigeria	4	40	-	97 - 99	-	Natural	
Mendes et al (1967) - Brazil	11	40	97	-	-	Natural	
Schoop et al (1967) - Germany	143	55	-	-	100	Natural	
Euzeby and Cottereau (1968) - France	10	30	69	-	-	Natural	
Fagasinski (1968) - Poland	38	26	-	-	100	Natural	
			PARBENDAZOLE				
Theodorides et al (1968) - U.S.A.	51	25 - 50	100	-	-	Natural	
Chang and Wescott (1969) - U.S.A.	10	20	100	-	-	Natural	
	85	20	-	-	99	Natural	

Table 4.3. - Comparison of the Average 'Strongyle' Egg-count of four groups of 15 sows during the two weeks previous to, and the two weeks following, treatment with Dichlorvos pellets

	Untreated group	Treated groups (grammes dichlorvos) per sow		
		1.15	1.44	1.96
Pre-treatment e.p.g.	788	907	925	982
Post-treatment e.p.g.	1,047	58	23	29
Percentage change	32.9 increase	96.3	97.5 decrease	97.0

Table 4.4. - Results of critical trial. Each sow given 1.44 g. dichlorvos

Sow No.	Total Nos. Helminths at risk			Total Nos. Helminths expelled			Anthelmintic efficiency %		
	Oes.	A.s.	T.s.	Oes.	A.s.	T.s.	Oes.	A.s.	T.s.
1	3,079	1	0	3,079	1	0	100	-	-
2	1,911	2	0	1 908	2	0	99.8	-	-
3	1,874	0	1	1 773	0	1	94.6	-	-
Totals:	6,864	3	1	6,760	3	1	98.5	(100)	(100)

(Oes. = Oesophagostomum spp., A.s. = Ascaris suum, T.s. = Trichuris suis.)

Table 4.5. - The effect of dichlorvos on Oesophagostomum spp. eggs in vivo. (Counts are averages of 20 sows)

Post-treatment sampling	Egg-count e.p.g.	Larvae recovered per gramme faeces			% Hatch	% Stunted	L_3 Infective?
		Total	Stunted	L_3			
after 0 hours	1,590	569	<1	569	35.8	0	yes
24 hours	1,280	546	134	412	42.7	32.5	yes

Table 4.6. - A comparison of the productivity (measured in terms of litter-size and growth-rate) of sows given a single pre-natal treatment of 1.44 g. dichlorvos and that of untreated controls.
All weights are in lbs.

The author wishes to thank Mr. M. Roberts and Mr. P. Hunt, Shell Research Limited, for performing the statistical analysis.

	Untreated Controls	Treated Group
No. of sows:	19	19
No. of piglets:		
born alive	212	197
born dead	13	15
No. of pigs surviving:		
to three weeks	181	180
to eight weeks	164	169
Average weight per pig adjusted for age:		
at birth	2.9	3.0
at three weeks	10.35	10.23
at eight weeks	33.59	33.85
Average litter weight adjusted for age:		
at birth	32.4	30.8
at three weeks	98.6	97.9
at eight weeks	295.4	306.4

DISCUSSION

4.3.1. Characteristics Desirable for an Anthelmintic for Adult Pigs

Spectrum of Activity:

The survey described at length in Chapter 1 of this thesis provides the data necessary for defining the required biocidal properties. The nematodes of paramount importance in British sows and boars are without doubt Oesophagostomum spp. and H. rubidus. Any medicament intended for this class of livestock must be capable of expelling these two genera. The sow is of minor significance in the epidemiology of the other worms important in growing pigs such as Ascaris and Trichuris. Consequently, it is not essential that the chosen anthelmintic should show efficacy against these, but such activity would be a useful bonus as a small minority of sows do act as carriers.

Safety:

In addition to the usual criteria of acceptability regarding acute and chronic hazards to the patient, the user and the consumer, any product under consideration must be shown to be free from teratogenic effects. It must not

provoke abortion and it must not interfere with spermatogenesis.

Earlier Candidate Compounds:

Until the introduction of the broad-spectrum anthelmintics less than a decade ago, carbon disulphide was the compound most often used to combat hyostrongylosis. Bozicevich and Wright (1935) showed that volumes of 0.027 to 0.22 ml/kg. body-weight gave a percentage control varying from 41 to 100. Dewes (cit. Dodd, 1960) reports good clinical responses after the use of this drug. Dodd recommends a dose of 3-5 ml./100 lbs. body-weight. Shanks (1965), however, found that two doses of 15 ml. produced no beneficial effect and he comments on the practical inconvenience of administering liquids to sows. Signs of toxicity have been seen at 0.2 ml/kg. (Bozicevich and Wright, 1935).

The piperazine compounds, which are extremely useful in the control of ascariasis, have little or no influence on H. rubidus (Enzie, Wilkens and Colglazier, 1958). These

authors also tested these salts against Oesophagostomum spp. At a dose-rate of 110 mg/kg. in drinking water, the citrate and dihydrochloride proved to be almost 100% effective, whilst other analogues were less satisfactory. There is widespread feeling, however, that even the dihydrochloride has a variable potency, perhaps because of the development of drug resistant strains of the parasite (Mendes, Rocha, Rocha, Serra, de Campos and Ribeiro, 1967).

Gibson (1965) lists a number of references to the use and toxic effects of phenothiazine in the therapy of Oesophagostomum infections. Quantities in excess of 100 mg/kg. give 64-100% control. Andrews and Connelly (1944) wormed pregnant sows a few days before farrowing at 220 mg/kg. Acute toxicity may occur with doses as low as 440 mg/kg., but many pigs will tolerate greater amounts than this.

Hygromycin B is an antibiotic with anthelmintic properties. If given as a feed additive it will depress or inhibit ova production by female oesophagostomes (Goldsby and Todd, 1957) and will expell the worms after a period of

weeks (McCowan, Gossett, Callender and Brandt, 1960).

Modern Generation Anthelmintics:

Clearly, none of the compounds described above even approaches the standards of performance required for the treatment of sows. Within recent years, however, much progress has been made in the field of chemotherapy. Anthelmintics are now available that possess a wide spectrum of activity against both adult and fourth stage nematodes yet have a low mammalian toxicity. The first compound to be exploited in this way was thiabendazole.

When Shanks (1963, 65) evaluated this drug against H. rubidus he recorded pretreatment egg-counts of up to 32,000 e.p.g. Although he found no Oesophagostomum at post mortem, the eggs he counted must have been produced primarily by this genus as Hyostrongylus has a very low biotic potential (Davidson, Murray and Sutherland, 1968). The highest H. rubidus egg-count recorded by Connan (1967a) was 220 e.p.g. in a sow that harboured over 10,000 adult worms. Later studies have, however, provided conclusive

evidence of the value of thiabendazole against Hyostrongylus (Tables 4.1. and 4.2.). Also tabulated is data supporting the manufacturer's claims for the action of thiabendazole against Oesophagostomum spp.

Davidson and Taffs (1965) state that thiabendazole may be safely given to pregnant sows soon before farrowing. This drug possesses a useful ovicidal property (Taffs and Davidson, 1967; Taffs, 1968c). This enables sows to be moved into disinfected premises as early as six hours after treatment. The main disadvantage of thiabendazole is that it is distasteful to a proportion of sows (Cairns and Hargreaves, 1966). The unpalatability noted in the present work was a consequence of the picadex rather than of the thiabendazole itself. Several farmers have reported verbally to the author that the thiabendazole-picadex mixture sometimes stimulates emesis. Newer formulations (Thiprazole Sow Wormer®) omit the piperazine salt thus rectifying these faults.

The other broad-spectrum anthelmintics appear to be

more palatable to pigs. Tetramisole, for example, is highly effective against Oesophagostomum and Hyostrongylus, although some field and laboratory trials have given disappointing results (Tables 4.1. and 4.2.). Good activity is shown against Ascaris and Metastrongylus, but not Trichuris (Walley, 1967). Slight signs of toxicity may sometimes be seen at 15-20 mg/kg. (Walley, 1967). This compound can be administered by injection as well as per os.

Dichlorvos in slow release formulation may be given to pregnant sows in massive overdoses without ill-effect as was shown in the present study. In addition to Oesophagostomum and Hyostrongylus, it is effective against Ascaris, Trichuris and Globocephalus but its performance against Strongyloides is variable. The present formulation is readily taken in meal or mash rations but its physical form (plastic granules) is not ideal for mixing with pelleted or cubed food.

Widespread use in the field has confirmed that

dichlorvos, thiabendazole and tetramisole are free from teratogenic and mutagenic influences.

At the time of writing, there is no published information, other than that supplied by Gitter, Gibson, Kidd and Davies (1966), concerning the properties of haloxon as a pig anthelmintic. Parbendazole is not yet sold for use in pigs but experimental work has shown promise (Table 4.2.).

It can be seen from this brief review that the new generation anthelmintics are a great improvement on their forerunners, but that the perfect chemotherapeutic agent has yet to be discovered.

4.3.2. Suppression of the Periparturient Egg-Rise

The nature of the periparturient rise and the potential role that it may play in the transmission of *Oesophagostomum* infections to suckling piglets has been discussed in Chapter 2. Fortunately, the present project has shown that the egg-rise can be completely eliminated by the administration of one dose of a suitable broad-spectrum

anthelmintic one week before the anticipated date of parturition. Identical results have been obtained by Ludvigsen (1967) who used a single dose of 14 or 28 g. thiabendazole per animal at the time that "pregnant sows are put into the farrowing pen". Thomas and Smith (1968) attempted a similar form of therapy. They treated with 50 mg/kg. body-weight at one month and one week before farrowing. The 'strongyle' egg-count was depressed by 73% when compared with controls (or 89% when compared with the pre-treatment values) but the control animals failed to show the typical periparturient rise.

Broad-spectrum anthelmintics have a variable effect on the 'spring rise' of sheep depending on whether the ewes are kept at grass or indoors (Dunsmore, 1965; Nunns, Rawes and Shearer, 1965; Brunsdon, 1966b and Connan, 1967b). Since sows are usually brought into a farrowing box before parturition, results from penned ewes provide the more analogous situation. Helle (1966) suppressed the rise with thiabendazole given at 75 mg/kg. or tetramisole at 20 mg/kg. given early in pregnancy. Connan (1967b) obtained similar

results with thiabendazole at 3 g. per ewe administered rather later in the period of gestation. If animals are at grass, effective control is obtained by a single dose provided four to six weeks after lambing. Such a regime would not be suitable for pigs as the periparturient rise is initiated even before farrowing takes place.

4.3.3. Enhancement of Piglet Productivity by Prenatal Treatments

The present trend in the British pig industry is towards specialization. Many large concerns restrict their activities to the production of piglets for sale as weaners which are sent to the corn-growing areas for fattening. The exchange price is calculated on a live-weight basis so that the litter-weight at weaning is a crucial factor affecting the livelihood of the pig breeder.

In the present study, the litters from the medicated sows were, on average, 11.0 lbs. heavier than those from the controls. The price given for weaners is around 2s. 6d. per lb. although this figure varies according to

supply and demand. Thus, treatment resulted in an increase in income of £1 7s. 6d. per litter at a cost of 2s. 3d. The animals under experiment were only lightly infested with gastro-intestinal helminths so that greater differences might be expected in the average herd.

The figures obtained were not statistically significant and it might be argued that the detected tendency, however obvious, was obtained by chance. It is, therefore, of interest to compare this study with those performed by other workers. Thomas and Smith (1968) used two groups of 15 sows that harboured a moderate infestation which included both Oesophagostomum and Hyostrongylus. At eight weeks of age, the average weight of the litters from the treated sows was 37.1 lbs. greater than that from the controls, but again these results were not statistically significant. Field observations by Davidson, Murray and Sutherland (1967) showed that the 56-day weights of piglets born to sows treated with piperazine on one farm averaged 36.5 lbs. during one farrowing period and 35.0 lbs. during the next. The following year, this therapy was replaced by the

thiabendazole-picadex mixture and the corresponding figures were 43.3 and 48.1 lbs. The deficiences in this experimental design are obvious, but it can be seen that all three investigations described in this section have given comparable results. Edwards (1965) and Nesvadba and Zavadil (1968), as a result of their experiences in the field also believe that prenatal treatments increase piglet productivity.

Nickel and Haupt (1964) experimentally infected growing swine with Oesophagostomum and observed profound clinical signs including anorexia and reduced weight-gains with doses of 20,000-90,000 larvae. In addition to these symptoms, Taffs (1966) recorded diarrhoea or faeces flecked with blood and mucus following a challenge with 30,000 larvae (Appendix 5, Table 12). Shorb (1948), too, observed diarrhoea and even death, whilst Prugelhof (1952) and Nesvadba and Zavadil (1968) observed severe retardation of the growth-rate of heavily infested weaners and porkers in the field. The view has been advanced that the invading oesophagostomes provide a

portal of entry for microbial pathogens such as Salmonella cholerae-suis (Kotlan, 1956; Nickel and Haupt, 1964), Pasturella spp. and Escherichia coli (Nesvadba and Zavadil, 1968).

Opinions concerning the influence of H. rubidus on growing pigs is divided. This worm does contribute to the periparturient rise (Connan, 1967a) but the number of larvae made available to the newly born piglets is comparatively small as a result of the low biotic potential of this genus. Clinical outbreaks of hyostrongylosis in young pigs have been recorded by Crocker and Biester (1920), Skrjabin and Bekenski (1925) and Clay (1938). Experimentally, Davenport (1967) was able to produce a significant depression of growth-rate by the administration of an unspecified number of H. rubidus larvae, whilst Porter (1940) did not find any difference in the weights of infected and worm-free control animals.

4.3.4. Additional Benefits of Prenatal Treatments

As well as the protection of piglets from the massive

onslaught of strongylate larvae resulting from the peri-parturient rise (vide Chapter 2), the prenatal treatment of sows also acts as a prophylactic measure against clinical parasitic gastro-enteritis in the dam herself. H. rubidus is capable of provoking a clinical picture resembling the 'thin sow' or 'fading sow' syndrome that has been recorded from many parts of Great Britain within the last few years. Stevens (1967) believes that a mis-interpretation of recommendations regarding modern husbandry and feeding methods have, in some cases, initiated a vicious malnutrition cycle in which gastro-intestinal parasitism plays a contributory role. Maclean (1968) incriminated helminthiasis in approximately one-third of the outbreaks that he investigated.

The significance of Oesophagostomum spp. infections in sows is less well defined. There are no well substantiated cases of clinical disease in sows where this worm has been the sole aetiological factor. Lamina and Bohnhardt (1964) believe that there is an association between heavy Oesophagostomum infections and

infertility in gilts. Gitter, Gibson, Kidd and Davies (1966) draw attention to the heavy worm burdens that are often present in herds with histories of unthriftiness, infertility and scouring. The interpretation of such data can, however, be the subject of much debate owing to the difficulty of differentiating between cause and effect. Melrose (1966) suggests that the depressed reproductive capacity might well be a sequel to the unthriftiness of heavily parasitized animals rather than a direct response to the presence of the worm. In Czechoslovakia, Nesvadba and Zavadil (1968) attribute nervous disorders, muscular weakness, agalactia and severe anaemia in sows to severe oesophagostomiasis. In western Europe, however, this genus must be regarded as almost innocuous for adult pigs although it may exacerbate outbreaks of the 'fading sow syndrome' or infertility in special circumstances.

In view of the suggestion that the gastro-intestinal strongylates may influence the fertility of breeding animals, it may be wise to treat sows before service i.e.

at about the time of weaning. Such a procedure would also be expected to minimize pasture contamination by pregnant stock. However, the periparturient rise is generally terminated soon after weaning by the spontaneous expulsion of Oesophagostomum (Chapter 2). If it can be shown that H. rubidus behaves in the same way, then this form of therapy may be superfluous. Connan (1967a) has shown that the egg-count of unthrifty sows often remains high after weaning, so in these particular instances the second dose would seem to be desirable.

Chemotherapy is no substitute for hygiene. Efforts must be made to keep buildings clean and more especially to prevent pastures becoming 'pig-sick'. Little is known of the ecology of the free-living stages of porcine oesophagostomes or H. rubidus in this country, but it is thought that the former, at least, can survive on pasture for ten months or more (Taffs, 1966). Thus, the resting of paddocks is of dubious value. It would be a better policy to plough and re-seed every second or third year. The transfer of the in-pig sows on Farm EC from grass to

concrete, along with single prenatal anthelmintic treatments, achieved a remarkable, albeit slow, improvement in the general condition of the herd (Chapter 2).

Clinical cases should be removed from the source of infection and should receive supportive therapy i.e. supplementary food enriched with extra protein, vitamins and minerals. Many of the worst cases will have developed abscesses. These are best slaughtered as the chances of a full economic recovery are slight.

4.3.5. Dichlorvos as an Anthelmintic for Sows

Suppression of 'Strongyle' Egg-counts:

After treatment, the 'strongyle' egg-counts fell to a very low level and remained depressed for a period of six weeks or more thereafter. The pattern of Oesophagostomum spp. egg production (Figs. 4.2. and 4.3.) seen during the two months following treatment is of interest. The fall shortly after treatment of over 98% is followed by a gradual increase which becomes more pronounced six weeks later. Perhaps the most satisfactory explanation is that

nearly complete Oesophagostomum expulsion, corresponding to the observed 98.5% worm elimination in the critical trial, is followed by the maturation of residual immature worms. Those already in the lumen might mature at any time during the five weeks after treatment, those in the histotropic phase in the following week. In addition, surviving mature worms would continue to ovulate, perhaps to an increased degree since small populations of this worm have been shown to have a higher individual production rate than large populations (Chapter 2). New infections acquired after treatment become patent and swell the egg-count six weeks after treatment, although some contribution may be made from the third week (Taffs, 1966, 1968a,c).

From the practical viewpoint, however, the important facts are that 17 of the 45 treated sows shed no Oesophagostomum eggs at all during the six weeks following treatment and that (with three exceptions at the lowest dose-rate) only very small numbers of eggs were excreted by those sows that did show patent infections (Appendix 4, Table 2).

Expulsion of Worms:

The critical trial demonstrated that between 94.6 and 100% of the total Oesophagostomum populations of three heavily infested sows were expelled from the host following treatment with 1.44 g. dichlorvos. Thus, it is reasonable to assume that the initial reductions in egg-counts following the administration of the anthelmintic were caused by the displacement of adult worms from their habitat rather than by the inhibition of ovulation.

Efficacy against H. rubidus:

Whereas Oesophagostomum is a prolific egg-layer, H. rubidus has a very low biotic potential (Connan, 1967a; Davidson, Murray and Sutherland, 1968). Hyostrongylus constituted such a small percentage of the total 'strongyle' egg-output from the sows under observation, that the egg-counts shown in Fig. 4.2. may be regarded as representing Oesophagostomum alone. Only qualitative results, therefore, have been presented for H. rubidus. Patent Hyostrongylus infections were carried by a number of the control animals throughout the greater part of the experi-

mental period. Larvae of this genus could also be cultured from the faeces of a proportion of the sows of the treated groups prior to the administration of the drug. After treatment with 1.44 g. or 1.96 g. dichlorvos, no further positive samples were detected during the following three weeks (the time taken for a new patent infection to be established from external sources; the prepatent period of H. rubidus being around 21 days). Thus, it would appear that dichlorvos is active against this nematode but this evidence is not conclusive. Further work is needed to confirm the present findings. Ikeme (1966) demonstrated 97-99% expulsion of H. rubidus in a series of critical trials after treatment with dichlorvos at 40 mg/kg. (Table 4.2.).

Optimal Dose-rate:

In the present study, three dose-rates were used: 1.15 g., 1.45 g. and 1.96 g. dichlorvos, these quantities representing one-fifth, one-quarter and one-third of the contents of one of the supplied packets, each of which contained 33 g. of formulated material. In practice, a

farmer rarely knows the weight of his sows and would be unwilling to follow an elaborate scheme relating dose to body weight. It is, therefore, necessary to simplify the dosage schedule as much as possible. The final recommendations will depend on three factors. Firstly, the biggest sow must receive an adequate amount of the product; secondly, the smallest sow must not be given a quantity approaching the toxic limit; thirdly, the price of treating the smaller animals must not be excessive.

On the basis of the egg-count data, there would appear to be no advantage in using 1.96 g. dichlorvos as equally good results were obtained by the administration of 1.45 g. A marginally poorer performance was, however, obtained when 1.15 g. was given. Three of 15 sows shed relatively large numbers of 'strongyle' ova soon after treatment at this level and larval culture revealed that control of H. rubidus was incomplete in some cases. The optimum dosage thus lies somewhere between 1.15 and 1.45 g.

Effect on 'Strongyle' Eggs:

The hatchability of the 'strongyle' eggs passed in the faeces of sows 24 hours after treatment was unaffected but there was a deleterious effect on the larvae that emerged from these. The number of eggs capable of producing infective third stage larvae was reduced by a factor of one-third. Since only a few Atgard pellets are being passed from sows at this time, the majority being eliminated after two or three days, eggs subsequently voided may have their infective potential much more greatly impaired. Nevertheless, until more information is available, it must be recommended that wherever possible, sows should be withheld from disinfected quarters for up to four days after treatment.

Application and Acceptability:

All the farms visited in connection with the recent studies fed meal rations. The Atgard mixed well with food of this nature and did not affect its palatability. One can envisage a slight inconvenience where pelleted

feed-stuffs are used, especially in the case of the large 'Jumbo' nuts. The anthelmintic pellets would fall between the food masses and accumulate on the floor of the trough. This can be easily overcome by crushing the nuts or substituting meal on the day that worming is to take place.

Toxicity:

No indication of toxicity was noted during the course of these trials. Acute intoxication did not occur even in those animals given a considerable overdose (66 g. formulated product). No harm was done to the unborn piglets.

Other Attributes:

It was shown in Chapter 3 that Oesophagostomum larvae can be disseminated by certain manure-breeding flies (Psychoda spp.). It is, therefore, of interest to note that dichlorvos is a powerful insecticide and that PVC formulations may retain this activity even when passed out in the faeces of treated cattle (Lloyd and

Mattysse, 1966). It is probable that psychodid flies are unable to breed in the faeces of pigs recently wormed with Atgard, and are thus unable to spread infection in this way.

Conclusion:

Dichlorvos in slow release formulation can be included in the list of products suitable for the anthelmintic treatment of sows on the basis of its low toxicity and spectrum of activity.

Chapter 5

STUDIES OF PROTEIN METABOLISM IN PIGS EXPERIMENTALLY INFECTED WITH *OESOPHAGOSTOMUM* OR *HYOSTRONGYLUS*

INTRODUCTION

Hypoalbuminaemia has been noted as a sequel to infections with the nematode genus Oesophagostomum in sheep (Dobson, 1967) and in cattle (Bremner, 1966). In experiments using ^{51}Cr-labelled protein molecules, Bremner (1968) has demonstrated that, in the case of Oe. radiatum infection in calves, decreased serum albumin levels are associated with an abnormally large loss of plasma protein into the gastro-intestinal tract.

Similar variations from the physiological norm are encountered in bovine and ovine ostertagiasis (Mulligan, Dalton and Anderson, 1963; Jarrett, 1966). Taxonomically, the genera Ostertagia and Hyostrongylus are very closely related and the predilection sites in their respective hosts are similar, as are the pathological responses they elicit (Davidson, Murray and Sutherland, 1968). By analogy, therefore, it might be anticipated that the physiological responses of the pig to hyostrongylosis would be similar to that of the ruminant to ostertagiasis. Nielsen (1966a), however, was unable to demonstrate hypoalbuminaemia or accelerated protein losses in heavily

infested sows.

It is generally believed that pigs carrying heavy strongylate infections have a slower growth-rate than worm-free swine (Davidson, Murray and Sutherland, 1967). In view of the ruminant work outlined above, it seemed possible that this retarded development might be a consequence of a similar strain on the host's protein reserves. Experiments were therefore designed to test this hypothesis.

Firstly, serum samples were examined to establish whether or not hypoalbuminaemia is a sequel of Oesophagostomum infections in pigs. The liver, however, has an enormous anabolic potential and can compensate for albumin losses up to a threshold level, so that a 'protein leak' can occur without obvious depletion of the intravascular pool. Methods reviewed by Jarnum (1963) for the detection of hypercatabolism in humans, and adapted for veterinary use by Professors Mulligan (Glasgow) and Nielsen (Copenhagen), were therefore adopted when results from the first study proved to be inconclusive.

Thus, turnover studies were performed by the introduction of serum albumin labelled with a radio-isotope into the circulating blood of worm-free and parasitized pigs. The distribution of radio-activity throughout the body and the rate of excretion enabled a series of physiological parameters to be measured and compared. Finally, the rate of passage of macromolecules into the gastro-intestinal tract of pigs harbouring Oesophagostomum or Hyostrongylus and normal control pigs was estimated by a technique involving the intravenous injection of a synthetic compound, ^{131}I-polyvinyl pyrrolidone.

MATERIALS AND METHODS

5.1.1. Animals Used

Worm-free twelve-week old Large Whites were purchased from Messrs. George Pinkerton Ltd., Houston, Renfrewshire. Litter-mates were used for the infected and control groups in each experiment. Castrated males were used wherever possible since this facilitated the separation of urine and faeces, although on one occasion a female had to be included in order to make up the number required. Autopsies on control animals confirmed that the pigs had been raised under worm-free conditions.

The worm-free pigs used in Experiment 5.1., also Large Whites, had been purchased from Research and Advisory Services (Agriculture) Limited, Wimborne, Dorset.

5.1.2. Experimental Designs

Experiment 5.1.

In order to study the effects of a heavy Oesophagostomum challenge on the main protein constituents of the serum, blood samples from four pigs infected on a single occasion with 30,000 larvae were taken at weekly intervals from the

8th week before, until the 16th week after infection. This project was undertaken in collaboration with Dr. L.F. Taffs of the Animal Health Trust who collected the samples and sent them to this laboratory for examination. The parasitology and clinical responses of the hosts have been described elsewhere (Taffs, 1966).

Experiment 5.2.

Three pigs were infected on the same day with 10,000 Oesophagostomum spp. larvae, and each was paired with an uninfected litter-mate control. Each couple were given ^{125}I-albumin solution by intravenous injection according to the following schedule:

Pig No.	Isotope given	Age of infection monitored
1234 (infected) 1233 (control)	4 days before infection	0 - 16 days
1237 (infected) 1235 (control)	14 days after infection	17 - 35 days
1238 (infected) 1236 (control)	32 days after infection	36 - 55 days

Three to four days are required for the labelled albumin to be distributed through the intra- and extra-vascular pools and for an equilibrium to be attained. Thus, the isotope was injected a corresponding time before the parasitic infection had reached the required age. Porcine oesophagostomes become fully mature at about 42 days after infection, and so the experiment spanned the whole parasitic life-cycle of this nematode. The moderate infecting dose of 10,000 larvae was chosen, though this would result in heavier burdens than are normally seen in the field, since no diarrhoea would be provoked to complicate the interpretation of the results. The total urine and faeces output of each pig were collected daily. Blood samples were taken ten minutes after injection, then twice daily for three days, once daily for a further two days and thereafter once every second day.

Experiment 5.3.

In this study, two pigs were each infected with 15,000 Oesophagostomum larvae. The infecting dose had

been raised by 50% in order to exaggerate any patho-physiological lesion, whilst still keeping below the number that would stimulate a loosening of the faeces. These experimental pigs, together with the three worm-free litter-mate controls, were given ^{131}I-polyvinyl pyrrolidone (^{131}I-PVP) solution by intravenous injection 15 days after infection.

PVP is a synthetic compound with an average molecular weight of 40,000. The amount of labelled material appearing in the faeces is a good indicator of the rate of passage of plasma macromolecules into the lumen of the alimentary tract. The timing of the injection was based on the work of Bremner (1968) who states that there is a marked increase in the protein catabolism of calves infected with Oe. radiatum from the 20th day after infection.

Faeces, urine and plasma samples were collected daily until the radio-activity of the faeces fell below the level of significance (vide infra) on the 13th day

after injection. This experiment therefore spanned the period from the 15th to the 27th days post-infection.

Experiment 5.4.

This was conducted in a manner similar to that in Experiment 5.3. but was based on infections with Hyostrongylus rubidus. Three pigs were infected with 2,400, 2,400 and 1,600 larvae respectively, and two kept as non-parasitized controls. The ^{131}I-PVP was injected intravenously on the 38th day after infection. The parasitic development of H. rubidus is normally completed in 21 days, and so the monitored worm population was composed of adult worms. The numbers administered to each pig were governed only by the availability of larvae.

The radio-activity of the consecutive daily plasma samples fell more rapidly in this experiment than in the last, and observations were only possible over a six-day period.

5.1.3. Housing and Feeding

Housing proved to be the factor that limited the scale of the investigations. Before the pigs were made radioactive they could be kept in traditional loose-boxes. Thereafter, the accommodation had to be such that the urine and faeces of each animal could be totally and separately collected in a manner that precluded contamination of the environment. For this purpose, Shinfield metabolism crates were constructed (Allen, Barber, Braude and Mitchell, 1963). These allow the pigs to be kept singly in a confined space so that they are unable to turn around. A mesh floor is used so that the faecal material collects at this level while urine flows through to be channelled by a urine-tray into a bottle. If male animals are used, the faeces will not become contaminated by urine as the latter is directed forwards (away from the dung collecting area).

In order to saturate the thyroid gland with iodine to ensure that the tracer would not accumulate at this site and that the isotope would be rapidly excreted follow-

ing catabolism of the labelled protein, a wet mash was made up by mixing a standard commercial growers meal with a 0.005% aqueous solution of sodium iodide to form a porridge-like consistency. Enough was offered to satisfy the hunger of each pig at a single meal. If the pigs became thirsty at other times of the day, further amounts of iodide solution were given. This regime was commenced at least five days before the introduction of the radio-iodine.

5.1.4. Larval Culture and Administration

Third stage infective Oesophagostomum larvae were produced by the method described in Chapter 3. The pure culture of H. rubidus was kindly supplied by Dr. D.W. Brocklesby of the Agricultural Research Council Laboratories at Compton.

The larvae were given to the pigs by mixing each inoculum with a 50 g. sample of pig meal. The treated food was offered to individual pigs in a plastic bowl held by the operator. As the pigs had been starved for

12 hours beforehand, their appetite was guaranteed. In this way, the dose could be quickly, surely and easily administered.

5.1.5. Preparation of Injections

I am indebted to the staff of the Department of Veterinary Physiology of the University of Glasgow who prepared and labelled the porcine albumin solutions. Blood was collected in sterile containers from pigs slaughtered at the Corporation of Glasgow Abattoir or at the Veterinary School. After clotting had taken place, the serum was pipetted off and centrifuged to remove the small numbers of erythrocytes still in suspension.

Pure albumin was recovered from the serum in two different ways. The first method involved the use of anhydrous sodium sulphate which precipitated the globulins (Appendix 5, Table 1). Better yields were, however, obtained by passing the serum through a four-metre Sephadex G100 column which separated the plasma proteins on the basis of their molecular weight. The

purity of the preparation was tested by electrophoresis.

A ^{125}I-tracer was purchased from the Radiochemical Centre, Amersham, in the form of radio-iodide, free of reducing agents. The ^{125}I was bound onto the tyrosine group of the albumin molecule by the method of McFarlane (1958) as described fully in Appendix 5, Table 2. The resultant solution was dialysed to remove unbound iodine, and more albumin added to reduce the activity to below 5 μc/mg. in order to minimize radiation decomposition. The 125 isotope of iodine was chosen as its relatively long half-life (60 days) enables observations to be continued over considerable periods.

The PVP was in the form of iodinated polyvinyl pyrrolidone-I^{131} injection as produced by the Radiochemical Centre. In this case, the 131 isotope was used as it was anticipated that a high proportion of the PVP would have disappeared from the body within days of its administration, and thus the shorter half-life (8 days) would be adequate.

Intravenous injection was performed via an ear-vein. Blood samples were, thereafter, taken from the opposite ear for at least a week. Each pig was sedated with a 5% solution of chlorpromazine hydrochloride (Largactil, May and Baker; 2 mg/kg. body weight), given intramuscularly one hour before the manipulation, then lightly anaesthetized by the intraperitoneal injection of pentabarbitone sodium (Nemutal, Abbott; $1\frac{1}{2}$ ml/lb. body weight). The intravascular route was avoided so that the ear-veins could be kept intact. If necessary, the anaesthesia was deepened by the administration of ether through a face mask. Deep narcosis was deliberately avoided as pigs are very susceptible to pulmonary oedema and should, therefore, be encouraged to regain consciousness as speedily as possible. Despite these precautions two pigs were lost in this way. These have been excluded from the present report, and account for the uneven distribution of animals between the treated and control groups in Experiments 5.3. and 5.4.

5.1.6. Collection of Samples

Faeces:

These gathered on the grid floor, and were collected once a day into plastic bags for transport to the laboratory. Care was taken to separate 'clean' faecal material from that known, or suspected to be contaminated with urine. As a check a small sample of dung was also taken directly from the rectum. The consistency of the dung was also noted.

Urine:

This flowed into a polythene bottle that could be closed with a watertight screw-on cap.

Blood:

Though the ideal method of collecting blood from the pig is by puncture of the anterior vena cava, because of the number of handlers necessary for this procedure, the writer was obliged to use the simpler but somewhat less satisfactory method of puncture of an ear vein. The samples were taken by inflicting a small stab-wound with

a number 24 scalpel blade and allowing the exuding blood to run into a tube containing sequestrine, or, in appropriate cases, into an untreated, sterile bottle. This procedure worked reasonably well as the pigs were confined to a small area, and would stand still if given a wad of cotton-wool to chew.

Worm Counts:

Where ^{131}I had been used, the worms were isolated, identified and counted at autopsy by the methods described in Chapter 1. In the experiments using ^{125}I, there was a remote possibility that the radio-activity of the pigs might have been still too high to allow this procedure, and therefore a vermifuge was administered to eject the worms from the host. The anthelmintic used was dichlorvos (Atgard, Shell International Chemical Company) at a dose rate of 40 mg/kg. Experiences related in Chapter 4 had shown that virtually total expulsion of Oesophagostomum populations occur after dichlorvos therapy. The worms that appeared in the faeces were intact and could be easily counted.

5.1.7. Preparation of Radio-active Samples

Faeces:

The total weight of the 24 hour sample was measured. The uncontaminated material was spread over a large tray, and small pieces were chosen at random from all parts of the surface and placed on the pan of a balance. When five grammes had been taken this was transferred to a lipless test tube and the material compressed into the bottom of the tube to the exclusion of any trapped air. This process was repeated with a second five gramme aliquot and a third was made up from the rectal sample.

Urine:

The volume of the 24 hour sample was measured, and the whole thoroughly mixed. Three five-millilitre portions were pipetted out.

Blood:

The sequestrinated whole blood samples were centrifuged for 15 minutes at 2,000 r.p.m. This sedimented the corpuscular elements of the blood,

leaving the plasma which was pipetted off. A 0.5 ml. portion was placed in the counting tube, and made up to 5.0 ml. with water containing a small quantity of sodium hydroxide to prevent precipitation of the proteins.

Standards:

When measuring the radio-activity of the samples, allowance had to be made for the decay of the isotope. This objective was achieved by preserving a portion of the labelled preparation intended for injection into the animal. This was diluted with a weak solution of sodium hydroxide so that the activity of a five-millilitre aliquot approximated that anticipated for the plasma during the experiment.

5.1.8. Examination of Samples

The measuring tubes were of such a size that they fitted into the well of the sodium iodide crystal of the scintillation counter (Ecko Electronics Ltd.). Five-millilitre samples were used in order to exploit the equipment under conditions known to excite its maximum efficiency. The crystal is sensitive to gamma emissions

and converts each into a light impulse. These are detected, magnified and passed through a discriminator, which 'filters out' all but the selected energy band, before being counted (Ecko model scaler M5200 and timer M5220). The majority of observations were made over a 30 second period and any counts less than three times the background were considered insignificant.

Background Count:

This is the natural radio-activity of the environment about the sodium iodide crystal. This was protected by a lead shield and so this figure was very small. The background was ascertained before any other measurements were made. It was repeated at intervals throughout the period that the apparatus was in use in order to ensure that the well had not become contaminated.

Standard Count:

After the background count had been monitored, the standard preparation was examined. Two separate readings were taken, and this procedure was repeated several times

during each series of observations to ensure that the sensitivity of the equipment remained constant.

Samples:

The preliminary readings having been performed, the sample tubes were scanned one by one, two counts being made on each.

5.1.9. Protein Estimations

The total serum protein values were measured by the biuret reaction (Weichselbaum, 1946). Duplicate samples of 0.1 ml. serum were added to 4.9 ml. of 0.85% sodium chloride solution. This was mixed with 5.0 ml. of biuret reagent. A blank was made up with 5.0 ml. salt solution and an equal volume of biuret. 'Versitol', a commercial preparation containing 4.3 gms. per cent protein, was used as a standard for calibrating the spectro-photometer (Unicam S.P.500) employed for reading the intensities of the resultant solutions.

Separation of the albumin and globulin fractions was effected by cellulose acetate electrophoresis using 'Oxoid'

strips across a Shandon electrophoresis tank (Kohn and Feinberg, 1965). The strips were floated on the surface of the buffer solution (Appendix 5, Table 3) which was drawn into the material by capillary action, then totally immersed and blotted. The strip was then laid across the supports on the tank, the ends covered by pads dipping into the reservoir of buffer solution. A small drop of the serum was placed across the width of the coarse upper surface of the strip at the cathode end. A constant voltage of 150 v. was applied across the strips for one hour. The lid of the tank was replaced during this time to minimize evaporation. The rate of migration of the different protein molecules is dependent upon the charges they carry and the procedures outlined above would, therefore, serve to separate the constituents of the serum samples. The strips were dried in hot air, stained with purified Ponceau S, washed with 5% acetic acid and the colour intensity of each protein band read by means of a 'Chromoscan' (Joyce, Loebl and Co.).

5.1.10. Disposal and/or Treatment of Radio-active Carcasses, Samples and Equipment.

The two pigs that died immediately after the administration of the isotope were buried six feet underground. At the termination of the experiment, very little activity remained in those given ^{131}I, and so these could be used for the production of meat-and-bone flour for agricultural use in the normal way. The pigs receiving ^{125}I were kept for a further two months to ensure that virtually all the activity had been excreted or had decayed before being subjected to the same fate.

The faecal material contained only very small amounts of activity and could be discarded through the usual channels without delay. The urine and blood were often highly active and were kept in store for a period of weeks until such times as the radio-isotope had decayed to negligible amounts. The fluids were then flushed, a little at a time, into the drain together with large quantities of water.

Wherever possible disposable apparatus was used. This was kept to one side after use until natural decontamination had taken place and then discarded. Other items such as pipettes were thoroughly cleaned, then stored until their activity was low enough to allow re-use.

5.1.11. Calculation of Results

Serum Proteins:

Electrophoresis and scanning gave the relative proportions of albumin and globulin. The latter could be further sub-divided into the alpha, beta and gamma constituents by this technique, but this was irrelevant for the present study. The albumin:globulin ratio (A/G) could be calculated from these data, and the absolute quantities of each per unit volume of serum could be obtained from the total serum protein results by proportion.

Isotope Studies - General:

Correction factors

The background activity was measured directly and expressed as counts per 30 or 100 seconds as appropriate.

The decay factor was calculated by dividing the original standard count by that taken at the time of examination.

Computation of Readings:

Plasma

The average was taken of the two counts made on each sample. From this value was subtracted the background count, and the result multiplied by the decay factor to give the figure that would have been registered if no radio-active decay had taken place. This was compared with the corresponding reading for the ten minute sample, and expressed as a decimal fraction.

Urine and Faeces

The mean of the averages of the three pairs of results for each collection were taken, and allowances made for background and decay. Further corrections were made to convert the figures to counts per millilitre of urine, or per gramme of faeces, per second. Lastly, consideration was taken of the size of the daily sample to give the total activity excreted over a 24 hour period.

^{125}I-albumin Calculations:

An example of these manipulations is given in Appendix 5, Tables 5 and 6.

Total injected activity

The total activity of the material introduced into each pig was easily calculated as known weights of the albumin solution were used for the injections (Appendix 5, Table 4) and for the preparation of the standard. The activity of the latter was monitored and the amount of labelled albumin given to each animal could, thereby, be quantitated in terms of counts per second:

Standard aliquot contains a g. albumin solution and produces n counts per second.

Thus, 1 g. solution gives $\frac{n}{a}$ c./sec.

Weight of albumin solution injected into the pig = (weight of syringe when full - weight of syringe after injection) = b g.

Thus, total injected activity = (b $\frac{n}{a}$) c./sec.

Plasma volume (Vp)

This is calculated by the application of the dilution principle. The total injected activity is known (vide supra), and the count-rate per unit volume of the ten minute plasma sample was measured. Within the ten minutes after injection, the labelled albumin will have become thoroughly distributed throughout the intra-vascular pool but very little will have entered the extra-vascular pools. Thus, the plasma volume expressed in terms of millilitres per kilogramme body weight may be estimated from the degree of dilution of the original solution that has taken place in this time:

$$\text{Vp (ml/kg.)} = \frac{\text{Total injected activity (c./sec.)}}{\text{Ten minute plasma activity (c./sec./ml.)} \times \text{Body weight (kg.)}}$$

Intra-vascular albumin pool (CA)

The quantity of albumin circulating in unit volume of serum was measured by the biuret reaction (vide supra), and this enabled the following step to be taken:

$$\text{CA (g/kg)} = \text{Vp} \times \text{Serum albumin concentration (g/ml.)}$$

Curve Analysis - Sterling (1951) Method:

Total body albumin pool (TA)

A graph is drawn of the plasma activities, expressed as decimal fractions of the ten minute sample result, on a semi-logarithmic scale against time (Fig. 5.1.). The first part of the curve falls rapidly as the equilibrium is established between the intra- and extra-vascular pools. Thereafter, the gradient decreases and merges into the second component of the curve, which is a straight line representing only the loss of label from the body.

The exponential part of the disappearance curve is extrapolated back to the time of injection. The intercept is given the symbol c, and this factor represents the relative distribution of albumin in the two main pools at the time that the plasma volume was measured. The total albumin pool is therefore obtained from:

$$TA = \frac{CA}{c} \text{ (g/kg.)}$$

Extra-vascular albumin pools (EA)

This value is simply the total body pool less the intra-vascular component:

$$EA = TA - CA$$

Catabolic rate

This can be expressed as the apparent half-life of the injected albumin after a state of equilibrium has been attained, i.e. by analysis of the exponential part of the curve.

Curve Analysis - Matthews (1957) Method:

Again the exponential part of the curve is extended backwards to the time of injection. At chosen points of time, the value indicated by the extrapolated line is subtracted from the corresponding figure on the curve obtained from the experimental observations. The results from this manipulation are also plotted on the graph (Fig. 5.1.). In all cases, a straight line was formed.

Albumin metabolism is thought to be a function of the intra-vascular compartment, and therefore the fractional catabolic rate (K) may be expressed as the fraction of the intra-vascular albumin pool degraded in a 24 hour period. This figure is obtained from the following formula (Matthews, 1957):

$$K = \frac{1}{\frac{c_1}{b_1} + \frac{c_2}{b_2}}$$

where c_1 and c_2 are the points of intersection on the zero time axis of the two straight lines, and b_1 and b_2 are the gradients of the two curves calculated thus:

$$b = \frac{\text{Natural log. of 2}}{\text{half-life in days}} = 0.693/t_{\frac{1}{2}}$$

Catabolic Rate - Campbell's Method (Campbell, Cuthbertson, Matthews and McFarlane, 1956)

Assuming once again that albumin catabolism is a function of the intra-vascular compartment, the fractional catabolic rate will be the proportion of the total plasma activity excreted in the urine and faeces during a 24 hour

period, or, expressed mathematically:

$$\text{Fractional catabolic rate} = \frac{\text{Total urine and faeces activity}}{\text{Total circulating activity}}$$

i.e. $$K = \frac{U + F}{Qp \times Vp}$$

where Qp is the counts per ml. plasma at the time that the excretory materials were produced, and Vp is expressed in mls.

The absolute degradation rate is the weight of albumin metabolized per day, which is given by: (K x CA) g/kg./day.

^{131}I-PVP Calculations:

An example of these calculations is given in Appendix 5, Table 7.

Total injected activity

This was calculated from the weight of the solution injected (Appendix 5, Table 4) and the activity of the standard as described above for the albumin turnover experiment.

Apparent plasma half-life

The plasma activities, expressed as a percentage of the original count taken after 24 hours, were plotted on a semi-logarithmic scale against time. In every case, the points fell along a straight line, and thus the apparent half-life could be read off (Fig. 5.2.).

Faecal 'plasma clearance'

Some activity was excreted in the faeces each day. The faecal 'plasma clearance' is the volume of plasma that would have contained that amount of ^{131}I-PVP. It is a convenient method of quantifying the magnitude of passage of this macromolecule into the gastro-intestinal tract for comparative purposes.

Faeces collected during a 24 hour period contain PVP that had entered the digestive tract some time previously. The faecal activities were therefore compared with the plasma count-rates for the previous day:

$$\text{Faecal 'plasma clearance' (ml.)} = \frac{\text{Faecal activity (c./sec./24 hour sample)}}{\text{Plasma activity (c./sec./ml.)}}$$

Total excretion in urine and faeces

The total excreted activity appearing in the urine and faeces was recorded as percentages of the injected dose.

RESULTS

5.2.1. Experiment 5.1.

The total serum protein values, A:G ratios, absolute globulin and albumin concentrations for each pig are displayed pictorially in Figs. 5.3. - 5.5. and are tabulated in Appendix 5, Tables 8-10. No obvious drop in albumin levels occurred in pigs P173 or P176. Low values were recorded from P175 for the three weeks immediately after infection and a similar, but a less well defined response was seen in P174. The latter pig showed a reduction in total serum protein concentration at this time, but otherwise no obvious abnormalities were displayed in these or in the absolute globulin results.

The clinical histories of these pigs as recorded by Taffs (1966) are summarized in Appendix 5, Table 11. The salient features are that P175 was the worst affected, being diarrhoeic from the 5th to the 29th day after infection, and that P176 showed few signs attributable to the parasitic burden it carried, whilst the responses of P173 and P174 were intermediate. The numbers of worms recovered varied from 1,942 (P174) to 6,344 (P175).

5.2.2. Experiment 5.2.

This project was intended as a pilot study, and the data presented should be interpreted with this in mind. One pair of animals was used for each phase of the investigation. The results thus obtained cannot be compared with confidence as no indication was given of the variation in values to be expected within populations of infected and worm-free pigs. Thus, no significance can be read into the absolute values obtained, but these are recorded as certain trends were suggested. One definite conclusion could be made: the rate of albumin catabolism in the parasitized pigs was not grossly accelerated.

One other important factor must be taken into account when considering the results of this experiment. This is the fact that the pigs used for these investigations were not sexually mature but were still growing rapidly. Thus, the weight of the pigs increased by up to 30% during the time that they were under test (Table 5.1.). This implies that the body pools were expanding, and consequently that

the residual labelled albumin was continuously being diluted. Thus, the gradient of the plasma disappearance curves will be too steep and all values that are calculated by graph analysis (EA, $t_{\frac{1}{2}}$, K^m and K^c - Table 5.2.) will be subject to error. These results are, therefore, suitable only for comparative purposes, and should not be regarded as absolute physiological values.

Each pair of animals had reached a different stage in their life-cycle when the isotope was injected. This explains the variations between groups seen especially in plasma volume values (Table 5.2.).

When purchased, the pigs, although not identical, were very evenly matched. They were divided into groups by means of random figure tables. On examination of the data in Table 5.1., it can be seen that the terminal weights of the experimental subjects was invariably lower than the initial values for the replacement animals. The growth-rate of the pigs in the metabolism crates was slower than that of their litter-mates kept in the conventional manner. This difference is probably accounted for by

temperature, since the pigs in the metal metabolism cages lacked straw and were unable to huddle together in groups. The infected animals of phases one and two of the experiment had a slower growth-rate than their normal companions. In the last study, the parasitized pig matched the control so that their weights were comparable 55 days after infection. No parasitized pig showed any sign of diarrhoea.

No worm-count was attempted at the termination of the first phase of this study because of the technical difficulties involved in recovering larvae from large quantities of faecal material. Worms 35 days old or more are easier to find and count.

In each study, the figures for the serum albumin concentration and extra-vascular albumin pool size were smaller in the infected animals than in the controls, whereas the opposite was true of the intra-vascular pool size. The plasma volumes were also larger in the parasitized pigs except in the first phase of the experi-

ment when they were equal. As mentioned above, these comparisons are not necessarily valid.

The fractional catabolic rates were identical in the parasitized and normal animals. There was, therefore, no increase in the albumin turnover rates of the pigs carrying heavy Oesophagostomum burdens. The apparent plasma half-lives were shorter in the infected subjects, but again these results are not statistically satisfactory and may have been coincidental.

5.2.3. Experiment 5.3.

Growing pigs were also used for this experiment and the next (Experiment 5.4.). The results are therefore subject to the qualifications outlined in the previous section. Larger numbers of experimental animals were used, so that, although still short of the statistical ideal, more meaningful comparisons could be made (Table 5.3.).

As in the previous experiment, the infested pigs had lower serum albumin values than did the non-parasitized animals. Control pig No. 1748 had an exceptionally high

reading. This animal developed a mild diarrhoea of unknown (non-parasitic) origin during the experimental period, and this resulted in a marked shortening of the plasma half-life and a substantial increase in the faecal output of ^{131}I-PVP. No such differences were revealed when the corresponding figures for the non-diarrhoeic controls and the parasitized animals were contrasted. No loosening of the faeces was observed in the latter group.

5.2.4. Experiment 5.4.

The numbers of adult Hyostrongylus recovered from the mucosal surface of the stomachs were 442, 278 and 353 respectively, i.e. 18, 12 and 22% of the numbers of larvae that were administered (Table 5.4.). No sign of diarrhoea was seen in any animal during the course of this experiment.

Although there were variations from pig to pig, the results show that a slightly greater amount of radioactivity was passed in the faeces of the parasitized animals than in that of the controls. The accumulative

'plasma clearance' over a six day period was 80.0 ml. per pig for the controls, and 114.7 ml. per pig for these carrying H. rubidus. This represents an increase of 43%. The same results expressed as the percentage of the total injected dose excreted in the faeces are: worm-free, 0.999%; infected, 1.153%; increase, 15.4%. Only one pig had an appreciably shortened half-life (No. 1865).

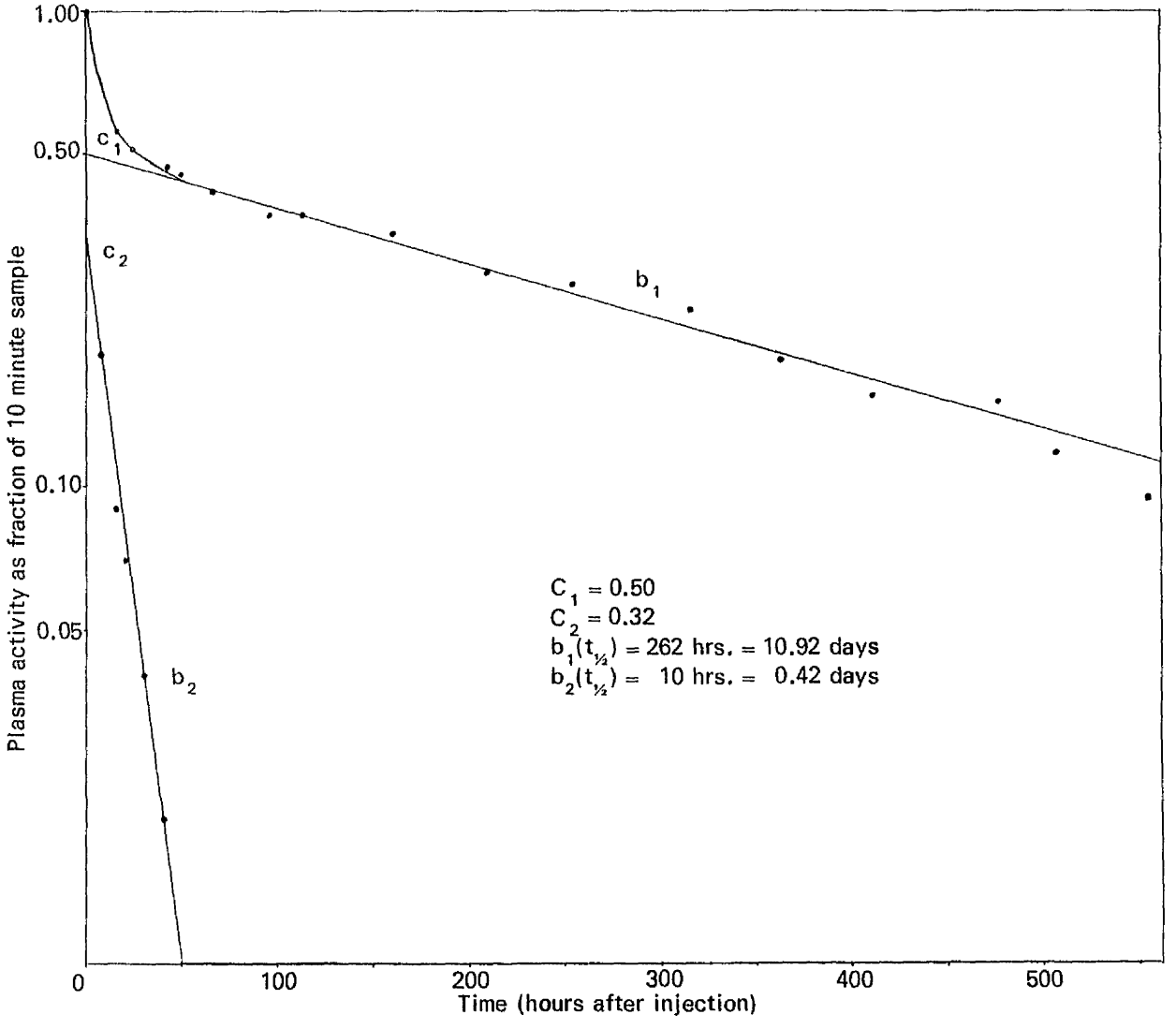


Fig 5.1 The rate of disappearance of radio-activity from the plasma of Pig 1233 and the Matthews method of curve analysis (*vide* Section 5.1.10. and Appendix 5 Table 5)

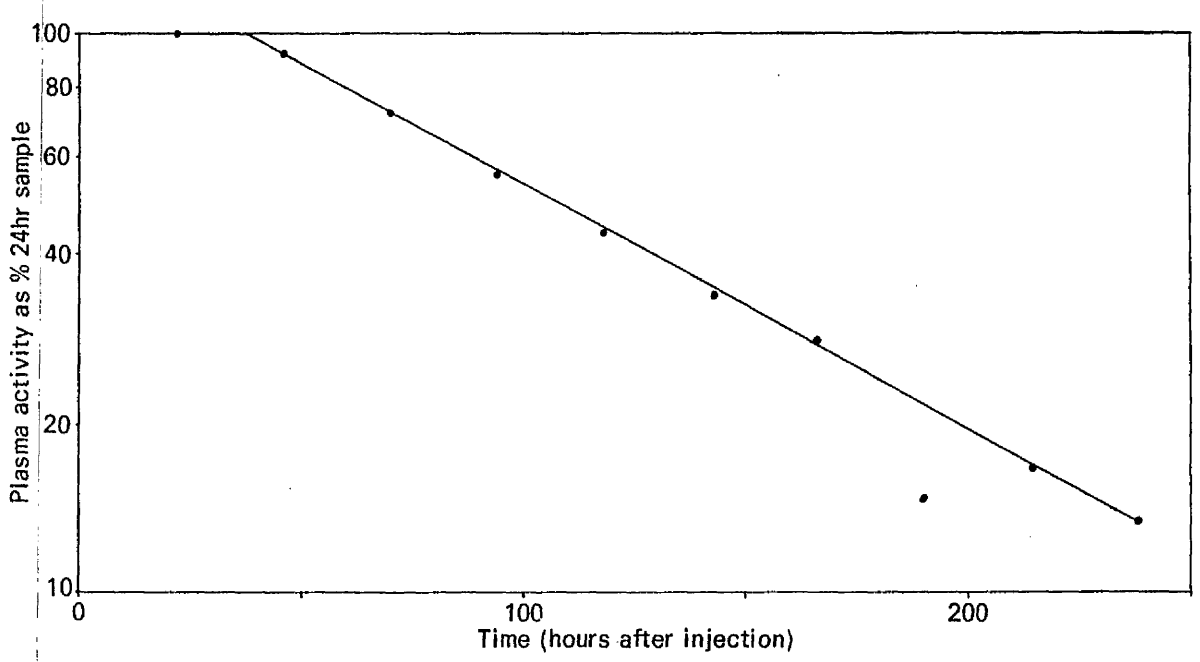


Fig 5.2 The rate of disappearance of ^{131}I-PVP from the blood of Pig 1871

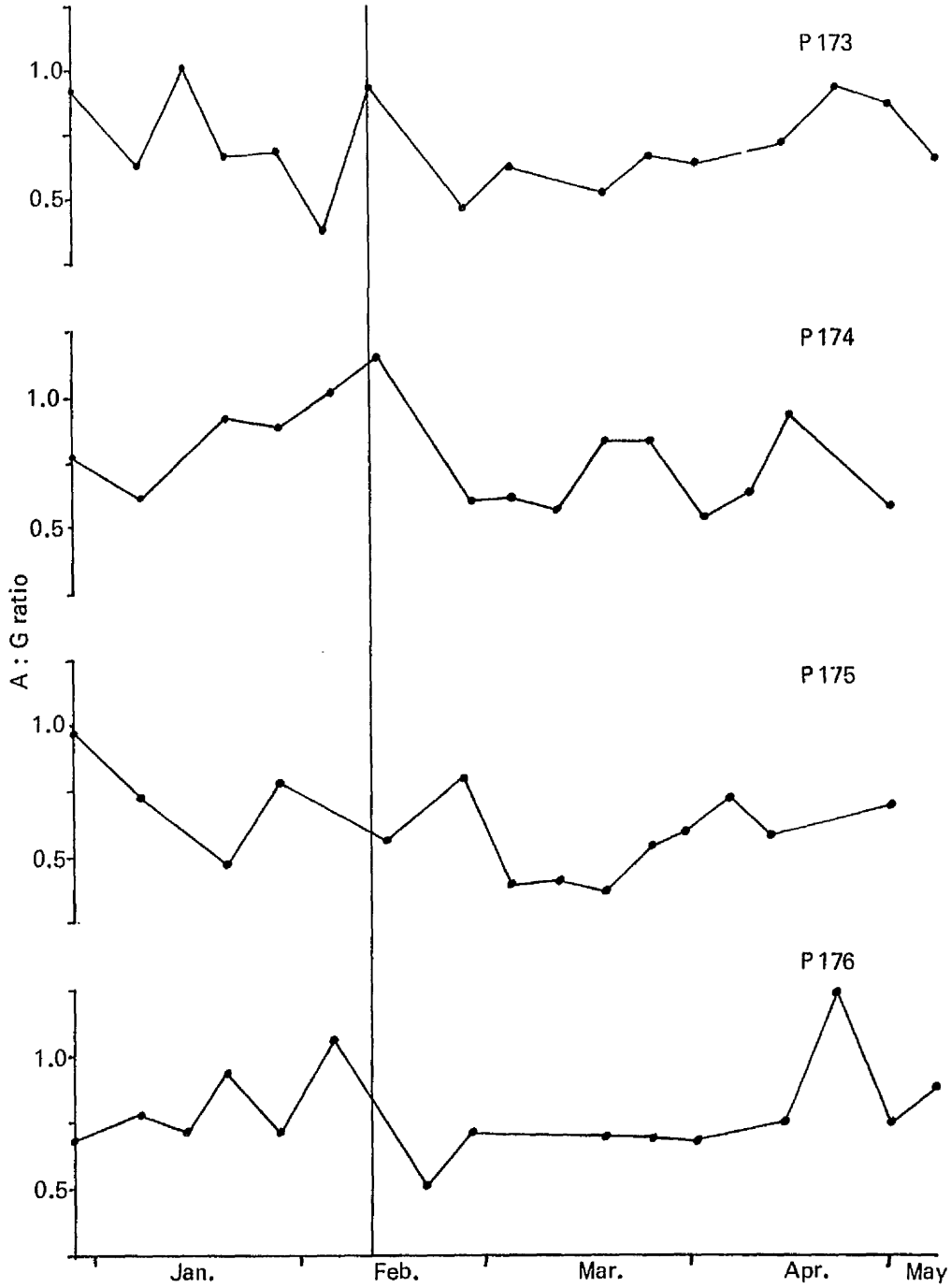


Fig 5.3 The albumin : globulin ratio of four pigs infected with 30,000 *Oesophagostomum* larvae on February 12th.

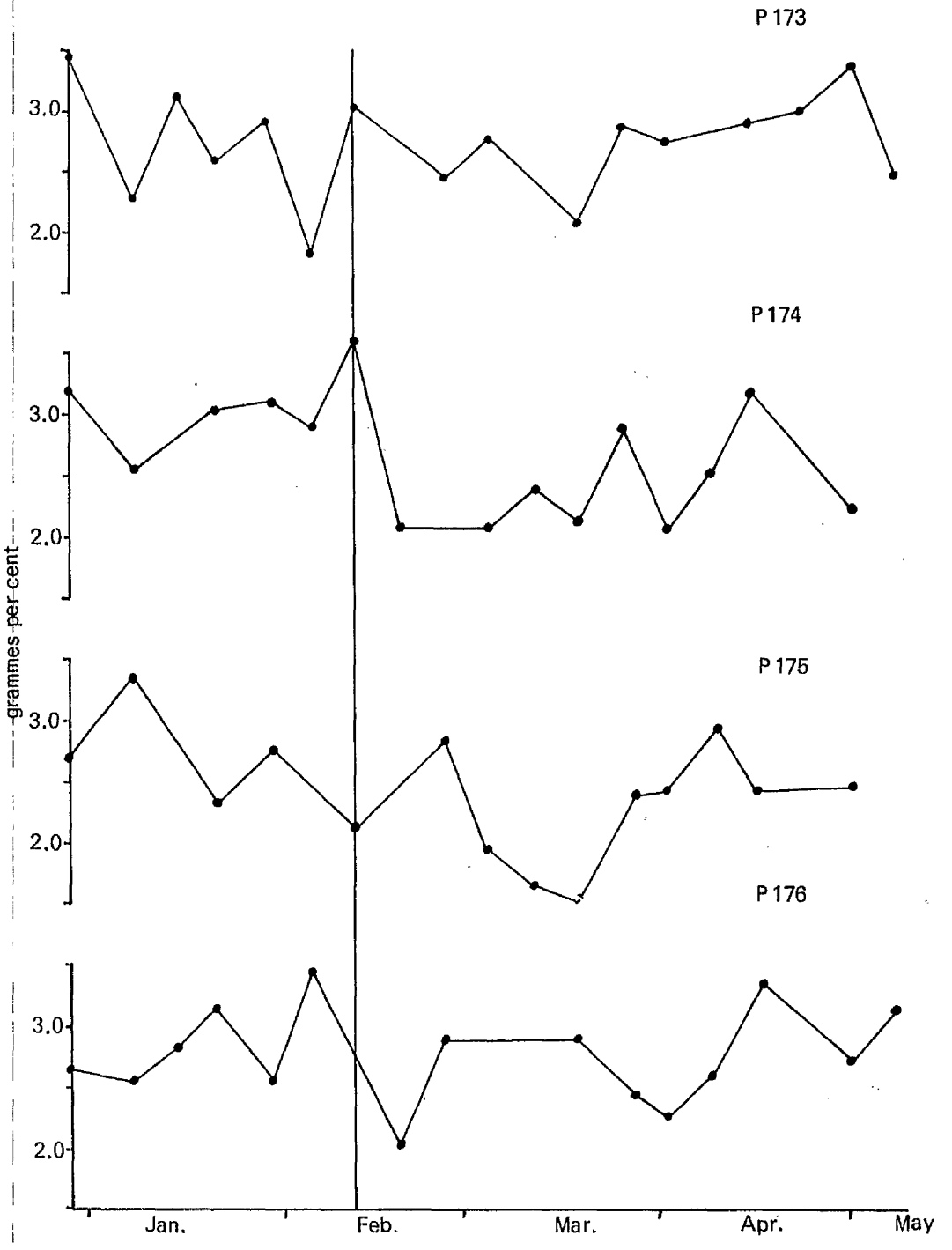


Fig 5.4 Serum albumin values of four pigs infected with 30,000 *Oesophagostomum* larvae on February 12th.

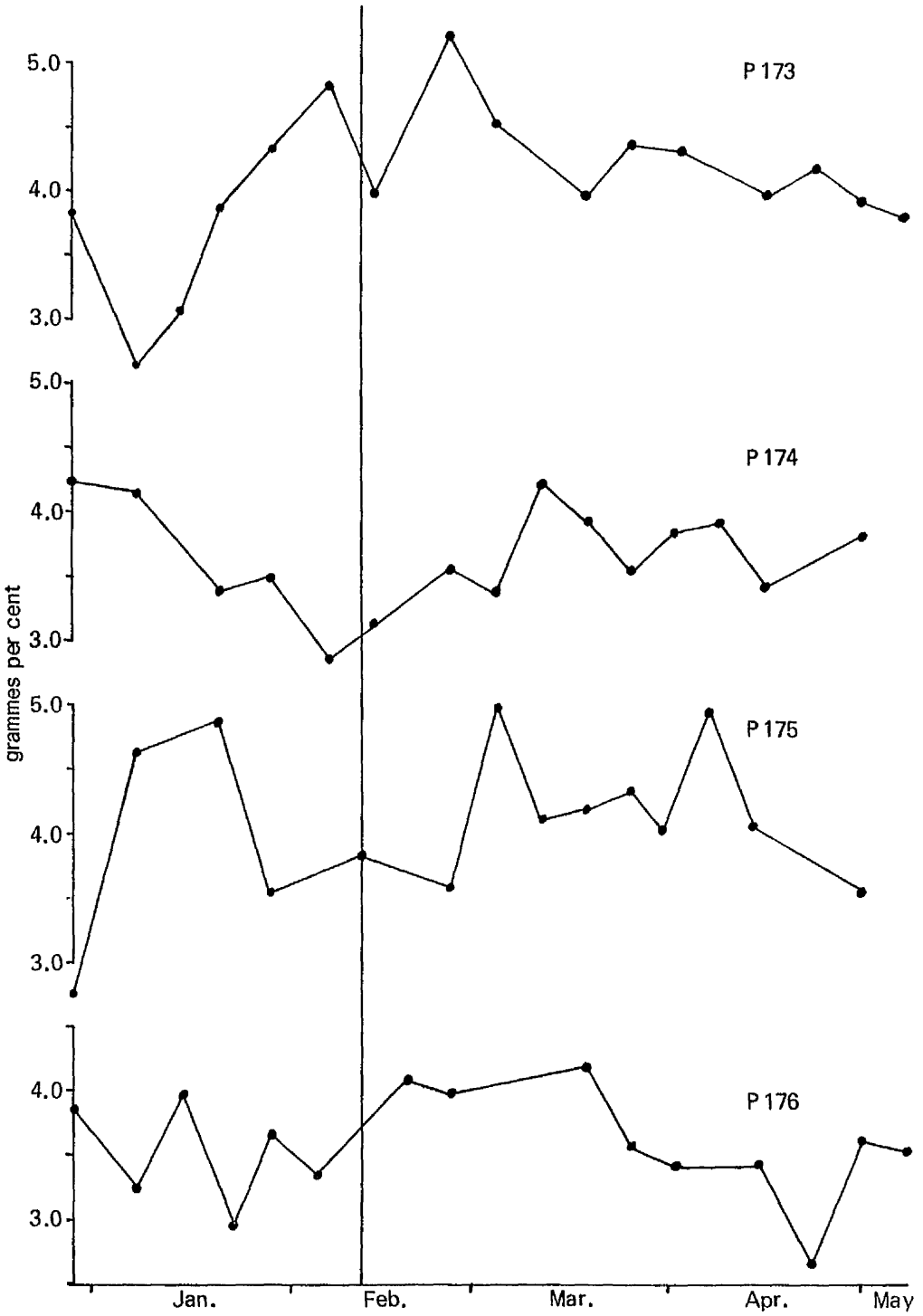


Fig 5.5 Serum globulin values of four pigs infected with 30,000 *Oesophagostomum* larvae on 12th February.

Table 5.1. - Experiment 5.2. (albumin turnover in pigs infected with Oesophagostomum).
Body weights of experimental pigs at time of injection of the ^{125}I labelled albumin and at the end of the subsequent period of observation

Phase	Pig No.	Initial Wt. lbs.	Final Wt. lbs.	Wt. Gain lbs.	Number of days
1	1234 (I)	32	42	10	21
	1233 (C)	$35\frac{1}{2}$	50	$14\frac{1}{2}$	
2	1237 (I)	80	92	12	20
	1235 (C)	87	104	17	
3	1238 (I)	111	150	39	22
	1236 (C)	118	148	30	

I = infected

C = control

Table 5.2. - Experiment 5.2. (albumin turnover in pigs infected with Oesophagostomum).
Synopsis of results

	Phase 1		Phase 2		Phase 3	
Pig No.	1234	1233	1237	1235	1238	1236
Age of infection (days)	0 - 16	control	17 - 35	control	36 - 55	control
Number of worms recovered at post-mortem	-	-	3,900	0	9,700	0
Serum albumin (gm./100 ml.)	2.80	3.11	2.10	2.78	2.44	2.70
Plasma volume (ml./kg.)	56	56	47	43	79	73
Intravascular albumin pool (gm./kg.)	2.09	1.75	0.99	1.19	2.93	2.85
Extravascular albumin pool (gm./kg.)	0.52	1.75	1.37	1.79	1.03	1.75
Fractional catabolic rate (Campbell)	0.13	0.09	0.19	0.21	0.07	0.10
Fractional catabolic rate (Matthews)	0.09	0.12	0.17	0.16	0.08	0.09
Absolute degradation of albumin (gm./kg./day)	0.16	0.16	0.19	0.21	0.19	0.26
Apparent plasma half-life (hr.)	240	262	224	240	244	270

Table 5.3. - Experiment 5.3. (the fate of ^{131}I-polyvinylpyrrolidone in pigs infected with Oesophagostomum).
Synopsis of results

Pig No.	1752	1751	1753	1749	1748
Status	infected		control		diarrhoeic control
Number of worms recovered at autopsy	2,900	10,800	0	0	0
Total serum protein concentration (gm./100 ml.)	6.46	5.70	6.60	5.52	5.98
Serum globulin (gm./100 ml.)	2.73	3.28	3.58	3.09	3.40
Serum albumin (gm./100 ml.)	2.58	2.43	3.02	2.42	3.73
Apparent plasma half-life (hr.)	45	40	40	48	33
Cumulative (12 day) faecal output as % injected dose	2.88	4.62	3.02	2.73	11.21
Mean daily faecal 'plasma clearance' (ml.)	31.3	58.6	66.3	25.3	270.9

Table 5.4. - Experiment 5.4. (the fate of ^{131}I-polyvinylpyrrolidone in pigs infected with H. rubidus).

Synopsis of results

Pig No.	1871	1869	1870	1865	1872
Status	control		infected		
Number of worms recovered at autopsy	0	0	442	278	353
Total serum protein concentration (gm./100 ml.)	6.00	5.66	6.51	6.35	6.39
Serum globulin (gm./100 ml.)	3.50	2.36	3.36	3.80	4.27
Serum albumin (gm./100 ml.)	2.50	2.30	3.15	2.55	2.12
Apparent plasma half-life (hr.)	70	56	77	39	60
Cumulative (6 day) faecal output as % injected dose	0.996	1.001	1.283	1.121	1.084
Mean daily faecal 'plasma clearance' (ml.)	11.3	15.4	18.7	18.1	14.2

DISCUSSION

5.3.1. Methods Used

The information that can be gained by the direct measurement of serum albumin concentrations is limited because the liver is capable of synthesizing proteins in greater quantities than usual should abnormally large losses drain the physiological pools. Thus, whilst hypo-albuminaemia signifies a definite dysfunction, a normal serum albumin value does not necessarily denote physiological normality. A similar result would be obtained if a smaller protein loss was being compensated by accelerated anabolism.

If an occult protein loss is suspected, one means of detection is the measurement of the rate at which these substances are being metabolised. As far as serum proteins are concerned, catabolism is easier to quantify than anabolism and Experiment 5.2. - 5.4 were planned accordingly. It is obviously more attractive to study the turnover of the substance under observation (e.g. albumin) than to utilize an indirect method (e.g. the injection of PVP) which necessarily involves interpretation by analogy. In this particular instance, however, each approach to

the problem has inherent difficulties, and so both techniques were applied in an attempt to compile a comprehensive picture.

One method of measuring the catabolic rate of a plasma protein directly is to inject a known amount into an experimental animal and then to follow the subsequent distribution throughout the body and observe its ultimate fate. In order to do this one must be able to identify the injected material, so that it must differ from the natural substance to some extent. On the other hand, the inoculum and the original must be sufficiently similar to behave identically within the body. Providing that certain conditions are observed, labelling with radio-isotopes provides a simple and convenient method of fulfilling these demands. Several isotopes may be used in this manner. One of these is ^{51}Cr as used by Bremner (1968), but it seems that protein treated in this way invariably undergoes a degree of denaturation so that the plasma half-lives of such preparations are misleadingly short. One member of the serum protein complex,

ceruloplasmin, has a copper atom in the molecule and this can be replaced by ^{67}Cu . This appears to be a successful preparation for studies of this nature, but sophisticated procedures are required for the isolation of the parent compound (Nielsen, 1967). In contrast, albumin can be prepared in large quantities by relatively simple techniques, and labelled by placing a radio-iodine atom in one tyrosine group of the molecule. Care must be taken that auto-irradiation does not denature the protein. This is achieved by ensuring that the mean ratio of radio-iodine to albumin is $\frac{1}{2}$ - 1 atom/molecule, and that after labelling inert albumin is added to reduce the specific activity of the solution to below 5 μc/mg. If these precautions are taken, the biological properties of the resulting product closely resemble those of native albumin. Niobium (^{95}Nb) also gives good results but is expensive (Dargie, Holmes, McClean and Mulligan, 1968).

Protein metabolism is a function of the intra-vascular, rather than the extra-vascular body pool, though a large part of the catabolic process takes

place within the gastro-intestinal tract. In dogs, 9% of the total metabolic clearance occurs in the stomach, 40% in the small intestine and 4% in the colon (Glenert, Jarnum and Riemer, 1964). Comparable figures are not available for the stomach and colon of the pig, but Dich and Nielsen (1964) give a figure of 10-30% for the small intestine. Albumin and other plasma proteins pass from the intra-vascular pool into the lumen of the digestive tract. There, they are broken down by enzymatic action into the constituent amino-acids which are reabsorbed, taken to the liver and resynthesized. The mechanism by which the transfer from plasma to lumen takes place is not yet fully understood. The protein molecules may be transported in the epithelial cells which are continually being sloughed off, or by migration of macromolecules through the mucosa. However this happens, degradation releases the radio-iodine label which is not utilized by the body in any way provided that the thyroid has been saturated beforehand with ^{127}I. A small quantity is incorporated into the substance of bacteria and other

micro-organisms which explains the slight radio-activity of the faeces of these experimental animals. Most of the label is reabsorbed and soon excreted in the urine. Thus, the study of albumin turnover rates by this method gives no direct evidence concerning the passage of albumin into the gastro-intestinal tract, since some catabolism may take place at other sites and contribute to the urine activity.

To overcome this difficulty in interpretation, the PVP test was introduced. Polyvinyl pyrrolidone is an inert synthetic polymer with a molecular weight varying from 20,000-75,000 (cf. albumin, 69,000). It is not broken down by the digestive processes of the gastro-intestinal tract and is not reabsorbed to any significant degree. It can be labelled with radio-iodine to form a stable complex. This substance does not resemble albumin either chemically or biologically, but when injected into the blood-stream it will pass into the intestine at a rate approximately proportional to that of the plasma albumin. The amount of radio-activity appearing in the

faeces per unit time is therefore proportional to the amount of protein that has passed into the intestinal lumen whilst the excrement was being formed. No information regarding pool sizes or catabolic rates in absolute terms can be obtained by this technique.

5.3.2. Protein Losing Gastro-enteropathy and Diarrhoea

Nielsen (1967) has put forward the hypothesis that the hypercatabolism of plasma proteins in certain diseases of domestic animals, including parasitic gastro-enteritis, is a function of the 'clinical picture of the disease' rather than the 'pathoanatomical nature of the lesions'. In other words, the excessive degradation rate is caused by the non-specific symptom diarrhoea rather than the accelerated leakage of macromolecules through the primary intestinal lesion.

The evidence for this belief is derived from a series of comparative studies (Nielsen, 1966b; Nielsen and Nansen, 1967). Albumin or gamma-globulin turnovers were measured in cattle suffering from clinical ostertagiasis during diarrhoeic and non-diarrhoeic phases of the disease. The

results are in turn contrasted with a similar data derived from sows naturally infested with massive numbers of H. rubidus and growing pigs with terminal ileitis (Nielsen, 1966a). More recently, pigs with chronic enterocolitis have been investigated during periods of overt disease and during periods of remission (Nansen and Nielsen, 1967). In all cases, hypercatabolism was associated with diarrhoea, whilst animals with similar pathological lesions but not showing loosening of the faeces were normal in this respect or even hypocatabolic.

These clinical observations are supported by an experiment in which the passage of labelled albumin and gamma-globulin into isolated re-entrant intestinal loops were measured in normal calves (Nielsen and Dich, 1965). The metabolic clearance was increased when intestinal ingesta or magnesium sulphate solution were passing through the loop.

Nielsen and Anderson (1967) and Nansen and Nielsen (1967) associate a specific lesion in the small intestine

(lymphangiectasia) with protein hypercatabolism, although the two are not inevitably found together (Nielsen, 1967). Such morphological changes are very common in diseases of the digestive system and as well as being found in bovine ostertagiasis are present in certain human conditions in which protein loss is an outstanding feature (Waldmann, Steinfeld, Dutcher, Davidson and Gordon, 1961). Nielsen and his co-workers believe that the protein loss is not confined to the organ damaged by the aetiological agent, but that it occurs universally along the gut and particularly from the small intestine. This view is shared by Ross and Todd (1965).

Opposed to this view are Cornelius, Baker, Keneko and Douglas (1962) who were unable to show any increased catabolism in calves with heavy mixed trichostrongylid infections until just before death, but their techniques have been criticized by Halliday, Mulligan and Dalton, (1968). In addition, Bremner (1968) found large protein losses occurring in bovine oesophagostomiasis whether or not the subjects were scouring.

There is evidence, at least in some conditions, to suggest that there is an increased passage of macromolecules through the epithelium in the area of parasitic invasion. Thus, in bovine and ovine ostertagiasis (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart, 1965; Armour, Jarrett and Jennings, 1966) and in porcine hyostrongylosis (Davidson, Murray and Sutherland, 1967), plasma pepsinogen levels are grossly elevated. This is probably the result of increased permeability, which would not only allow protein molecules to leak through into the lumen, but also enable pepsinogen to pass in the opposite direction. Murray and Jarrett (cit. Jennings, Armour, Kirkpatrick and Murray, 1967) have shown by means of a series of electron photomicrographs that the junctional complexes between many epithelial cells in these regions were broken down and that macromolecules do pass between the cells. Mulligan, Dalton and Anderson (1963) have demonstrated increased permeability to ^{131}I-PVP in bovine ostertagiasis. The excessive protein passed into the lumen in this way would, however, be broken down into the constituent amino-acids and reabsorbed

lower down the digestive tract unless secondary changes such as diarrhoea hindered this. It seems likely, therefore, that both increased permeability and diarrhoea play a part in many protein losing gastro-enteropathies.

5.3.3. Albumin Metabolism in Porcine Oesophagostomiasis

These studies were performed with small numbers of pigs and should be repeated on a larger scale to confirm and expand the results obtained. However, sufficient data have been collected to enable one conclusion to be advanced with confidence. That is, that any decreased productivity in Oesophagostomum-infested fattening pigs is unlikely to have been caused by a 'protein leak' of the type demonstrated in bovine oesophagostomiasis.

The information presented in Chapter 1 of this thesis gives a good estimate of the parasitic status of three age groups within the Scottish pig population. Included in the abattoir survey were porkers (four to five months old) and baconers (six to eight months old). The maximum number of oesophagostomes found in the former group was

1,129 (average: 138), whilst only two baconers harboured more than 1,000 (average: 243). The pigs used in Experiment 5.1. were slaughtered at bacon weight, and those in Experiments 5.2. and 5.3. at rather less than porker weight. By comparing the worm burdens of the naturally and experimentally infected hosts, it can be seen that the induced infections were more severe than those commonly found in practice. This was confirmed in Experiment 5.1. in which the administration of 30,000 larvae produced a loosening of the faeces. Oesophagostomum is not considered to be a direct cause of diarrhoea in the field.

In spite of the size of the artificial infections, the effect on the albumin turnover as compared with worm-free controls was minimal. An apparent depression of serum albumin values occurred in only two of the four pigs receiving the highest larval dose level, and it is interesting to note that the most pronounced effect was seen in the pig with protracted diarrhoea. Each infected pig in Experiments 5.2. and 5.4. had a lower serum albumin concentra-

tion than the control animals, but the statistical significance of this observation is in doubt. The rate of albumin catabolism and the quantities of PVP entering the gastro-intestinal tract were similar in the infested and worm-free subjects. If the parasitized pigs were, in fact, hypoalbuminaemic, a 'protein leak' into the digestive tract was not responsible.

Why is it that infections of 10,000-30,000 larvae have such a small influence on albumin metabolism in the pig when 2,000 Oe. columbianum or 10,000 Oe. radiatum produce spectacular responses in their respective hosts? Bremner (1968) states that the apparent plasma half-lives of his infected calves were in the order of three days as compared with seven days in his worm-free controls. Nielsen (1967) records a range of between 16.5 and 28.0 days for the apparent plasma half-lives of his normal cattle. The large discrepancy between the authors can be accounted for by the different techniques used, chromium labelled proteins having a shorter half-life than those tagged with radio-iodine (vide supra). No tracer work

has been attempted in sheep carrying Oe. columbianum, but Dobson (1967) has demonstrated a steady fall in the serum albumin concentrations of infected sheep reaching minimal values approximately 25-30% below those of the controls after the third week.

Oesophagostome infections in pigs differ from those in ruminants in several respects, the outstanding difference being that one of the main clinical signs of heavy Oe. columbianum or Oe. radiatum infections is scouring. Bearing in mind Nielsen's hypothesis (vide supra), it would seem that this would provide an adequate explanation for the question posed above. The increased permeability of the gastro-intestinal tract of the diarrhoeic control pig in Experiment 5.3. adds weight to this argument. Bremner's infecting dose, however, did not invariably precipitate frank diarrhoea, and yet he was unable to show any differences in the rates of protein loss between calves showing clinical symptoms and those that were not scouring.

The parasitic life-cycles of the species of porcine and ruminant oesophagostomes under discussion are not identical. In the pig, the histotropic larvae stimulate the production of small nodules, less than five millimetres in diameter (vide § 0.8), that protrude in towards the lumen of the caecum and colon. The others, however, cause much larger lesions that often become caseated and are frequently seen on the peritoneal surface of the intestine (Chapter 6). A proportion of the larvae penetrate the wall of the ruminant digestive tract and proceed on an abortive migration into various tissues of the body. But perhaps the most significant point is that Oe. columbianum and Oe. radiatum larvae invade the mucosae of the small intestine as well as that of the large bowel. Information based on canine studies has shown that less than 5% of protein catabolism takes place in the colon (vide supra) and therefore increased permeability to macromolecules in this region alone is not likely to have a pronounced effect on the overall catabolic rate. The large lesions formed in the wall of

the small intestine, where 40% of the daily degradation occurs, may very well account for the relatively large protein losses. The apparent normality of albumin metabolism in the pig and the profuse protein losses in ruminants may perhaps be explained in these terms.

5.3.4. Albumin Metabolism in Porcine Hyostrongylosis

Hyostrongylosis of the pig is analogous to Ostertagia infections of ruminants in many ways. The histology of the lesions is practically identical, but whereas diarrhoea is a feature of clinical ostertagiasis, this symptom is not seen even in the most severely affected pigs. The relationship between O. ostertagi and protein losing gastro-enteropathy has been discussed earlier. If the findings of Murray and Jarrett cited above are correct, the permeability of the pig's stomach to PVP will be increased after infection with H. rubidus. Conversely, if such changes do take place, Nielsen's hypothesis would not be valid for the pig.

The results of the present study suggest that there is a leakage of macromolecules, but do not prove this conclusively. The experiment should be repeated on a larger scale with pigs using a larger infecting dose.

Unfortunately, Hyostrongylus is an exceptionally difficult nematode to culture because of its low fecundity, and the number of larvae made available for this experiment fell considerably short of the ideal. The parasitic status of the experimental animals bears little relationship to field conditions as this worm was never seen in pigs of this age group during the course of the slaughterhouse survey (Chapter 1). In Britain, H. rubidus is primarily a parasite of the breeding stock, being harboured by 50.7% of adult pigs. In many cases, the worm burdens are considerably in excess of those recorded in the present study.

Chapter 6

THE PARASITOLOGY OF *OESOPHAGOSTOMUM* INFECTIONS IN AN ABNORMAL HOST, THE GUINEA-PIG

INTRODUCTION

The study of disease in farm animals is handicapped by the difficulties of performing laboratory investigations with such large experimental subjects. Field trials such as those described in Chapter 2 and Chapter 4 may yield useful information, but in general there are so many variables to influence the results that interpretation often proves difficult. The measurement of certain parameters demands a precise knowledge of the 'disease status' (in the present context: the size and nature of the parasitic burden), as well as the inclusion of a group of 'disease free' controls for comparative purposes. Such conditions are rarely attainable in the field and laboratory studies become a necessity. Yet farm animals, especially if 'disease free' are expensive to obtain and to maintain. Accommodation occupies large surface areas and requires capital expenditure. There are great advantages, therefore, if the disease condition can be reproduced wholly or partly in smaller animals such as rodents or rabbits. These are the considerations that, together with the occurrence of the 1967/68 epidemic of Foot-and-Mouth Disease, when work with susceptible animals proved im-

possible, stimulated the studies described in this chapter.

The choice of the experimental host was based on the work of Alicata (1934a). He infected a small number of guinea-pigs and rabbits with porcine oesophagostomes and reported larval invasion of the intestinal mucosa with nodule formation and the appearance of liver lesions. He also mentions the occasional recovery of Oesophagostomum that had undergone the first parasitic moult to become fourth stage larvae.

Guinea-pigs were, therefore, infected with Oesophagostomum larvae cultured from pig faeces and observations were made of the subsequent behaviour and development of the worms. This information could then be compared with published work relating to the characteristics displayed by this parasite in its natural host and an assessment thereby made of the suitability of the guinea-pig as an experimental host for porcine oesophagostomes.

MATERIALS AND METHODS

6.1.1. Animals Used

Eighty-seven guinea-pigs weighing between 160 and 360 g. were studied during the course of the experiment. The guinea-pigs originated from the breeding unit of the Department of Veterinary Pathology of the University of Glasgow, and were genetically heterologous. They were not specific-pathogen-free and were reared in an animal-house in which pseudotuberculosis was endemic.

6.1.2. Experimental Design

The animals were randomly allotted to four groups each receiving a different treatment and were killed at intervals from the 18th hour to the 48th day after infection.

Group No.	No. of guinea-pigs	Status	Inoculum received
1	57	Parasitized	10,000 *Oesophagostomum* larvae.
2	15	Control	None
3	11	Control	Fluid from larval suspension.
4	4	Control	*Pasturella pseudotuberculosis*

The inoculum used for the Group 1 guinea-pigs consisted of larvae suspended in an aqueous medium. The Group 3 controls were included in order that pathological changes initiated by the worms and those provoked by the supporting fluid could be differentiated. Similarly, the need to distinguish hepatic lesions caused by the experimental procedure and those resulting from the endemic pseudotuberculosis necessitated the inclusion of the Group 4 controls.

6.1.3. Preparation of Oesophagostomum Larvae

This procedure has been described earlier (Chapter 3).

6.1.4. Infection of Guinea-pigs

The techniques applied were similar to those already outlined for the administration of infective larvae to laboratory rats (Chapter 3). Each member of Group 1 received a single dose of 10,000 third stage ensheathed Oesophagostomum larvae.

6.1.5. Preparation of Inoculum for Group 3

Samples of the larval suspension were subjected to light centrifugation (1,500 r.p.m. for three minutes), or allowed to stand in a vertically held centrifuge tube for at least two hours. The supernatent fluid was drawn off and examined under the stereo-microscope (x 23 magnification). No larvae were seen. The Group 3 guinea-pigs were each given approximately 1.5 ml. of this liquid using the methods recorded in Chapter 3.

6.1.6. Infection with P. pseudotuberculosis

Broth suspension of the bacterium were kindly supplied by the Department's bacteriology laboratory. Two strains were used: one that had been maintained in the laboratory for a number of years, and a field strain newly isolated from a rabbit. One guinea-pig was infected with the laboratory strain. Organisms isolated from the liver tissue of this animal at autopsy were used to prepare a new batch of broth for injection into a further two guinea-pigs. In addition, one guinea-pig was inoculated with the wild strain. In all cases,

the bacterial cultures were administered by the intramuscular route.

6.1.7. Measurement of Body Weights

All guinea-pigs were weighed at the time of infection and again when they were sacrificed. In addition, a normal growth curve was compiled by weighing six Group 2 control animals three times a week for the duration of the experiment.

6.1.8. Euthanasia of Guinea-pigs

All the experimental animals and controls were killed by the intracardiac injection of a solution of sodium pentabarbitone ("Nembutal", Abbott Ltd.). Group 1 subjects were sacrificed after 18 hours and 1, 3, 5, 7, 9, 11, 13, 15, 17, 23, 24, 29, 39, 47 and 48 days. Group 2 after 18 hours and 1, 5, 7, 10, 15, 17, 23 and 42 days; and Group 3 after 7, 13, 22 and 31 days.

6.1.9. Post-mortem Methods

The gastro-intestinal tract was removed and separated from the mesenteries. It was divided into the

following parts: stomach, small intestine, caecum and colon. The latter was further subdivided into three parts: I - the portion extending distally from the ileocaecal valve for approximately 3 cms. This is thick walled and sacculated, in contrast to Section II which is relatively thin walled and regularly cylindrical. The junction between the second and final parts was deemed to be the point where the first distinct faecal pellet occurred. Section III terminated at the anus (Plate 6.1.). All organs in the abdominal and thoracic cavities were examined visually for macroscopic lesions. The intestines were opened longitudinally, and the contents examined for nematode parasites using the methods described in Chapter 3 for rats.

6.1.10. Detection of Larvae in Tissues

Host tissues were crushed between glass plates to facilitate the counting of larvae by direct microscopical inspection (full description Chapter 3). No systematic search was made for larvae in organs other than the intestine, but their presence in other sites frequently became obvious as a result of the macroscopic lesions that they initiated.

Larvae were also found in some histological sections.

6.1.11. Histopathology

Tissues were taken from the large intestine, mesenteric and other lymph nodes associated with the intestinal tract, liver and kidney. This material was fixed in corrosive formol. Sections were cut at 5μ and stained with haematoxylin and eosin.

6.1.12. Bacteriological Examination

Material was taken aseptically from liver lesions and submitted to the Department's bacteriology laboratory with a request for the isolation and identification of any micro-organisms that were present.

6.1.13. Examination of Larvae from Tissues

Morphological studies were performed on larvae isolated by the digestion of tissues in a solution containing 1% pepsin and 1.2% hydrochloric acid, incubated overnight at 32°C. The larvae were recovered from the resulting suspension by repeatedly allowing the heavier particles to settle out, discarding the overlying liquid and re-

constituting with water, followed by a final screening under the stereoscopic microscope (X23 magnification). The larvae were removed with a pasteur pipette and placed on a microscope slide under a cover-slip. An opisometer was then used to measure the dimensions of camera lucida drawings (X550 magnification) of the larvae.

RESULTS

6.2.1. Clinical Effects

There were great individual variations in the clinical response to the administration of 10,000 Oesophagostomum larvae. The average weight gain of the growing guinea-pig was reduced (Fig. 6.1., Table 6.1.). Several animals even lost weight during the early part of the experiment, although others developed as rapidly as the controls throughout the period of observation. The information available for Group 3 indicated that the weight gains of these animals were only very slightly reduced.

Three animals from Group 1 died: on the second, seventh and thirteenth days post infection. Severely affected animals were listless and emaciated with a staring coat. Diarrhoea was not a feature.

6.2.2. Parasitology

Eighteen hours after infection, approximately 4.2% of the administered nematodes were present in the intestinal mucosa (Plate 6.2., Table 6.2.). Thereafter, the numbers of larvae detectable in the wall of the digestive tract

fell rapidly. At the 48th day, only 0.4 (maximum 0.8) % remained. A remarkable feature of these infections was the longevity of some larvae in the tissues of this abnormal host. Even at Day 48, most of the intact larvae were capable of vigorous movement when warmed on the microscope stage.

The greatest concentration of larvae occurred in the three cms. of the colon immediately distal to the ileocaecal valve ('I' - Plate 6.1.) where 20.7 per sq. cm. were recorded eighteen hours post-infection. The corresponding figure for the caecal wall was 11.0 per sq. cm., and for Sections II and III of the colon: 0.5 and 4.6 respectively, (Table 6.2.). In all parts of the large intestine, aggregations of larvae could be seen in the lymphatic patches, with smaller numbers scattered throughout the surrounding mucosa. After the second week, the surviving larvae were evenly distributed along the length of the large intestine.

When the squash preparations were examined microscopically, the structure of the larvae was partially obscured by the surrounding structures (Plate 6.2.), but

all appeared to be exsheathed third stage. The larvae liberated from the host tissues by digestion could, however, be examined in detail. Without exception, these were all exsheathed third stage larvae. Thus, no development had occurred. The mean length of the larvae recovered on Days 1 and 3 was 505μ (SE 3.9μ, maximum recorded 580μ), and from the 13th to the 24th days 496μ (SE 5.6., maximum 555μ) (Table 6.4.). No fourth or fifth stage Oesophagostomum were isolated from the host tissues or the intestinal contents. No other helminth genera were observed in the infected or control animals.

6.2.3. Pathology

The earliest consequences of larval invasion obvious at gross inspection were punctate haemorrhages and a more generalized hyperaemia in the wall of the large intestine. Evidence of nodule formation at this site was not visible macroscopically until the seventh day after infection when slightly raised patches could be seen in the mucosal wall of approximately half of the guinea-pigs (Table 6.3.). By the ninth day, discrete protrusions, sometimes

pedunculated, projected from the serosal surface of the mucosa of every animal (Plate 6.3.). These nodules varied in magnitude from barely visible to pin head size. In the latter stages of the pathogenesis (after the fifth week), these lesions became less prominent, and those in near proximity tended to coalesce. The parasitic nodules were especially numerous in the first two to three cms. of the colon (Table 6.2.).

In addition to Oesophagostomum larvae, the presence of a second parasite became apparent when the histological sections of the large intestine were screened (Plate 6.12.). This was a ciliated protozoon identical in size and appearance with Balantidium coli. It was often present in large numbers in the intestinal contents of the Group 1 guinea-pigs, but was not seen in the Group 2 or 3 controls. On occasion, the protozoan was found to have invaded the intestinal mucosa of the parasitized animals. In these cases, it was seen lying just under the epithelium lifting it from the underlying tissue. No obvious host reaction was evoked during the period of study.

Oesophagostomum nodules also occurred in, or on other organs including small intestine, lymph nodes, peritoneum, liver, diaphragm and lungs (Table 6.3.). With the exception of the liver lesions, the gross pathological appearance approximated that described above. Larvae were demonstrated in crush preparations, or in histological sections from atleast a proportion of the lesions from each site. Microscopic and/or macroscopic examination of the kidneys, spleen and other organs not listed in Table 6.3. failed to reveal any sign of the presence of parasites.

In the liver, lesions became noticeable as minute white spots on the surface on the third day after infection. From the seventh to the 24th day, two types could be distinguished. The one form consisted of a small (less than one mm. diameter) compact spot, the other being twice this size with diffuse borders gradually merging into the surrounding tissues. After the 24th day only the smaller type was seen. Neither lesion extended more than a millimetre or so into the liver parenchyma. The numbers of hepatic lesions increased to reach a

maximum around the 13th day (Table 6.2.). The largest number of white spots noted in one liver was 29. Microbiological tests consistently failed to demonstrate the presence of P. pseudotuberculosis or any other species of bacteria in the livers of parasitized guinea-pigs. All but one of the control animals (Group 2 and Group 3) were free from nodular and hepatic lesions at autopsy. The exception was one Group 2 control animal that had one small white spot on the surface of the liver. Bacteriological tests were not performed in this instance.

6.2.4. Pseudotuberculosis

The guinea-pig that had been given the original laboratory strain of P. pseudotuberculosis was killed after 21 days. Autopsy revealed that the mesenteric lymph nodes were swollen, and that the surface of the liver was sparsely covered by small discrete white spots. When the hepatic tissue was incised more lesions were seen within the parenchyma. The spleen was not obviously involved.

Of the two guinea-pigs receiving bacteria cultured from the animal described in the previous paragraph, one died on the 14th day and the other was sacrificed on the following day. Both had enlarged mesenteric lymph nodes and mottled splenic lesions. The sacrificed animal displayed hepatic lesions resembling those seen in the donor. The liver of the other contained large irregular necrotic patches, as did that of the guinea-pig infected with the wild strain.

Material from the liver lesions of all these animals gave rise to colonies of P. pseudotuberculosis when plated on blood agar and McConkey's medium.

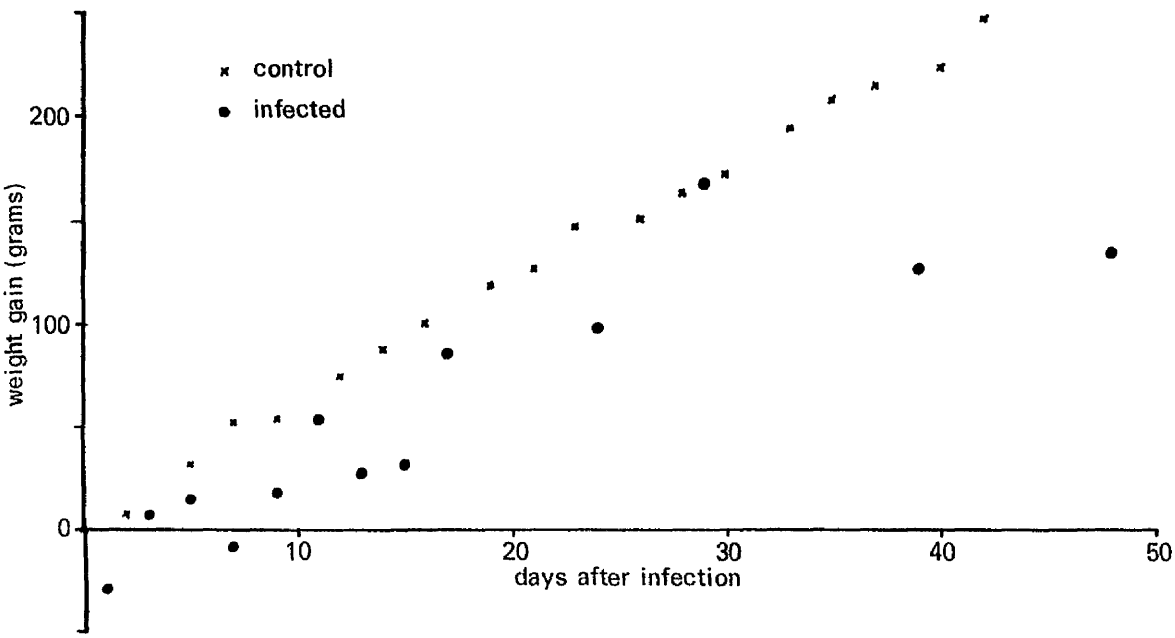


Fig 6.1 The average weight-gains of groups of guinea-pigs that had been infected with 10,000 *Oesophagostomum* larvae compared with a growth curve compiled from normal control animals

Table 6.1. - Growth rate of guinea-pigs infected with 10,000 Oesophagostomum larvae (Group 1), uninfected controls (Group 2) and a further group of uninfected controls given a portion of the liquid in which the larvae had been suspended

Days post infection	Increase in body weights (gms.)		
	Group 1	Group 2	Group 3
1	-28		
2		7	
3	7		
5	15	32	
7	-8	52	39
9	18	54	
11	54	75	
13	28		89
14		88	
15	32		
16		101	
17	87		
19		119	
21		127	
22			108
23	99	148	
24	99		
26		151	
28		164	
29	168		
30		172	
31			219
33		194	
35		208	
37		214	
39	127		
40		222	
42		243	
48	133		

Table 6.2. - Average number of larvae and nodules in the intestine and lesions on the liver of guinea-pigs infected with 10,000 Oesophagostomum larvae

Days post infection	Total No. of larvae in intestine	Larvae per sq. cm.				Total No. of nodules in intestine	Nodules per sq. cm.				Total No. of white spots on liver
		Cae	I	II	III		Cae	I	II	III	
1	417	11.0	20.7	0.5	4.6	0	0	0	0	0	0
3	272	4.5	11.6	1.8	2.6	0	0	0	0	0	2
7	91	3.1	2.1	0.3	0.5	16	0.3	1.5	0.2	0	4
13	215	6.1	4.6	1.8	0.7	44	0.7	2.5	0.4	0	16
17	107	0.8	1.8	0.7	2.8	33	0	2.3	0.1	0.7	8
24	64	1.1	1.2	0.7	0.7	63	0.8	5.3	0.2	0.6	11
48	38	0.7	0.3	0.3	0.7	+	+	+	+	+	10

+ = confluent, impossible to count

Cae = caecum

I, II, III, = sections of colon (vide Plate 6.1.)

Table 6.3. - The frequency of occurrence of nodules in various tissues of guinea-pigs infected with 10,000 Oesophagostomum larvae

No lesions were found in organs not listed

Days post infection	Nos. of guinea-pigs examined	Nos. of guinea-pigs with nodules in						
		Large intestine	Liver	Lymph nodes	Peritoneum and omentum	Diaphragm	Small intestine	Lung
1	6	0	0	0	0	0	0	0
3	5	0	2	0	0	0	0	0
5	3	0	1	0	1	0	0	0
7	7	3	4	3	0	0	1	0
9	3	3	3	1	0	0	0	0
11	3	3	3	1	0	0	0	0
13	5	5	5	0	2	1	0	0
15	3	3	3	1	1	1	0	0
17	5	5	5	1	1	0	0	0
23/24	5	5	5	4	2	1	0	1
29	3	3	3	1	1	0	0	0
39	1	1	1	1	0	0	0	0
48	2	2	2	0	2	1	0	0

Table 6.4. - Length of larvae isolated from the intestinal mucosa of guinea-pigs infected with Oesophagostomum

Days post infection	No. Measured	Average Length	Standard error	Range
1 - 3	41	505μ	3.9	470 - 580
13 - 24	27	496μ	5.6	445 - 555

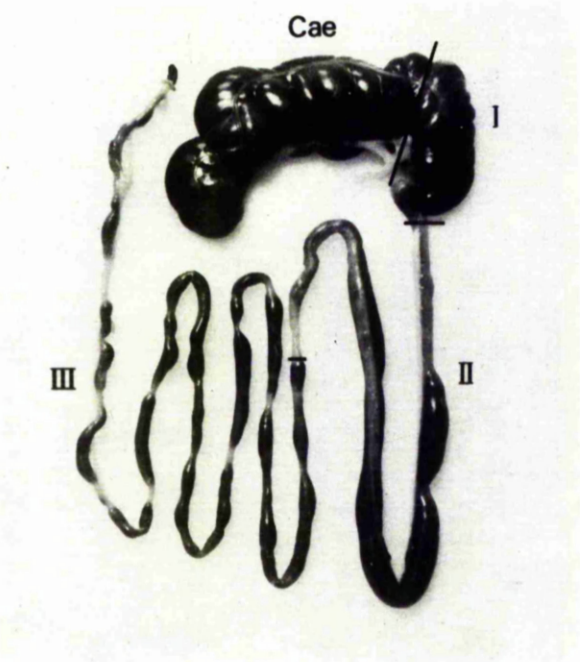


Plate 6.1 The large intestine of a normal guinea-pig. Cae = caecum, I = Section I of the colon, etc. For explanation see 6.1.12..

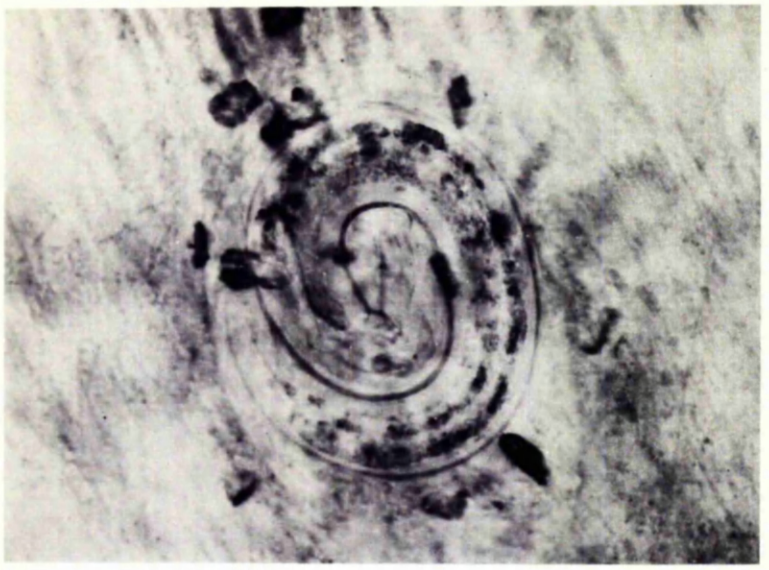


Plate 6.2 An *Oesophagostomum* larva in the wall of the large intestine of a guinea-pig photographed seven days after infection

Plate 6.3 Subserosal *Oesophagostomum* lesions on the large intestine (Section I) of a guinea-pig that had been infected 15 days earlier

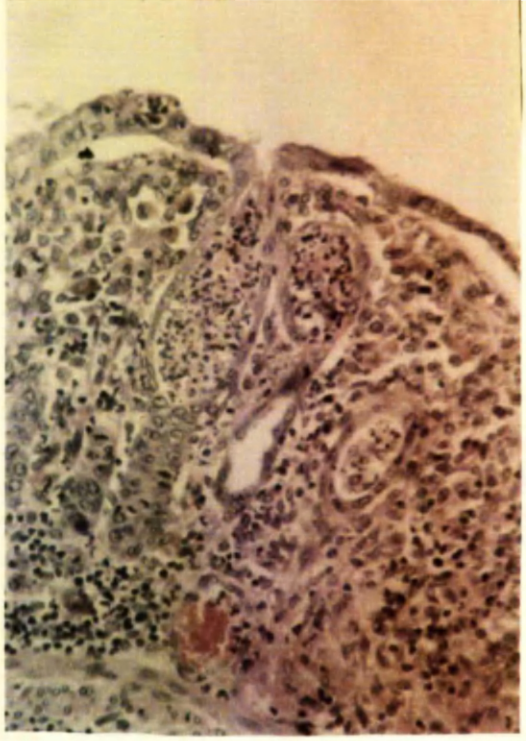


Plate 6.4 **Guinea-pig large intestine 18 hours after infection with *Oesophagostomum* larvae. The mucosal crypts are partly distended with an exudate rich in cells.** *(Haematoxylin and eosin x360)*

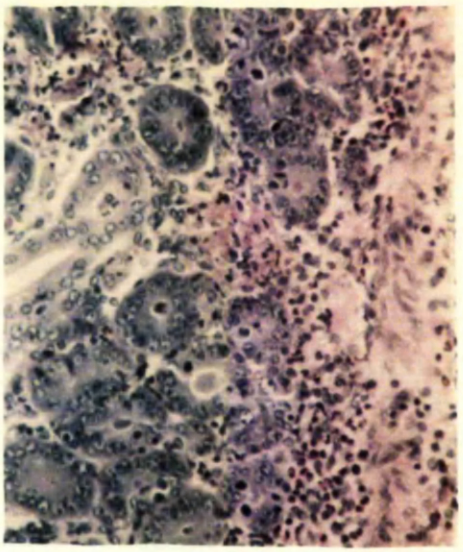


Plate 6.6 **Guinea-pig large intestine 18 hours after infection with *Oesophagostomum* larvae. Localised tissue necrosis and granulocyte accumulation caused by the passage of invading larvae.**
(Haematoxylin and eosin x360)

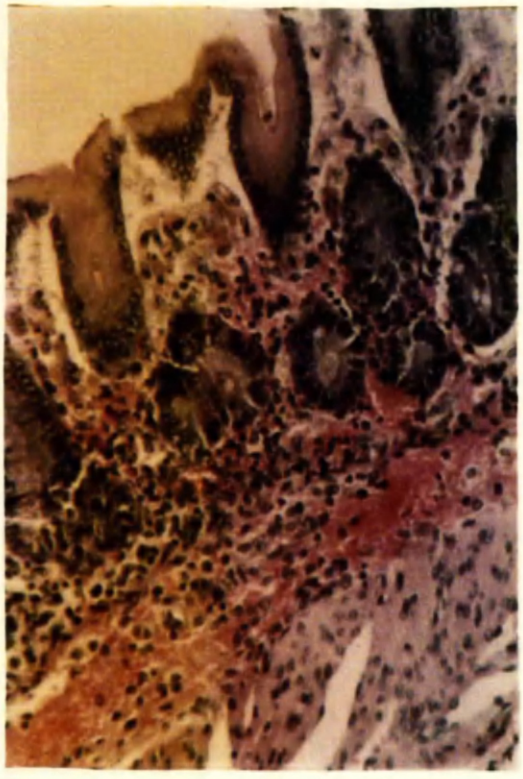


Plate 6.5 **Guinea-pig large intestine 18 hours after infection with *Oesophagostomum* larvae. Recent haemorrhage into the lamina propria and submucosa.** *(Haematoxylin and eosin x360)*

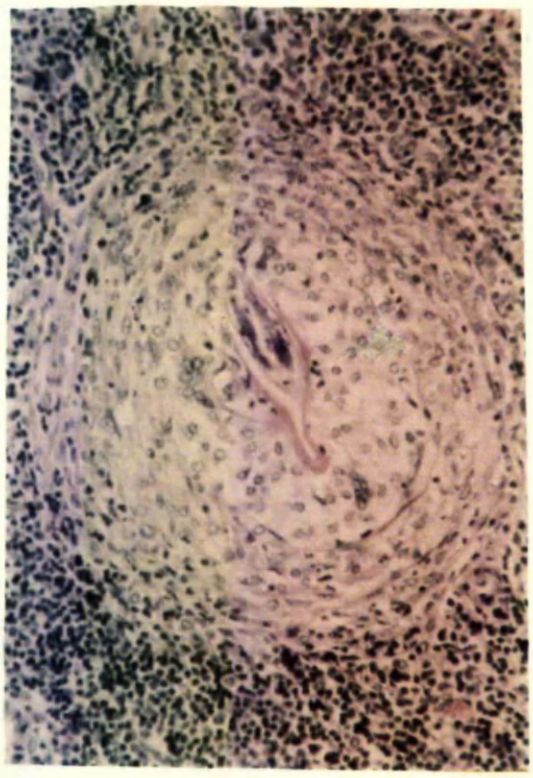


Plate 6.7 **Guinea-pig large intestine 9 days after infection with *Oesophagostomum* larvae. The larva has come to rest in a lymph follicle and is surrounded by a thin layer of amorphous eosinophilic material. This is encompassed by a loose matrix containing reticulo-endothelioid cells and occasional giant cells which, in turn, is encircled by a zone of fibroblasts.**
(Haematoxylin and eosin x360)

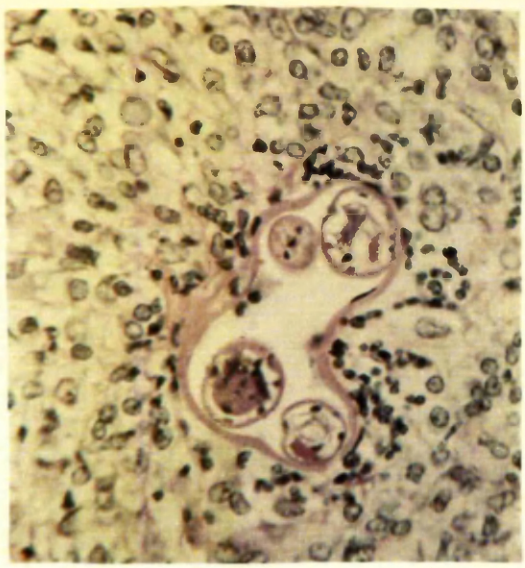


Plate 6.8 **Guinea-pig large intestine 23 days after infection with *Oesophagostomum* larvae. The nematode in the centre of a nodule is cut transversely in several places. It lies in a space limited by a wall of eosinophilic material beyond which is the reticulo-endothelioid layer.** *(Haematoxylin and eosin x 1200)*

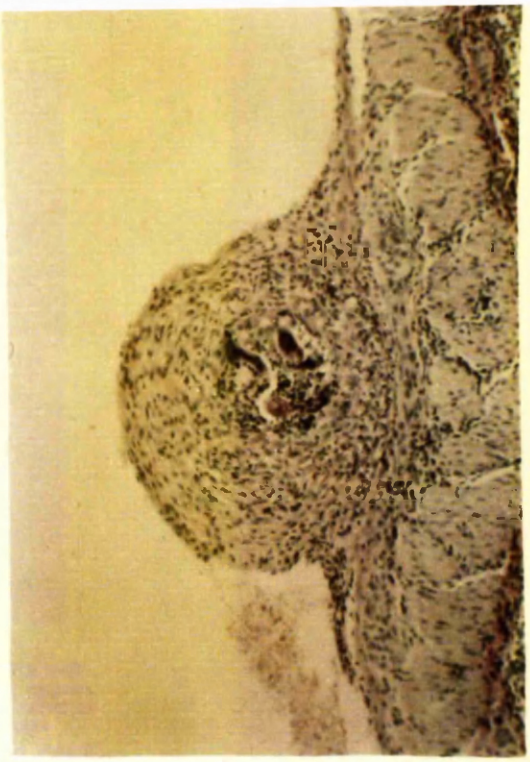


Plate 6.10 **Serosal surface of guinea-pig large intestine 23 days after infection with *Oesophagostomum* larvae showing a protuding subserosal nodule, the wall of which is composed almost exclusively of fibrous tissue. The worm appears to be dead.** *(Haematoxylin and eosin x 360)*

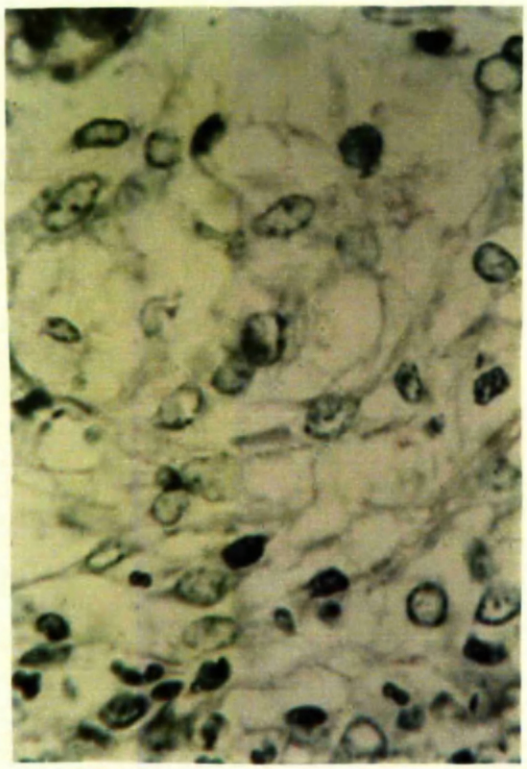


Plate 6.9 **High power view of the loose matrix containing reticulo-endothelioid cells in the *Oesophagostomum* nodule shown in Plate 6.8.** *(Haematoxylin and eosin x 2400)*

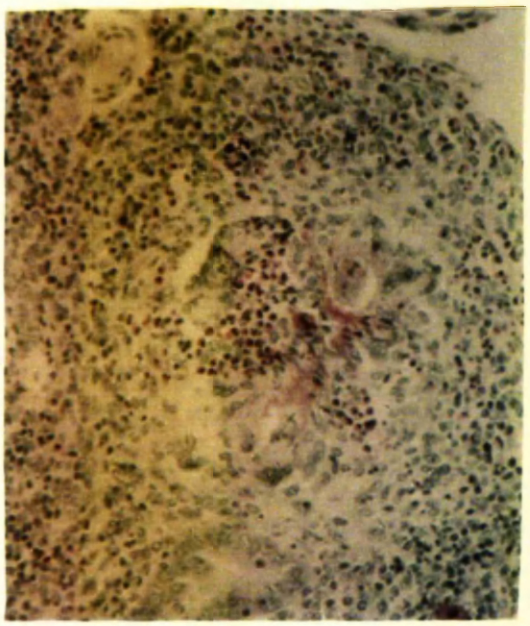


Plate 6.11 **Mucosa of guinea-pig large intestine 5 days after infection with *Oesophagostomum* larvae. The position of a dead larva is marked by eosinophilic debris and numerous eosinophils.** *(Haematoxylin and eosin x 360*

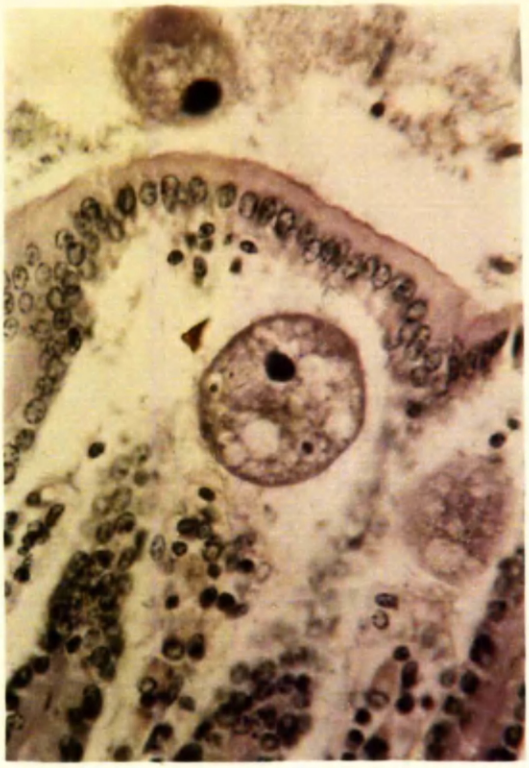


Plate 6.12 **Guinea-pig large intestine 39 days after infection with *Oesophagostomum* larvae. *Balantidium coli* trophozoites are seen beneath the epithelium and free in the intestinal lumen.** *(Haematoxylin and eosin x 1200)*

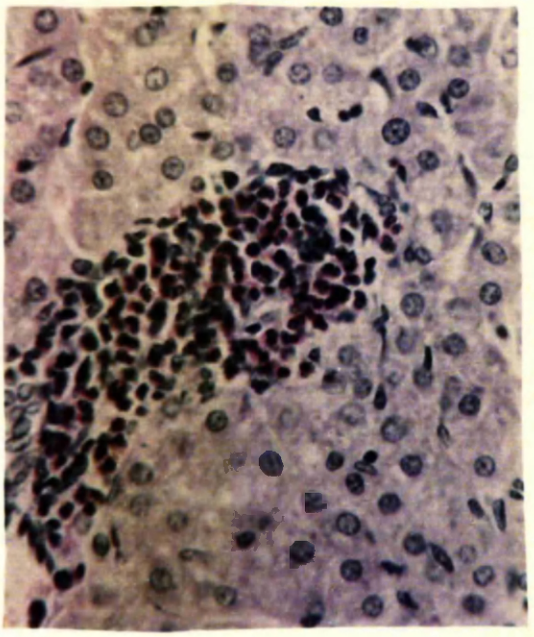


Plate 6.14 **Guinea-pig liver 17 days after infection with *Oesophagostomum* larvae. The path taken by a migrating larva is marked by proliferation of macrophages and infiltration of eosinophils.** *(Haematoxylin and eosin x 1200)*

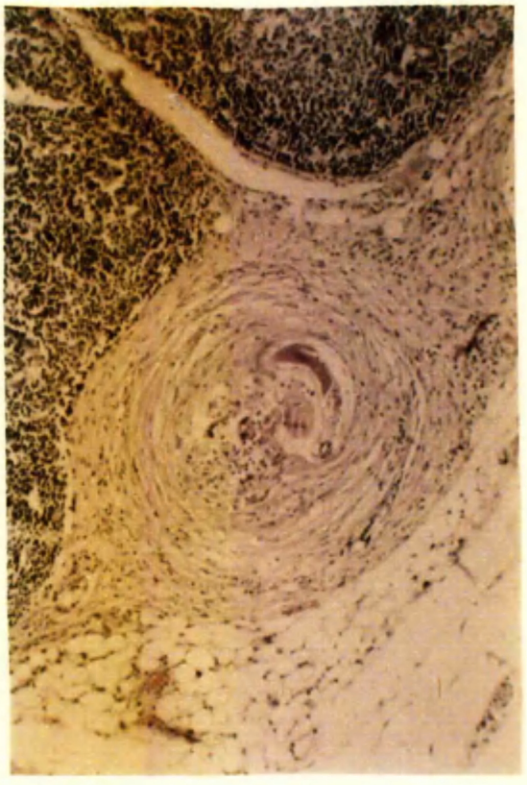


Plate 6.13 **Mesenteric lymph-node from a guinea-pig infected with *Oesophagostomum* 39 days previously. A parasitic nodule is seen in the peripheral stroma.** *(Haematoxylin and eosin x 360)*

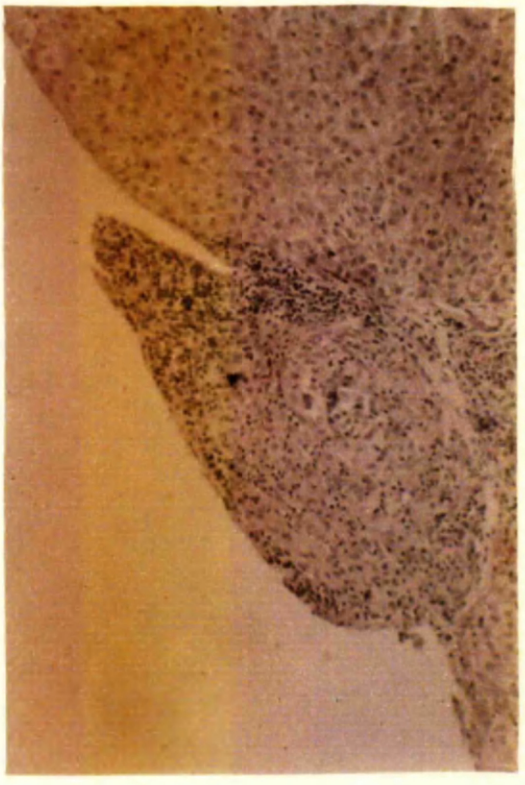


Plate 6.15 **Guinea-pig liver 23 days after infection with *Oesophagostomum* larvae. A pedunculated nodule is attached to the surface of the liver.** *(Haematoxylin and eosin x 360)*

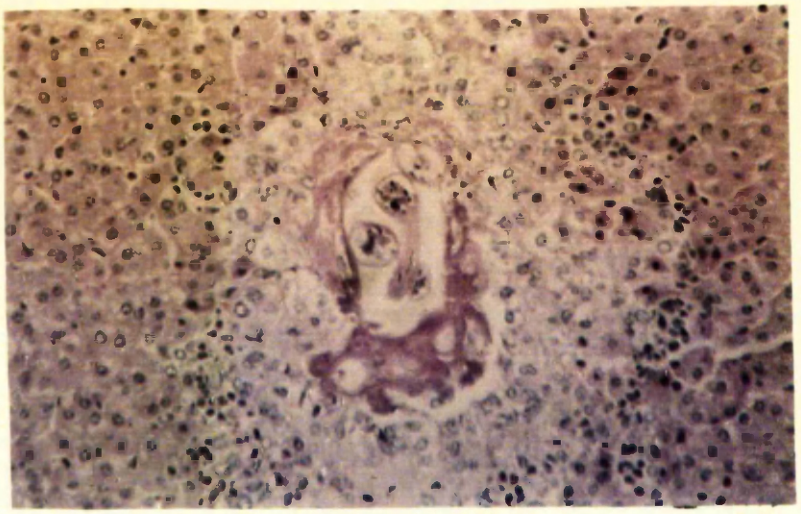


Plate 6.16 **Guinea-pig liver 9 days after infection with *Oesophagostomum* larvae. A nodule within the parenchyma of the liver. The worm is cut in transverse section.**
(Haematoxylin and eosin x 1200)

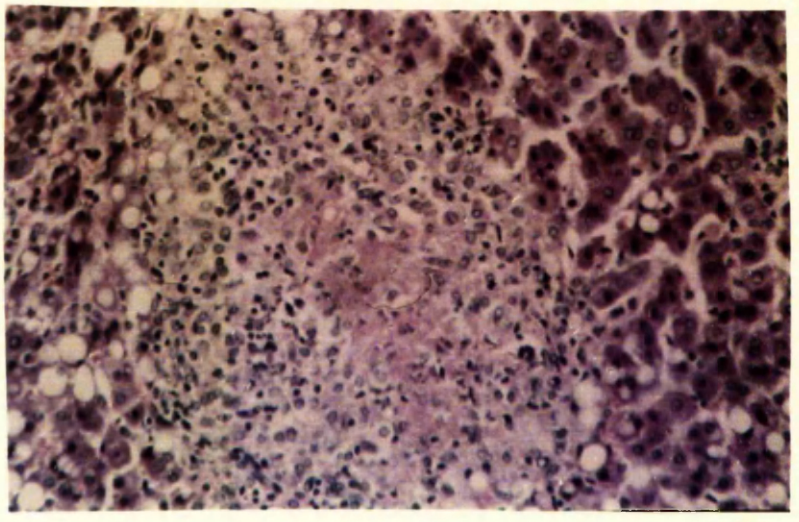


Plate 6.17 **Liver of guinea-pig infected with *Pasturella pseudotuberculosis*. The lesion is composed of a mass of weakly eosinophilic necrotic tissue, surrounded by macrophages with occasional granulocytes and fibroblasts. There are no eosinophils present. Nearby viable hepatic cells are vacuolated.** *(Haematoxylin and eosin x 1200)*

DISCUSSION

6.3.1. General Considerations

Data obtained from animal experimentation are subject to an inconvenient, albeit inevitable, degree of variability. At present, it is not practicable to produce clones of genetically identical vertebrates for routine research purposes. Thus, mammalian studies invariably involve the comparison of a series of 'treated' genetically dissimilar units with a group of dissimilar 'normal' entities. The outcome is, of course, that the mathematical expression of each biological function will give not one value for each group but a range of results, even in the case of 'normal' animals. An examination of the raw data assembled at the end of this thesis will show that the present study is not exempt from the consequences of this concept. In general, the results for a group of similarly treated animals are scattered about a mean in a predictable manner, and statistical methods have been developed for concisely defining such variability.

When planning laboratory experiments an attempt is

made to minimize the differences within and between groups. In the present instance, for example, the guinea-pigs should have been closely related (although not in-bred), specific-pathogen-free and all of the same age, weight and sex. However, such factors as the availability of stock and budgetary considerations sometimes make the ideal difficult to attain.

The experimental intervention should be simple and well-defined if the responses are to be understood. In this present study, this objective was also thwarted to a certain degree since a protozoan parasite, B. coli, was accidentally transmitted along with the two species of nematode larvae. A further complication was the presence in the environment of at least one other pathogen, P. pseudotuberculosis.

However, good use can be made of such an imperfect situation provided that:

(a) a sufficiently large number of animals is used.

(b) suitable control groups are included

(c) the differences between the groups are not too subtle.

Although not ideal, the experimental model used for this study of Oesophagostomum infections in guinea-pigs proved adequate.

6.3.2. Parasitology

Up to 5.4% of the administered Oesophagostomum larvae were able to exsheath and invade the tissues of this abnormal host. This figure is some ten times greater than that recorded when laboratory rats were infected with 20,000 larvae (Chapter 3). In both mammalian species, penetration appeared to be confined to the large intestine as it is in the natural host. The oesophagostomes of ruminants also enter the wall of the small intestine (Dobson, 1966; Elek and Durie, 1967). Most larvae were seen in the region of the lymphatic patches which are a prominent feature of the intestinal wall of the guinea-pig. The caecum and first part of the colon are particularly rich in lymphatic tissue and this may account for the comparatively high larval densities seen in these

locations. Alternatively, the majority of larvae able to infect the host may have done so at the earliest opportunity, so that only a few capable larvae would reach the more distal part of the intestinal tract. Presumably, the 94.6% or more that were unaccounted for at the 18th hour had been killed and digested or had passed out in the faeces.

The larvae entered the mucosal crypts, passed through the epithelium and continued into the lamina propria, submucosa, muscularis mucosa or lymphatic aggregations. Many went no further becoming encapsulated to form the nucleus of the macroscopic nodules (Plates 6.7. and 6.10.).

The numbers of larvae that could be counted in the mucosa declined rapidly during the days after infection. Some perished in this situation. Others, however, continued their migration venturing away from the digestive tract. The route or routes were not readily identifiable, but the final resting place of each larva was marked by nodule formation.

The lymph nodes lying close beside the caecum and the first part of the colon were frequently invaded, and this may be a consequence of the localization of larvae in the intestinal lymphatic patches. Larvae were seen in the lumen of lymphatic vessels on two occasions. In general, however, histological observations do not support this hypothesis as most nodules were associated with the peripheral stroma of the lymph node rather than with the parenchyma (Plate 6.13.). It is possible, however, that the larvae did arrive via the lymphatic vessels but that their progress was arrested and diverted by the narrow dimensions of the passages within the nodes.

Nodules were also seen in the mesenteries and omenta, but on these occasions there was no obvious association with lymph or blood vessels. Active migration on or between the serosae did, therefore, take place and probably accounts for the lesions seen on the abdominal surface of the diaphragm. The comparative infrequency of these lesions (Table 6.3.) discredits the possibility that this was the main migratory pathway.

From the ninth day onwards, lesions were found with equal frequency in the intestine and liver. That is to say, larvae invariably found their way to the latter organ. White spots first appeared on the surface three days after infection indicating a speedy journey from the site of penetration. Yet other visceral organs (spleen, pancreas, kidneys and adrenals) never displayed signs of parasitic invasion. Bearing in mind the paucity of peritoneal nodules, there is a strong suggestion that carriage may be by the hepatic portal system. The same picture would result if the serosae provided almost unimpeded passage and the larvae were attracted or channelled towards the liver. Larval tracts were seen within the liver parenchyma (Plate 6.14.) but the majority of the lesions occurred superficially rather than in the parenchyma (Plate 6.15.). The peritoneal route may, therefore, be favoured but, as in the case of the lymphatic lesions, this picture may represent the arrest of larvae migrating <u>out</u> from within the organ.

The lymphatic route would not take larvae to the liver

but to the vena cava. Thereafter the first capillary network to be encountered would be in the lungs. Yet only one larva was ever recovered from this site, and the possibility that this may have been inhaled during administration cannot be excluded.

A remarkable feature of these infections was the extreme longevity of a proportion of the larvae that had become encapsulated in the intestinal mucosa. Despite the foreign environment and the tissue reaction, many were capable of vigorous movement even at the 48th day. In the natural host, the pig, the first parasitic moult takes place after approximately four to five days and the resultant fourth stage larvae begin their return to the lumen of the intestine on the 6th day (Kotlan, 1948; Shorb, 1948), although many are inhibited in their development and may or may not emerge from the nodules (Shorb and Shalkop, 1959). In the present experiment, no development of the larvae took place after exsheathment. The nematodes neither grew nor moulted, and none returned to the lumen. In contrast, Alicata (1934) was

able to recover two fourth stage larvae from the intestinal wall of his infected guinea-pigs.

6.3.3. Pathology

Existing knowledge of the nature and appearance of Oesophagostomum nodules in the pig has already been summarized in the introductory chapter of this thesis (§ 0.8). Macroscopically, the lesion is a circumscribed swelling that protrudes into the lumen of the large intestine measuring up to 4 - 5 mm. in diameter. It may be raised or flattened, with or without a central depression. The larvae do not regularly venture into the submucosa, and, with only one exception (Bolle, 1951), have never been found outside the intestinal tract.

The lesions initiated by Oe. venulosum in sheep and goats parallel those of the pig oesophagostomes in their natural host (Goldberg, 1952) in that they also are relatively inconspicuous. In sharp contrast is Oe. columbianum in these hosts. This parasite often wanders into the submucosa and beyond into the abdominal cavity, mesenteric lymph nodes and liver (Carne and Ross,

1932; Dobson, 1966; Horak and Clark, 1967; Sarles, 1944; Shelton and Griffiths, 1967). Srivastava and Singh (1964) and Tewari and Iyer (1961) describe similar behaviour in an unnamed Oesophagostomum spp. which is probably Oe. columbianum. Large protruding nodules directed into the peritoneal cavity form around the larvae that come to rest in the submucosa (Dobson, 1966; Fourie, 1935; Sarles, 1944).

The behaviour of porcine oesophagostome larvae in the guinea-pig as shown in the present study and that of Alicata (1934), resembles that of Oe. columbianum in sheep and goats rather than Oe. dentatum and Oe. quadrispinulatum in pigs. Can it be that the 'wanderlust' of certain Oesophagostomum species is a consequence of poor or incomplete adaptation to the host? It is unlikely that the aberrant larvae of Oe. columbianum ever return to the lumen of the intestine, and they have thereby forfeited the opportunity to contribute to the perpetuation of their species. It is difficult to envisage any biological advantage for such activity within the normal host, but should the larvae be eaten accidentally by another animal species, such behaviour could be a decided

advantage - especially if the natural host is a carnivore or omnivore. This principle was well illustrated in Chapter 2 where the potential ability of the rat to act as a paratenic host for pig oesophagostomes was demonstrated. It seems possible, therefore, that the association between Oe. columbianum and sheep and goats may have been founded later in evolutionary history than was the case for Oe. venulosum, which appears better adapted to its host. Oe. apiostomum in primates also causes large nodules and shows tendencies to migrate in an apparently haphazard fashion (Lie Kian Joe, 1949; Weinberg, 1909), whereas Oe. radiatum in cattle occupies an intermediate position, confining its presence to the intestinal wall and stimulating a smaller host reaction (Anataraman, 1942; Elek and Durie, 1967; Koch, 1949; Mayhew, 1948).

The course of the Oesophagostomum infection in guinea-pigs is not, however, entirely analogous to that of the other nodule forming oesophagostomes. In the latter instance, of course, a proportion of the worms undergo normal parasitic development and attain sexual

maturity. The nodules are often filled with large quantities of caseous material that may later become calcified, reactions that were not observed in the guinea-pig material. A common factor in all Oesophagostomum infections, however, is the strongly eosinophilic wall that forms around the histotropic larva (Plates 6.7., 6.8., 6.13. and 6.16.).

6.3.4. Aetiology of Liver Lesions

White spots could be seen on the livers of the parasitized guinea-pigs three days after infection, and were invariably present after the ninth day. It was known that pseudotuberculosis was endemic in the animal-house, yet all but one of the Group 2 and Group 3 controls were free of this type of lesion. The spots were, therefore, associated with the nematode infection, and yet Oesophagostomum spp. in the pig are known to be non-migratory. Were the larvae behaving in an atypical manner and entering the hepatic tissue or were they only an indirect cause of the lesions? It was conceivable that the oesophagostomes could have released a hepatotoxin or that they created a state of stress allowing a latent

bacterial infection to exacerbate. Examination of the evidence collected during the investigations showed that many of the lesions were certainly initiated by the presence of larvae in or on the liver.

Firstly, a number of histological preparations revealed larvae coiled within the lesions (Plate 6.16.). Other sections had cut the periphery of the small spots and showed no direct sign of the presence of nematode structures. In these cases the pathological picture was identical to that of the larval lesions and quite different from that seen in the Group 4 guinea-pigs (cf. Plates 6.15., 6.16. and 6.17.). Infection with P. pseudotuberculosis produced relatively large areas of coagulative necrosis. There was no eosinophilic infiltration and the fibroblastic response was minimal. The areas of necrosis marking the pathways taken by the migrating oesophagostomes were considerably smaller than those following the bacterial invasion, and were infiltrated by numbers of eosinophils.

One Group 2 animal did display signs of pseudotuberculosis but this was an exceptional case. Every Group 1 hepatic spot that was subjected to bacteriological examination proved sterile. In contrast, the Group 4 lesions invariably yielded P. pseudotuberculosis. The diagnostic procedures were thus proved to be sound.

6.3.5. Clinical Observations

At autopsy, the caecum and colon often only contained a small fraction of the volume of food material usually seen in healthy guinea-pigs. The negative weight gains that occurred in the early part of the experiment were caused primarily as a result of the normal evacuation of faecal pellets by anorexic animals. The reduction in growth-rate seen later was at least partially caused by reduced food intake. The presence of the nodular lesions in the wall of the large intestine may have interfered with the absorption of nutrient substances, whilst another effect may have been an alteration of the luminal environment with a secondary influence on the composition of the commensal or symbiotic microflora and microfauna.

6.3.6. Origin of Balantidium coli Infections

The occurrence of a ciliated protozoon with a peristome, kidney shaped macronucleus and cytoplasmic vacuoles in the large intestine of guinea-pigs inoculated with material of porcine faecal origin prompted a presumptive diagnosis of Balantidium coli. The similarity of the dimensions of this organism and those of B. coli strengthened this belief. B. coli infections have also been established experimentally in guinea-pigs by Andrews (1930), Schumaker (1930) and Westphal (1957).

Since the presence of this potential parasite was diagnosed in retrospect, the opportunity to perform exhaustive tests to establish its absence in the Group 2 controls was lost. This is particularly unfortunate as Rees (1927) and Scott (1927) have recorded naturally occurring Balantidium spp. in the guinea-pig. It was not, however, seen in any tissue section made from material from the latter group.

The B. coli trophozoite is a very frequent commensal

of domestic pigs, being found amongst the contents of the large intestine. No comprehensive survey has been performed in the United Kingdom to establish the prevalence in the host population. The organism can become parasitic if the intestinal mucosa is damaged by some other aetiological agent. In these cases, it often lies just under the epithelium as in the case of the guinea-pig material (Plate 6.12.). It sometimes penetrates more deeply and may even reach the lymphoid follicles, but this was not seen in the present context.

Reproduction of the ciliate can take place by binary fission within the host, and so large numbers can build up following the ingestion of comparatively few organisms. Transmission from animal to animal occurs by ejection of encysted forms in the faeces and subsequent accidental ingestion by the next host. The cysts are quite dense structures since they do not float readily in saturated saline, although they rise to the surface of saturated zinc sulphate solution.

The occurrence of B. coli in the Group 1 guinea-pigs was probably due to contamination of the larval culture with cyst forms of the protozoon. The larvae were washed by repeated sedimentation and resuspension in clean water, and it is probable that the cysts were of sufficient density to layer out with the larvae. The Group 3 inoculum was prepared by allowing the larvae to settle by gravity or with the aid of a centrifuge and then withdrawing the supernatent fluid for injection. The cysts would have accumulated in the rejected portion.

One other possibility exists. This is that the nematode larvae themselves transmitted the protozoon in a manner analogous to the carriage of Histomonas by Heterakis (Gibbs, 1962) or Toxoplasma by Toxocara (Hutcheson, 1967; Dubey, 1968; Jacobs, 1967). However, there was no experimental evidence available to suggest that this was the case. In view of the controversy concerning nematode transmission and the potential importance of this phenomenon in the field of animal and human disease, an experiment designed to confirm or reject the hypothesis that

Oesophagostomum may act in this way would be justified.

6.3.7. Conclusion

Oesophagostomum infections in guinea-pigs are characterised by the formation of subserosal nodules on the wall of the caecum and colon, and by the migration of larvae to other organs of the body. Larvae do not grow nor do they develop to the fourth stage.

In the natural host, the nodules are formed on the luminal surface of the intestine. The larvae are non-migratory, undergo the first parasitic moult on the 4th or 5th days after infection and start their return to the lumen on the next day.

The behaviour of Oesophagostomum larvae in the guinea-pig is thus so abnormal that, although some intriguing information has been gained from the present study, this host-parasite system is of little value as a laboratory model for the study of porcine oesophagostomiasis.

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APPENDIX 1

Table 1 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual porkers during period November, 1964 - January, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
29	4	3	0	0	0	0
30	0	0	0	2	0	0
35	0	0	0	0	0	0
36	13	0	0			
41	66	0	0	164	14	0
42	78	0	0	0	0	0
48	0	0	0	27	1	0
49	8	0	0	0	0	0
55	1	0	0	0	0	0
56	4	0	0	18	0	0
59	0	0	0	0	0	0
60	0	0	0	0	0	0
61	1	1	0	124	89	0
62	17	0	0	173	67	0
63	1	1	0	27	0	0
68	5	0	0	14	0	0
69	0	0	0	7	12	0
70	0	0	0	3	0	0
71	0	1	0	———	———	0
72	0	0	0	0	0	0
73	9	0	0	22	53	0
74	4	5	0	77	59	0
75	0	0	0	0	0	0
76	3	11	0	86	116	0
77	3	8	0	197	545	0

APPENDIX 1 (Continued)

Table 2 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual baconers during period-November, 1964 - January, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
26	0	5	0	0	0	0
27	0	0	0	0	2	0
28	0	0	0	12	6	0
31	0	0	0	4	4	0
32	0	0	0	5	19	0
33	15	0	0	0	0	0
34	0	0	0	0	0	0
37	0	0	0	0	0	0
38	0	0	0	0	0	0
39	2	0	0	0	0	0
40	0	0	0	0	0	0
43	0	0	0	0	0	0
44	2	0	0	6	0	0
45	0	0	0	6	0	0
46	0	1	0	0	0	0
47	4	7	0	0	0	0
50	0	4	0	0	0	0
51	2	0	0	0	0	0
52	0	0	0	0	0	0
57	0	5	0	3	0	0
58	1	3	0	3	0	0
64	0	0	0	3		0
65	1	0	0	0	0	0
66	1	0	0	18	0	0
67	1	0	0	3	0	0

APPENDIX 1 (Continued)

Table 3 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual sows during period November, 1964 - January, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
1	0	0	0	0	35	0
2	0	0	0	53	307	0
3	0	0	15	——— 20 ———		0
4	0	0	5	——— 45 ———		0
5	0	0	120	6,294	1,971	0
6	0	0	0	264	16	0
7	0	0	0	4,444	66	0
8	0	0	0	59	6	0
9	0	0	0	——— 450 ———		0
10	0	0	50	0	30	0
11	0	0	5	95	0	0
12	5	0	0	15	2,050	0
13	0	3	15	2,043	12	0
14	0	0	70	755	246	0
15	0	0	0	15	5	0
16	0	0	0	273	167	0
17	0	0	0	115	0	0
18	0	0	30	59	1,106	0
19	0	0	20	586	219	0
20	0	0	5,200	5,113	67	Globocephalus urosubulatus: 447 Trichostrongylus axei: 31 T. colubriformis: 158 T. vitrinus: 31
21	0	0	810	1,305	0	0
22	0	0	90	1,501	249	0
23	0	0	14,878	2,445	0	0
24	0	0	10	0	6	0
25	0	0	0	0	0	0

APPENDIX 1 (Continued)

Table 4 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual porkers during period February, 1965 - April, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
101	0	0	0	0	0	0
102	0	1	0	3	0	0
117	0	0	0	3	0	0
118	1	7	0	0	0	0
119	0	0	0	148	174	0
120	0	0	0	0	0	0
121	0	1	0	35	2	0
122	0	1	0	6		0
123	0	0	0	0	0	0
124	14	0	0	3	1	0
129	0	2	0	0	0	0
130	0	0	0	6	22	0
131	2	0	0	13	18	0
132	0	0	0	27	6	0
133	0	0	0	0	6	0
134	0	2	0	3	0	0

APPENDIX 1 (Continued)

Table 5 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual baconers during period February, 1965 - April, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
125	0	0	0	3	1	0
126	0	1	0	0	0	0
136	0	0	0	0	0	0
137	0	0	0	0	9	0
138	1	0	0	0	0	0
139	0	1	0	0	0	0
140	0	0	0	12	3	0
141	0	0	0	15	39	0
142	0	0	0	0	0	0
143	0	3	0	0	0	0
144	0	3	0	0	0	0
145	0	0	0	0	0	0
146	0	0	0	1		0
147	0	0	0	51	0	0
148	1	0	0	9	0	0

APPENDIX 1 (Continued)

Table 6 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual sows during period February, 1965 - April, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
87	0	2	1,048	1,172	236	0
88	0	0	294	1,118	242	0
89	0	1	5,640	574	332	0
90	0	0	0	141	507	0
92	0	0	600	508	82	0
93	0	0	12	4,439	152	0
94	0	0	144	11,797	1,449	0
100	0	1	0	5,097	5,735	0
103	90	0	18	714	492	0
104	0	0	48	1,614	999	0
105	9	0	95,680	4,962	240	0
106	0	0	1,200	91		0
107	0	0	216	1,522	162	0
109	0	0	0	948		0
110	0	0	0	0	0	0

APPENDIX 1 (Continued)

Table 7 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual porkers during period May, 1965 - July, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
150	0	0	0	15	6	0
151	-	0	0	-	-	0
152	2	0	0	121	151	0
153	3	0	0	3		0
173	1	0	0	0	0	0
174	0	0	0	24	0	0
175	0	0	0	0	2	0
176	2	0	0	0	3	0
179	0	2	0	0	0	0
184	0	0	0	1		0
185	0	0	0	0	0	0
186	0	0	0	0	0	0
192	3	0	0	0	3	0
193	0	0	0	372	22	0
194	-	0	0	-	-	0
195	-	1	-	-	-	0
196	-	3	-	-	-	0
197	-	3	-	-	-	0
198	-	4	-	-	-	0
199	-	2	-	-	-	0

APPENDIX 1 (Continued)

Table 8 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual baconers during period May, 1965 - July, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
160	0	0	0	180	0	0
161	0	0	0	1,528	1,160	0
162	0	0	0	0	0	0
163	77	5	0	0	0	0
177	0	0	0	12	0	0
178	2	0	0	3	0	0
180	1	2	0	0	0	0
181	0	9	0	0	0	0
182	6	0	0	0	0	0
183	0	0	0	0	0	0
187	0	1	0	0	0	0
188	0	0	0	0	0	0
189	0	0	0	20	29	0
190	0	0	0	3		0
191	0	0	0	0	0	0

APPENDIX 1 (Continued)

Table 9 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual sows during period May, 1965 - July, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
154	0	0	0	0	0	0
155	0	0	0	18	2	0
156	0	0	60	282	298	0
157	0	3	3,240	3,331	101	0
158	0	0	66	9,678	8,009	0
159	0	0	0	633	312	0
164	0	0	24	1,134	117	0
165	6	0	150	921	0	0
166	9	0	-	373	311	0
167	0	1	0	336	8	0
168	24	0	0	3,155	2,672	0
169	0	0	1,140	123	3	0
170	0	0	0	0	0	0
171	0	0	5,640	456	14	0
172	0	0	4,320	283	76	0

APPENDIX 1 (Continued)

Table 10 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual porkers during period August, 1965 - October, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
216	0	0	0	910	48	0
217	0	0	-	8	1	0
218	33	0	0	0	0	0
219	0	0	0	81	108	0
235	1	0	0	0	0	0
236	4	11	0	3	0	0
237	24	1	-	138	51	0
238	0	3	0	1	0	0
239	0	0	-	0	0	0
240	0	0	0	0	0	0
241	3	2	0	3		0
242	0	7	-	14	0	0
243	0	2	-	0	0	0
244	1	0	0	0	986	0
245	0	3	0	15	0	0

APPENDIX 1 (Continued)

Table 11 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual baconers during period August, 1965 - October, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
205	1	0	0	0	0	0
206	0	2	0	0	0	0
207	1	0	0	0	0	0
208	2	18	-	563	126	0
209	0	0	0	1,797	1,067	0
210	0	0	0	3	0	0
211	0	3	0	117	0	0
212	1	0	0	0	0	0
213	-	0	-	-	-	0
214	0	2	0	0	0	0
215	0	2	0	0	0	0
226	0	0	0	113	22	0
227	1	4	0	38	19	0
228	-	0	0	-	-	0
229	0	0	0	0	0	0

APPENDIX 1 (Continued)

Table 12 - Scottish survey: numbers of gastro-intestinal helminths recovered from individual sows during period August, 1965 - October, 1965

Pig No.	Nos. of helminths					
	T. suis	A. suum	H. rubidus	Oe. dentatum	Oe. quadrispinulatum	Others
200	0	0	0	432	82	0
201	0	0	0	6		0
202	0	0	0	417	62	0
203	0	0	7,680	210	290	0
204	0	0	0	42	2	0
220	35	9	0	11,918	293	0
221	0	1	0	386	787	0
222	1	0	0	97	59	0
223	13	0	0	1,011	218	0
224	0	0	0	178	79	0
230	0	0	0	48	22	0
231	0	0	0	75	122	0
232	1	0	0	55	20	0
233	0	0	0	9	0	0
234	0	0	432	6,344	4	0

APPENDIX I (Continued)

Table 13 - Scottish survey: numbers of Metastrongylus apri recovered from individual pigs during 1965

Month	No. baconers examined	No. sows examined	Individual worm counts: negative values not recorded					
			Baconers				Sows	
January	113	12	13	86	13	3	10	16
February	170	3	6 15 1	3 1	75 22	21 5	7	
March	224	1	4 257 1 1 5	15 12 13 65	1 372 2 3	26 45 19 5		
April	260	4	12 1 82	36 1 9	7 3 22	3 1	1	
May	279	2	6 54 89	1 19 90	122 233 8	35 47	3	9
June	353	0	14 49 3	59 2 197	5 14 1	3 1 20		
July	244	4	359	1	2			
August	192	1	1 1 2 1 8 1 3	10 1 33 1 1 1	11 31 6 4 40 2	2 20 6 2 3 5		
September	210	0	19 15 1	4 42 2	2 30 51	25 2		
October	119	2	5 7	3 1	8	20		
November	167	2	3 15 1	18 70	14 8	95 133		
December	182	2	26 6 5 3 1	37 7 2 35* 7	4 35 1 2 1	30 12 2 3 1		

* includes 1 M. pudendotectus

APPENDIX 1 (Continued)

Table 14 - The distribution of two Oesophagostomum species along the intestine of three Scottish sows. The nematode population of P158 was undergoing spontaneous expulsion

Pig No.	P94		P156		P158	
	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.
Cae	168	252	0	45	88	1,379
1	381	120	5	25	36	792
2	7,114	790	6	45	36	92
3	1,713	35	37	92	35	185
4	1,950	0	44	34	37	148
5	270	0	45	0	109	137
6	0	0	34	17	449	175
7	0	0	33	0	1,188	112
8	0	0	27	0	2,358	590
9	0	0	18	0	799	226
10	0	0	30	0	667	28
11	0	0	15	0	646	29
rectum	0	0	18	0	6,928	288

Cae = Caecum 1,2,3 etc. = sections of large intestine

Oe. d. = Oe. dentatum Oe. q. = Oe. quadrispinulatum

APPENDIX 1 (Continued)

Table 15 - Danish survey: numbers of helminths recovered from individual sows

Sow No.	Ascaris	Trichuris	Oe. dentatum	Oe. quadrispinulatum
SX 1	-	0	1,784	5,496
2	0	0	3,223	191
3	2	0	4,930	4,380
4	0	20	630	830
5	0	2	93	4
6	-	0	3,670	680
7	-	0	70	1,970
8	0	0	7,200	250
9	0	0	6,050	1,400
10	0	0	290	0
11	5	0	2	0
12	0	17	21	6
13	0	0	6,970	8,210
14	-	0	2,300	1,080
15	0	0	2	8
16	-	0	8,750	950
17	0	0	5,050	450
18	-	0	1,010	300
19	-	30	100	30
20	0	0	0	220
21	-	0	2,360	560
22	-	0	190	10
23	1	0	1,480	560
26	-	10	80	60
27	-	0	2,140	0
28	-	10	1,240	1,180
29	-	0	840	0
30	-	0	30	10
31	-	0	10	0

APPENDIX 1 (Continued)

Table 16 - Distribution of two Oesophagostomum spp. along the large intestine of Danish sows

Pig No.	SX1		SX2		SX3		SX4		SX5		SX6		SX7	
	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.	Oe. d.	Oe. q.
Cae	20	1,080	170	105	70	2,390	10	180	0	0	370	310	10	1,060
1	200	2,160	280	20	420	1,080	0	270	0	0	690	120	40	460
2	260	1,180	670	30	840	640	30	190	5	0	1,260	160	0	220
3	320	540	880	20	1,340	160	110	270	0	0	1,240	90	20	170
4	380	340	450	10	1,340	60	320	380	8	0	100	0	0	60
5	200	60	270	0	830	40	100	110	16	0	0	0	0	0
6	340	0	280	0	60	0	50	50	22	0	0	0	0	0
7	20	0	120	5	20	0	10	10	32	0	10	0	0	0
8	0	0	64	0	10	10	0	0	10	0	0	0	0	0
9	10	10	25	1	0	0	0	0	0	0	0	0	0	0
10	0	0	14	0	0	0	0	0	0	0	0	0	0	0

Cae = Caecum 1,2,3 etc. = sections of colon

Oe. d. = Oe. dentatum Oe. q. = Oe. quadrispinulatum

APPENDIX I (Continued)

Table 17 - Distribution of male and female Oesophagostomum along the large intestine and the distribution of the Oesophagostomum species in relation to the mucosal surface area

Sow No. SX4

	Oe. d.		Oe. q.		Area of mucosal surface (sq. cms.)	No. Oe. d. per sq. cm.	No. Oe. q. per sq. cm.
	No. Male	No. Female	No. Male	No. Female			
Cae	0	10	60	110	1,141	0.009	0.148
1	0	0	130	140	1,040	0	0.260
2	20	10	90	70	1,105	0.027	0.145
3	20	90	40	120	1,300	0.085	0.123
4	130	190	20	40	1,040	0.308	0.058
5	30	70	0	10	745	0.134	0.013
6	20	30	0	0	715	0.070	0
7	0	10	0	0	710	0.011	0
8	0	0	0	0	780	0	0
9	0	0	0	0	745	0	0
10	0	0	0	0	715	0	0

Cae = Caecum 1,2,3 etc. = sections of colon

Oe. d. = Oe. dentatum Oe. q. = Oe. quadrispinulatum

APPENDIX I (Continued)

Table 18 - The lengths of worms recovered from different points along the intestine

	Oe. quadrispinulatum Males							Oe. dentatum Males						
Pig No.	SX1	SX2	SX3	SX4	SX5	SX6	SX7	SX1	SX2	SX3	SX4	SX5	SX6	SX7
SI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cae	36.8	33.7	37.2	38.2	-	36.8	36.7	-	34.3	41.7	-	-	42.8	-
1	38.3	39.0	35.5	37.0	-	35.7	35.2	40.0	35.9	34.6	-	-	42.5	36.0
2	38.4	47.0	36.9	36.9	-	34.5	34.1	37.4	38.1	40.1	39.0	-	42.2	-
3	38.8	39.0	35.3	38.3	-	31.5	33.4	38.7	42.1	38.6	43.0	-	42.1	33.0
4	36.8	22.0	37.8	38.0	-	-	31.5	39.6	36.7	37.1	38.2	45.0	36.1	-
5	37.0	-	33.0	-	-	-	-	42.0	35.1	37.6	38.7	44.0	-	-
6	-	-	-	-	-	-	-	38.0	40.4	42.0	38.5	45.7	-	-
7	-	-	-	-	-	-	-	-	39.1	-	-	42.0	45.0	-
8	-	-	37.0	-	-	-	-	-	43.6	-	-	-	-	-
9	-	-	-	-	-	-	-	-	39.3	-	-	-	-	-
10	-	-	-	-	-	-	-	-	47.7	-	-	-	-	-
Average	37.9	34.5	36.7	37.3	-	35.7	35.2	39.2	39.0	38.2	38.8	43.7	42.1	34.5
No. examined	91	518	226	32	-	21	106	29	167	214	22	14	204	2

SI = small intestine Cae = Caecum 1,2,3 etc. = sections of large intestine

SX1, SX2, SX3 etc. = code numbers of Danish sows.
Measurements are in opisometer units (45 units = 10 mm.)

APPENDIX 1 (Continued)

Table 18 - The lengths of worms recovered from different points along the intestine

	Oe. quadrispinulatum Males							Oe. dentatum Males						
Pig No.	SX1	SX2	SX3	SX4	SX5	SX6	SX7	SX1	SX2	SX3	SX4	SX5	SX6	SX7
SI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cae	36.8	33.7	37.2	38.2	-	36.8	36.7	-	34.3	41.7	-	-	42.8	-
1	38.3	39.0	35.5	37.0	-	35.7	35.2	40.0	35.9	34.6	-	-	42.5	36.0
2	38.4	47.0	36.9	36.9	-	34.5	34.1	37.4	38.1	40.1	39.0	-	42.2	-
3	38.8	39.0	35.3	38.3	-	31.5	33.4	38.7	42.1	38.6	43.0	-	42.1	33.0
4	36.8	22.0	37.8	38.0	-	-	31.5	39.6	36.7	37.1	38.2	45.0	36.1	-
5	37.0	-	33.0	-	-	-	-	42.0	35.1	37.6	38.7	44.0	-	-
6	-	-	-	-	-	-	-	38.0	40.4	42.0	38.5	45.7	-	-
7	-	-	-	-	-	-	-	-	39.1	-	-	42.0	45.0	-
8	-	-	37.0	-	-	-	-	-	43.6	-	-	-	-	-
9	-	-	-	-	-	-	-	-	39.3	-	-	-	-	-
10	-	-	-	-	-	-	-	-	47.7	-	-	-	-	-
Average	37.9	34.5	36.7	37.3	-	35.7	35.2	39.2	39.0	38.2	38.8	43.7	42.1	34.5
No. examined	91	518	226	32	-	21	106	29	167	214	22	14	204	2

SI = small intestine Cae = Caecum 1,2,3 etc. = sections of large intestine

SX1, SX2, SX3 etc. = code numbers of Danish sows.
Measurements are in opisometer units (45 units = 10 mm.)

APPENDIX 2

Table 1 - The recognition of the 3rd stage larvae of H. rubidus and Oesophagostomum spp.

(From Kotlan and Vajda (1939) and Alicata (1935)).

	H. rubidus	Oesophagostomum spp.
Length:	780 - 850 μ [1] 715 - 736 μ [2]	560 - 600 μ 500 - 532 μ
Breadth:	24 - 26 μ [1] 22 μ [2]	28 - 30 μ 22 - 26 μ
Appearance of cuticle:	finely striated	coarsely striated
Tail 3):	digitiform process	rounded point
Movement:	very active, can swim	comparatively sluggish, does not swim

1) Kotlan and Vajda

2) Alicata

3) "Tail" refers to the larva itself which lies within the cuticle shed at the second moult.

APPENDIX 2 (Continued)

Table 2 - Egg-counts from sows on Farm B

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Sows - 1st-14th weeks of pregnancy	100	0	100	100	50	50	85	480
	0	100	0	1,300	0	0		
	0	200	0	0	0	200		
	0	1,200	0	300	0	100		
	800	0	200	2,000	900	1,300		
	0	500	0	0	100	300		
	600	0	200	500	700	0		
	0	1,000	0	0	100	0		
	1,900	0	300	0	0	0		
	100	600	600	0	0	400		
	0	3,200	400	600	2,900	200		
	900	0	0	300	1,700	400		
	2,100	3,200	100	200	0	0		
	1,000	0	2,100	100	3,000	1,800		
Sows - 15th week of pregnancy	1,000	500					2	750
Sows - 16th week of pregnancy	7,400	100	200	2,400	200	0	26	1,731
	300	1,900	0	900	200	100		
	200	1,100	600	1,400	700	100		
	1,800	0	100	14,000	7,600	2,700		
	300	900						
Sows - 1st week after parturition	400	4,900	1,200	1,000	1,300	4,500	30	1,607
	800	500	800	0	100	300		
	0	200	1,000	800	0	1,700		
	1,000	5,800	800	1,000	1,100	3,200		
	500	300	8,500	0	1,100	5,400		
Sows - 2nd week after parturition	5,300	1,100	8,400	2,000	600	2,600	28	3,032
	100	200	2,500	900	1,800	0		
	600	0	0	25,000	1,000	400		
	800	1,900	200	0	400	11,200		
	1,000	10,300	2,000	4,600				
Sows - 3rd week after parturition	2,700	2,200	1,400	4,600	7,200	500	15	2,987
	3,000	100	800	400	12,200	3,700		
	0	3,200	2,800					
Sows - 4th week after parturition	7,500	2,800	100	1,400	100	400	20	2,865
	2,100	3,000	2,200	800	20,200	2,200		
	3,900	1,100	2,200	3,100	2,700	1,500		
	0	0						

APPENDIX 2 (Continued)

Table 2 (Continued)

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Sows - 5th week after parturition	5,400 1,600 200	200 11,200 0	5,600 300 100	5,700 5,100 200	0 12,900 3,500	2,900 5,200 2,300	18	3,467
Sows - 6th week after parturition	2,400 1,200	1,900 8,000	300 2,100	10,400 0	8,400 1,000	2,200 400	12	3,192
Sows - 7th week after parturition	200	30,600	0	34,500	4,200	100	6	11,600
Sows - 8th week after parturition	600	900					2	750
Sows - 9th week after parturition	800						1	800
Boars	300 0	0 0	700 300	0 700	100 600	0 300	12	250
Piglets - up to 1 week old	0	0	0	0			4	0
Piglets - up to 2 weeks old	0 0 0	0 0 0	0 0	0 0	0 0	0 0	14	0
Piglets - up to 3 weeks old	0 0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	19	0
Piglets - up to 4 weeks old	0 0 0	0 0 0	100 0	0 0	0 0	0 0	14	7
Piglets - up to 5 weeks old	0 0 0	0 0 0	0 0	0 0	0 0	100 0	14	7
Piglets - up to 6 weeks old	0 0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	19	0
Piglets - up to 7 weeks old	100 0 0	0 100 0	0 100	0 0	100 0	100 0	14	36

APPENDIX 2 (Continued)

Table 2 (Continued)

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Piglets - up to 8 weeks old	0	0	0	0	0	0	6	0
	300	0	0	0	0	0		
	100	300	0	0	0	100		
	0	0	0	0	0	0		
	1,300	0	0	0	0	0		
	100	0	0	0	0	0		
	0	0	100	0	0	0		
	0	100	0	0	0	0		
	0	0	0	0	0	0		
	400	0	0	0	0	0		
Fatteners - 8 weeks-5 months	0	100	0	0	0	0	128	114
	0	100	0	0	0	0		
	0	0	200	0	0	0		
	0	0	0	6,000	100	100		
	100	0	600	1,100	0	0		
	0	0	200	0	0	0		
	500	0	200	100	0	500		
	0	0	0	0	0	300		
	100	0	100	0	0	0		
	0	0	0	0	0	100		
	0	0	0	100	0	0		
	0	0	0	200	0	100		
	0	900						

APPENDIX 2 (Continued)

Table 3 - Egg-counts from sows on Farm C

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Sows - 1st-14th weeks of pregnancy	50	100	100	50	200	100	107	301
	300	0	0	0	200	100		
	0	0	0	200	500	300		
	0	300	0	0	0	400		
	0	200	0	0	0	0		
	0	0	0	100	0	0		
	0	0	100	0	0	0		
	0	300	0	0	0	0		
	300	0	0	0	100	200		
	0	0	0	0	0	700		
	100	900	2,200	1,400	300	50		
	400	300	300	200	0	700		
	100	3,500	0	600	800	800		
	300	300	500	200	500	200		
	900	1,600	1,600	0	0	0		
	800	0	0	0	0	700		
	100							
Sows - 15th week of pregnancy	700	400	500	200	500	0	7	714
	2,700							
Sows - 16th week of pregnancy	1,400	0	100	200	100	200	9	444
	700	800	300					
Sows - 1st week after parturition	300	300	0	300	800	1,200	13	538
	800	100	500	900	1,100	500		
	200							
Sows - 2nd week after parturition	100	400	2,700	100	700	4,500	8	1,275
	1,700	0						
Sows - 3rd week after parturition	1,300	700	4,300	700	400	0	12	959
	0	1,500	500	1,500	500	100		
Sows - 4th week after parturition	400	0	1,200	1,500	400	200	12	609
	0	1,900	200	0	1,400	100		
Sows - 5th week after parturition	1,700	1,000	2,100	700	5,500	100	10	1,250
	700	500	100	100				
Sows - 6th week after parturition	0	0	0	1,800	2,700	0	14	407
	300	400	0	0	300	100		
	0	100						

APPENDIX 2 (Continued)

Table 3 (Continued)

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Sows - 7th week after parturition	700 1,100	700 1,100	1,100 900	300 600	50 1,200	900	11	786
Sows - 8th week after parturition	3,200 0	100 0	800	100	500	200	8	613
Sows - 9th week after parturition	800 300	300 100	0	400	600	4,400	8	863
Boars	200 0 0	0 0	0 300	0 100	0 0	0 0	13	30
Piglets - up to 2 weeks old	0	0	0				3	0
Piglets - up to 4 weeks old	0	0	0	0	0		5	0
Piglets - up to 5 weeks old	0	0	0	100	0	0	6	17
Piglets - up to 6 weeks old	0	0	0	0			4	0
Piglets - up to 7 weeks old	0 0	0 0	0	100	100	0	8	25
Piglets - up to 8 weeks old	0	0	0	0	0		5	0
Piglets - up to 9 weeks old	0	200	0	0	0		5	40
Fatteners - 9 weeks - 5 months	0 0 100 0 0 400 0 300 100 0 7,200 400 0 0 0 200 700	0 100 0 200 0 0 600 0 0 300 200 100 0 0 0 0	2,300 100 0 200 0 0 0 0 0 300 100 300 0 0 0 0	0 100 200 0 0 0 0 0 0 0 0 300 500 0 0 0 300	200 0 0 0 0 0 0 100 0 300 0 3,100 0 0 0 0	100 0 0 0 100 100 100 0 100 200 12,700 0 0 0 0 0	96	341

APPENDIX 2 (Continued)

Table 4 - Egg-counts from sows on Farm EC

Reproductive Status	No. 'strongyle' eggs per gramme faeces						No. of Samples Examined	Average e.p.g.
Sows - 1st week after service	3,300 3,100 900	19,500 9,800 27,400	6,400 12,000 3,200	1,100 0 14,000	19,800 300 4,400	9,900 2,400	17	8,100
Sows - 2nd week after service	1,500 200 10,000	600 27,500 19,900	8,200 22,700 700	9,500 7,200	7,100 1,600	10,700 12,000	15	9,300
Sows - 3rd week after service	800 1,600	6,700 900	12,300 8,100	5,400 11,600	23,800 7,900	10,800	11	8,200
Sows - 4th week after service	8,000 1,200 1,100	3,400 3,700 4,500	2,300 33,200 8,500	3,800 7,100 100	5,300 7,600 5,700	4,700 700	17	6,500
Sows - 5th week after service	4,700 8,800 500	7,900 13,600 7,100	1,100 10,400 1,100	3,000 18,700 3,600	23,600 2,100 400	7,100 100 14,100	18	7,100
Sows - 6th week after service	1,600 6,500 15,000 16,900	9,100 26,700 11,800 21,000	2,200 4,800 4,200 9,800	7,100 6,100 2,900 2,900	11,700 100 9,400	2,900 100 600	22	7,900
Sows - 7th week after service	1,100 4,600 1,700 1,600	1,600 1,400 300 5,500	23,600 3,800 1,700 6,700	5,800 100 4,100 400	4,300 12,400 2,300 400	11,600 6,500 21,700 10,700	24	5,600
Sows - 8th week after service	3,000 11,200 3,000	5,900 14,800 11,900	12,700 6,800 39,200	24,900 300 8,700	9,000 8,400 2,800	2,900 7,600 3,200	18	9,800
Sows - 9th week after service	17,700 19,700 2,000 400	3,000 11,400 600 5,300	6,400 31,400 6,000 5,200	35,400 1,000 700 1,000	7,500 6,300 3,500	800 17,900 1,000	22	8,300
Sows - 10th week after service	1,300 4,800 16,100	900 6,600 200	8,800 11,700 5,600	27,600 2,800 7,000	10,400 5,800 6,500	31,500 8,700 7,800	18	9,100
Sows - 11th week after service	5,100 6,900 1,100 12,100	4,700 21,800 1,000 17,800	9,000 4,800 1,500 200	14,300 4,700 1,400	1,900 500 3,300	11,100 4,700 4,700	21	6,300

APPENDIX 2 (Continued)

Table 4 (Continued)

Reproductive Status	No. 'strongyle' eggs per gramme faeces						No. of Samples Examined	Average e.p.g.
Sows - 12th week after service	5,500	2,100	5,300	1,500	4,300	22,400	20	7,400
	5,100	6,600	30,600	7,500	7,100	5,400		
	8,900	3,100	14,400	700	4,000	300		
	10,600	2,400						
Sows - 13th week after service	25,900	3,600	20,100	1,700	2,400	5,200	24	7,300
	4,900	11,300	7,400	1,700	15,300	4,600		
	11,100	6,100	1,300	11,000	6,100	3,600		
	9,200	2,300	2,500	9,700	1,900	9,200		
Sows - 14th week after service	1,000	5,600	15,600	6,600	19,500	6,400	21	7,300
	2,200	4,600	800	4,400	12,100	3,800		
	13,300	14,300	4,900	4,000	1,700	1,200		
	13,300	5,800	4,600					
Sows - 15th week after service	100	200	1,200	600	4,300	15,800	26	7,500
	2,000	4,400	27,900	2,200	9,100	4,500		
	7,800	2,000	6,100	600	27,200	38,400		
	12,100	1,900	2,300	7,300	2,100	12,800		
	2,900	1,100						
Sows - 16th week after service	2,000	24,400	16,600	4,000	6,800	22,200	18	10,600
	2,100	29,600	4,700	700	16,800	2,800		
	6,000	15,500	2,200	9,300	4,300	100		

APPENDIX 2 (Continued)

Table 5 - 'Strongyle' egg-counts from Sow MJK 52

Date	Average e.p.g.	Date	Average e.p.g.
1965			
3.11	75	10.2	600 (+ 175 A. suum)
4.11	150	15.2	4,200
8.11	50	17.2	7,725
11.11	125 (+ B. coli)	21.2	13,800
12.11	100 (+ B. coli)	24.2	9,900
13.11	100	28.2	7,925
15.11	250		
16.11	300	3.3	2,000 (+ 125 A. suum + B. coli)
17.11	100		
18.11	125	7.3	5,325 (+ 225 A. suum + oocysts)
22.11	650		
23.11	400	10.3	4,100 (+ 125 A. suum)
24.11	175	14.3	15,975 (+ 25 A. suum + 25 T. suis)
25.11	275		
26.11	200	17.3	1,375 (+ 150 A. suum)
		21.3	19,450 (+ 75 A. suum + 25 T. suis)
2.12	25 (+ 25 A. suum)		
6.12	175 (+ 25 A. suum)	24.3	5,850 (+ 25 A. suum)
9.12	50 (+ 650 A. suum)	28.3	5,100
13.12	3,225 (+ 175 A. suum)	31.3	15,200 (WEANED)
20.12	150 (+ 200 A. suum)		
23.12	1,350 (+ 475 A. suum)	4.4	17,900
27.12	2,125 (+ 175 A. suum)	6.4	6,550 (ON HEAT?)
30.12	2,725 (+ 275 A. suum)	12.4	650
		15.4	550
1966		18.4	450
		21.4	900
3.1	2,225	22.4	900
10.1	1,850 (+ 300 A. suum)	25.4	225
15.1	4,250 (+ 75 A. suum)		
17.1	5,750 (+ 650 A. suum)	28.4	250 (+ 50 T. suis)
20.1	1,125	29.4	550 (ON HEAT?)
24.1	925 (+ 175 A. suum)	30.4	2,925
27.1	9,075 (+ 200 A. suum)		
31.1	7,075 (+ 175 A. suum)	1.5	2,975
		4.5	350
3.2	1,750 (FARROWED)	7.5	1,650 (+ oocysts)
7.2	1,958 (+ 125 A. suum)	12.5	850 (+ oocysts)

APPENDIX 2 (Continued)

Table 6 - 'Strongyle' egg-counts from Sow MJK 53

Date	Average e.p.g.	Date	Average e.p.g.
1965			
2.11	4,500	28.12	175
3.11	2,925	29.12	300
4.11	10,625	30.12	225
8.11	1,700	31.12	100
11.11	800		
12.11	1,425	1966	
13.11	575	3.1	100
15.11	825	10.1	250 (+ 25 *T. suis*)
16.11	700	11.1	400
17.11	675	12.1	625 (+ 50 *T. suis*)
18.11	450	13.1	425
22.11	550	14.1	825 (OESTRUS)
23.11	800	17.1	1,225 (OESTRUS)
24.11	1,350	18.1	1,300 (OESTRUS)
25.11	2,025 (+ 25 *T. suis*)	19.1	600
26.11	1,925	20.1	275
29.11	1,950	21.1	175
30.11	1,900 (FARROWED AND WEANED)	24.1	275
		25.1	500
1.12	1,375	26.1	275
2.12	1,300	27.1	300 (+ 25 *T. suis*)
3.12	1,425	28.1	225
6.12	975	31.1	275
7.12	925 (+ 25 *T. suis*)		
8.12	225 (+ 25 *T. suis*)	1.2	300
9.12	825 (+ 25 *T. suis*)	2.2	75
10.12	1,325	3.2	200
13.12	350	4.2	275
14.12	25	7.2	275
15.12	150	8.2	400
20.12	25 (+ 25 *T. suis* + *B. coli*)	9.2	25
21.12	100	10.2	75
22.12	0	11.2	75
27.12	200	15.2	950

APPENDIX 2 (Continued)

Table 6 (Continued)

Date	Average e.p.g.	Date	Average e.p.g.
16.2	375 (+ 25 *T. suis*)	18.4	775
17.2	275	19.4	1,250
18.2	725	20.4	2,350
21.2	550	21.4	2,025
22.2	675	22.4	1,975
23.2	1,150	25.4	625
24.2	625	26.4	1,875 (GONADOPLEX)
25.2	25	27.4	550
28.2	100	28.4	225
		29.4	175
1.3	50 (+ 25 *T. suis*)	30.4	325
2.3	0 (+ 25 *A. suum*)		
3.3	0	1.5	525
7.3	400 (+ oocysts)	2.5	550
8.3	575	3.5	300
9.3	975	4.5	325
10.3	2,225	5.5	300
14.3	2,350	7.5	525
15.3	2,525	9.5	350
16.3	325	12.5	825
17.3	125	15.5	400
21.3	200	18.5	1,100
22.3	400	20.5	1,300
23.3	175	24.5	350
24.3	50	25.5	400
28.3	1,525	26.5	475
29.3	1,050	27.5	2,525
30.3	1,075	28.5	1,250
31.3	1,050	30.5	1,750
		31.5	1,650
4.4	450		
5.4	425		
7.4	200	1.6	1,525
12.4	75	2.6	2,500
13.4	225	3.6	2,075
15.4	875	6.6	3,200 (+ 25 *T. suis*)

APPENDIX 2 (Continued)

Table 6 (Continued)

Date	Average e.p.g.		Date	Average e.p.g.
7.6	4,975		1.8	325
9.6	950		2.8	775
13.6	1,350		3.8	375
14.6	975		4.8	1,200
16.6	1,650		5.8	1,150
20.6	3,700		6.8	675
21.6	1,375		7.8	475
22.6	650			
23.6	875			
24.6	1,375			
27.6	5,850			
28.6	1,175			
29.6	5,100			
30.6	400	(+ oocysts)		
1.7	600	(+ 25 A. suum)		
4.7	75	(PROGESTERONE THERAPY STARTS)		
5.7	200			
6.7	0			
7.7	75			
8.7	0			
12.7	0			
13.7	0			
15.7	0			
18.7	75			
20.7	0			
21.7	500			
23.7	500			
24.7	800	(PROGESTERONE + ACTH. THERAPY STARTS)		
25.7	275			
26.7	300			
27.7	150			
28.7	200			
31.7	1,100			

APPENDIX 2 (Continued)

Table 7 - Casualty sows slaughtered at 'Kødfoderfabrikken Sjælland'

Post mortem reports suppled by Chief Veterinarian U. Biering-Sørensen

Sow No.	
SX2	Pregnant foetus 15.8 cms. long. Uraemia.
SX3	Metritis following difficult farrowing.
SX4	Prolapsed uterus.
SX5	Pregnant, foetus 19.6 cms. long. 'Heart failure' associated with muscle degeneration.
SX8	'Heart failure' during parturition.
SX9	'Heart failure' after parturition.
SX10	Haemorrhage from spleen soon after parturition.
SX11	Pregnant, foetus 32 cms. long. 'Heart failure' associated with muscle degeneration.
SX12	'Heart failure' during parturition. Fatty liver.
SX13	'Heart failure' during parturition.
SX15	Metritis. Foetuses dead and decomposing, but fully formed.
SX17	'Heart failure' during parturition.
SX20	Post operative shock after Caesarian section.
SX23	'Heart failure' during parturition. Pulmonary oedema.

APPENDIX 2 (Continued)

Table 8 - Fecundity of female Oesophagostomum

From non-pregnant sows

Sow No.	'Strongyle' e.p.g.	No. of female worms	Fecundity index*
SX16	3,900	4,900	0.80
SX29	1,200	2,200	0.55
SX21	1,000	2,080	0.48
SX 6	1,100	2,030	0.54
SX28	1,600	1,980	0.81
SX14	1,000	1,740	0.58
SX27	450	1,260	0.35
SX 7	500	930	0.53
SX18	1,000	360	2.78
SX22	300	130	2.31
SX31	250	130	1.92
SX19	300	70	4.29
SX26	50	60	0.83
SX30	100	20	5.00
SX24	0	0	-
Average	917	1,192	-

From parturient sows

SX13	400	7,760	0.05
SX 3	5,700	4,890	1.17
SX 9	2,900	3,950	0.73
SX 8	5,500	3,825	1.44
SX17	1,300	3,150	0.41
SX 2	1,100	1,813	0.61
SX23	100	1,220	0.08
SX 4	500	900	0.56
SX10	200	160	1.25
SX20	100	70	1.43
SX 5	100	49	2.04
SX12	0	14	-
SX15	0	2	-
SX11	0	0	-
Average	1,279	1,841	-

* Fecundity index = $\frac{\text{'Strongyle' e.p.g.}}{\text{No. of female worms}}$

APPENDIX 2 (Continued)

Table 9 - Length of female worms

From non-pregnant sows

Sow No.	Oe. dentatum		Oe. quadrispinulatum	
	No. measured	Average length*	No. measured	Average length*
SX 1	54	45.84	125	44.28
SX 6	159	32.88	39	36.13
SX 7	5	34.8	77	36.53
SX14	61	39.89	26	41.42
SX16	86	44.20	7	37.71
SX18	29	41.45	7	42.14
SX19	5	38.00	-	-
SX21	20	29.55	4	30.25
SX22	12	21.33	-	-
SX26	3	26.67	3	29.67
SX27	63	42.32	-	-
SX28	53	41.42	45	39.18
SX29	45	33.38	10	39.10
SX31	13	29.69	-	-
		From parturient sows		
SX 2	192	42.10	12	35.42
SX 3	279	40.93	178	39.57
SX 4	41	40.98	48	42.69
SX 5	15	54.80	14	43.71
SX 8	59	45.41	-	-
SX10	12	46.66	-	-
SX12	-	-	4	41.25
SX13	56	33.88	117	38.15
SX17	52	44.56	6	40.83
SX20	-	-	7	46.57
SX23	41	37.88	14	37.72

* measured in opisometer units (45 units = 10 mm.).

APPENDIX 2 (Continued)

Table 10 - Statistical analysis of comparison between Oesophagostomum spp. populations from non-pregnant and parturient sows

The following is an English translation of a report prepared by Mr. Nørgaard and Miss Gerløv of the Statistics Unit of the Danish Meat Research Institute. I am most grateful for their co-operation.

1) Studies on the relationship Fecundity Index: Numbers of female worms.

   The scatter of the results from the Ortved series is greater than the Roskilde, i.e. the Ortved females can lay more eggs but do not invariably do so.

2) No statistical difference in the lengths of Oe. dentatum and Oe. quadrispinulatum females could be shown, but the variation within the groups was so great that it was not thought wise to use Oesophagostomum spp. for future calculations.

3) Oe. dentatum females from Ortved are longer than those from Roskilde ($p < 0.01$).

4) There was a similar tendency for Oe. quadrispinulatum females but this was not statistically significant.

5) The examination of a further 20 sows would be desirable.

APPENDIX 4

Table 1 - The faecal 'strongyle' egg-counts of sows treated with thiabendazole plus picadex one week before parturition

	No. 'strongyle' eggs per gramme faeces						No. of Samples	Average e.p.g.
Sows - 1st-14th weeks of pregnancy	See Appendix 2, Table 4.							
Sows - 15th week of pregnancy	100 2,000 7,800 12,100 2,900	200 4,400 2,000 1,900 1,100	1,200 27,900 6,100 2,300	600 2,200 600 7,300	4,300 9,100 27,200 2,100	15,800 4,500 38,400 12,800	26	7,612
Sows - 16th week of pregnancy	600 4,000 700 300	2,000 6,800 16,800 15,500	24,400 22,200 2,800 2,200	0 2,100 0 9,300	900 29,600 100 4,300	16,600 4,700 600	23	8,284
Sows - 1st week after parturition	0 0 0	0 11,700 0	0 2,400	2,100 0	6,800 200	700 1,400	14	1,736
Sows - 2nd week after parturition	0 0	0 0	100 200	500 0	7,200 0	100 0	12	675
Sows - 3rd week after parturition	400 0 0	0 0 0	0 2,900	200 0	2,300 0	500 0	14	450
Sows - 4th week after parturition	0 200	500 100	0	0	700	1,300	8	350
Sows - 5th week after parturition	800 400	3,100 2,300	400 1,900	0 0	6,500	1,300	10	1,670
Sows - 6th week after parturition	300 0	300 1,000	100 300	10,200	0	1,000	9	1,367
Sows - 7th week after parturition	200 2,000	900 3,400	400	400	100	10,700	8	2,263
Sows - 8th week after parturition	400	3,900	600	0			4	1 225

APPENDIX 4 (Continued)

Table 2 - A comparison of the faecal 'strongyle' egg-counts of sows treated with dichlorvos and untreated controls. Treatment was given on Day 0

CONTROL GROUP

'Strongyle' egg-count (e.p.g.)

Week No.	Sow No. 1273	4826	669	4449	4915	1634	1668	1545	1245	4451	808	1626	4129	4420	4130
-1½	300	0	100	100	100	1,200	2,000	2,400	2,000	700	800	900	400	1,100	900
-1	200	0	0	0	0	1,800	-	700	-	500	500	1,400	700	800	1,600
-½	1,300	0	100	200	100	1,000	200	1,100	1,500	2,000	900	400	300	900	1,400
0	-	100	100	0	0	2,800	1,400	3,200	2,400	400	1,000	300	200	300	100
+½	700	0	200	0	100	1,100	1,000	500	1,700	800	1,100	800	0	700	-
+1	500	0	0	300	300	1,700	1,100	100	6,300	700	1,300	400	500	800	1,300
+1½	3,400	400	400	300	400	3,200	3,800	1,300	900	600	800	1,000	0	600	800
+2	4,600	0	100	500	200	1,900	3,100	1,000	-	-	-	600	200	3,200	1,500
3	6,100	100	500	700	500	1,600	1,200	2,900	500	-	600	1,100	200	2,600	-
4	900	400	100	400	200	1,200	-	1,400	300	500	400	800	0	600	900
5	-	-	400	500	-	1,200	-	3,000	-	2,200	600	1,100	0	900	700
6	-	-	0	6,400	-	100	-	1,400	1,000	400	500	1,300	0	600	500
7	-	-	300	300	-	-	-	800	900	700	800	500	0	100	100
8	-	-	200	400	-	-	-	800	200	700	400	100	0	200	-
9	-	-	100	300	-	-	-	500	-	1,000	2,200	500	-	800	-

Presence of Hyostrongylus

-1½															
-1															
-½															
0															
+½						+		+						+	
+1						+								+	
+1½						+		+			+	+			
2								+				+			
3												+			
4												+		+	
5						+									
6				+											
7															
8														+	
9															

APPENDIX 4 (Continued)

Table 2 (Continued)

TREATED - 1.15 g. DICHLORVOS

Week No.	Sow No. 1328	4356	548	6498	4571	4701	2367	807	1202	1412	564	4088	1195	4131	4719
-1½	200	500	0	900	2,100	1,300	600	1,000	1,000	300	500	1,600	1,100	300	100
-1	700	200	200	900	1,300	600	300	600	1,100	0	200	2,800	700	300	0
-½	1,100	300	100	600	2,600	3,200	600	1,400	1,500	100	100	1,900	900	100	300
0	2,200	100	600	1,200	4,600	1,400	300	4,100	800	0	700	1,800	300	0	100
+½	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
+1	0	0	0	0	0	0	0	100	0	100	0	0	-	0	0
+1½	0	0	0	0	100	2,500	100	0	0	0	0	0	0	0	0
+2	0	0	0	0	0	0	300	100	0	0	0	0	0	0	0
3	0	0	0	0	0	0	1,300	0	0	-	0	0	0	0	0
4	0	0	100	100	500	1,300	-	0	0	0	0	0	-	0	0
5	1,800	-	500	-	600	700	-	0	0	0	0	100	0	0	0
6	0	0	0	-	1,400	0	-	0	0	0	0	100	0	0	-
7	100	0	100	-	1,300	0	-	0	100	0	0	0	0	0	-
8	-	100	100	-	1,000	100	-	0	0	0	-	0	0	0	-
9															

Presence of Hyostrongylus

-1½							+								
-1							+								
-½					+				+					+	
0				+						+					
+½					+										
+1															
+1½					+		+								
+2															
3															
4															
5															
6					+										
7	+														
8															
9															

APPENDIX 4 (Continued)

Table 2 (Continued)

TREATED - 1.44 g. DICHLORVOS

Week No.	Sow No. 1633	5347	4863	4452	741	1479	1359	5467	2564	743	771	4353	4172	5118	5802
-1½	300	100	100	100	200	600	2,600	400	1,500	6,800	800	400	800	400	1,400
-1	400	700	500	0	1,000	700	4,000	400	700	300	400	1,700	200	900	2,400
-½	600	200	700	1,200	1,100	1,700	1,300	300	600	2,700	900	500	300	500	600
0	700	0	600	0	0	1,100	3,500	100	900	2,800	2,100	100	100	500	200
+½	0	0	200	0	0	0	0	0	0	0	0	0	200	0	0
+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+1½	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0
+2	0	100	0	100	100	0	100	0	200	0	100	0	100	0	0
3	0	0	100	0	100	0	0	0	100	0	200	0	600	0	0
4	-	-	0	-	-	0	200	0	100	0	100	0	600	0	0
5	800	-	300	-	-	0	0	-	0	200	0	0	0	0	100
6	0	-	400	-	-	-	0	-	0	0	400	0	200	0	1,000
7	0	-	1,000	-	-	-	1,100	-	0	200	400	0	200	0	500
8	0	-	1,100	-	-	-	300	-	0	600	200	0	100	-	400
9	0	-	1,800	-	100	-	200	-	100	200	2,100	100	0	-	700

Presence of Hyostrongylus

-1½															
-1	+														
-½	+										+				
0															
+½															
+1															
+1½															
+2															
3															
4							+								
5															
6															
7															
8															
9															

APPENDIX 4 (Continued)

Table 2 (Continued)

TREATED - 1.96 g. DICHLORVOS

Week No.	Sow No. 5367	4345	4838	5506	4703	2214	84	4861	4378	1230	1772	3516	4356	1631	5182
-1½	400	0	200	4,900	0	600	900	900	800	2,500	700	1,000	100	600	300
-1	1,300	400	100	2,000	900	1,100	500	400	400	2,000	300	2,300	100	800	1,300
-½	800	300	300	3,400	3,500	700	100	300	900	1,100	0	800	300	200	400
0	300	1,300	600	3,500	700	300	200	1,700	400	2,300	600	4,500	300	500	300
+½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+1	0	0	100	200	0	0	0	0	0	0	0	0	0	0	0
+1½	0	0	200	100	0	0	0	0	0	100	0	0	0	0	0
+2	0	0	0	0	0	-	0	300	0	100	0	600	0	0	0
3	200	400	0	0	0	100	0	100	200	0	0	0	0	0	0
4	-	-	0	-	-	100	0	0	0	0	0	0	0	0	0
5	-	-	-	-	-	100	100	0	0	0	0	0	0	0	0
6	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0
7	-	-	-	-	-	-	100	200	100	100	100	0	0	0	-
8	-	-	-	-	-	-	0	0	0	200	200	-	-	0	-
9	-	-	100	-	500	-	0	400	0	0	800	-	-	-	-
						Presence of Hyostrongylus									
-1½															
-1		+													
-½		+													
0															
+½															
+1½															
+2															
3															
4															
5															
6															
7															
8															
9															

APPENDIX 4 (Continued)

Table 3 - A comparison of the productivity (measured in terms of litter-size and growth rate) of sows given a single pre-natal treatment of 1.44 g. dichlorvos and that of untreated controls

Dam Sow No.	Sire Boar No.	No. of Pigs	At birth. Litter Weight lbs.	"3 week" weighing			"8 week" weighing		
				Age in Days	No. of Pigs	Litter Weight lbs.	Age in Days	No. of Pigs	Litter Weight lbs.
UNTREATED SOWS									
1	A	14	43	20	13	130	55	11	360
2	B	11	36	18	10	102	53	9	326
3	F	12	32	21	10	80	57	8	230
4*	F	8	24	24	10	140	59	10	476
5	C	16	42	21	11	121	56	11	415
6	B	10	28	19	8	70	54	6	117
7	D	13	34	19	9	111	54	9	313
8	E	12	38	18	10	76	53	10	267
9	E	14	42	18	11	114	53	11	337
10	F	12	30	22	11	112	55	11	396
11	B	12	28	22	9	76	55	7	169
12	F	10	30	22	8	78	57	6	177
13	F	10	28	21	8	77	56	8	242
14	D	9	36	19	9	104	54	9	360
15*	F	7	26	18	8	101	53	8	314
16	F	8	28	19	8	89	54	8	314
17	E	9	30	24	8	70	59	6	164
18	B	11	32	23	10	136	58	9	364
19	F	12	28	21	10	68	56	7	201
TREATED SOWS									
21	C	14	38	24	10	130	59	10	452
22*	B	12	30	24	10	108	59	10	370
23*	A	9	31	20	10	106	56	9	288
24	B	13	34	24	10	120	59	10	379
25*	A	11	36	21	13	138	56	12	381
26*	E	7	24	22	8	90	57	7	276
27	F	10	28	19	10	112	54	10	389
28	A	11	28	18	10	104	53	9	327
29	B	14	32	22	12	104	57	8	225
30	B	12	36	22	9	126	57	9	446
31	A	11	26	21	11	97	56	11	346
32**	B	12	34	22	10	88	57	9	214
33	C	13	48	21	12	158	56	12	539
34	F	9	22	20	5	35	55	5	162
35	B	9	26	18	8	56	53	6	135
36	A	11	28	18	9	76	53	9	238
37	E	11	32	24	8	80	59	8	296
38*	A	5	20	23	6	68	58	6	169
39	C	16	32	21	9	84	56	9	261

* includes pigs fostered from dams of the same group

** dam treated with antibiotics

APPENDIX 5

Table 1 - Method for the separation of albumen from the serum of pigs for the albumen turnover experiment (Experiment 5.2.)

1) Anhydrous sodium sulphate was added to the serum until a concentration of 18% was reached.

2) When all the Na_2SO_4 was dissolved, globulins were precipitated.

3) The supernatent was poured off and more Na_2SO_4 added to bring the concentration up to 12%. More globulin precipitated.

4) The supernatant was dialysed against 0.85% sodium chloride solution overnight.

5) If no sulphate remained (calcium chloride test), purity of albumin solution checked by cellulose acetate electrophoresis.

APPENDIX 5 (Continued)

Table 2 - Method for the labelling of pig albumen with radio-iodine (after McFarlane, 1958) for the albumen turnover experiment (Experiment 5.2.)

Reagents

1) Iodine monochloride. Solution containing 0.42 mg.I/ml.

2) Radio-active iodine. Carrier-free radio-iodine as $Na^{*}I$ free from thiosulphate.

3) Glycine buffer A pH 8.5: 9 ml. molar glycine in M/4 NaCl solution + 1 ml. N.NaOH.

4) Glycine buffer B pH 9.0: 8 ml. molar glycine in M/4 NaCl solution + 2 ml. N.NaOH.

Method

1) Add 1 ml. glycine buffer B to 2 ml. of serum albumin (approximately 2% solution).

2) To 0.5 ml. of an ICl solution containing 12.0 µc $^{*}I$, add 1 ml. glycine buffer A and immediately add to the buffered protein solution.

3) Add carrier albumin (in this instance bovine albumin was used) to bring activity below 5 µc./mg.

APPENDIX 5 (Continued)

Table 3 - Weights of ^{125}I-labelled albumin solution (approximately 2%) injected into each pig (Experiment 5.2.)

Pig No.		
1234	-	5.1962 gm.
1233	-	4.7547 gm.
1237	-	11.3336 gm.
1235	-	11.2272 gm.
1238	-	15.745 gm.
1236	-	17.120 gm.

Weights of ^{131}I-PVP solution (1.21%) injected into each pig (Experiments 5.3. and 5.4.)

Pig No.		
1752	-	2.0278 gm.
1751	-	2.0759 gm.
1753	-	1.7230 gm.
1749	-	1.7987 gm.
1748	-	2.0329 gm.
1871	-	2.7139 gm.
1869	-	2.6804 gm.
1870	-	2.9882 gm.
1865	-	3.0034 gm.
1872	-	2.9020 gm.

APPENDIX 5 (Continued)

Table 4 - The buffer solution used for the electrophoretic separation of serum proteins

Barbitone	7.36 gm.
Sodium Barbitone	41.2 gm.

Make up to 4 litres

Add 20 ml. 5% thymol in isopropyl alcohol.

APPENDIX 5 (Continued)

Table 5 - An example of the mathematic treatment of the data collected from the albumen turnover experiment (Experiment 5.2.)

Pig 1233 (Control)

Total injected activity:

Weight of syringe containing albumin solution: 14.8870 gm.
Weight of syringe after albumin injected into pig: 9.6908 gm.
Weight of albumin solution injected into pigs: 5.1962 gm.

Weight of albumin solution used to make standard: 1.3593 gm.
Made up to 250 ml.

Activity of standard: 3029 cts./sec./ml.
Total standard activity = 3029 x 250 = 757,250 cts./sec.

$$\text{Total injected activity} = \frac{757{,}250 \times 5.1962}{1.3593} = 2{,}894{,}742 \text{ cts./sec.}$$

Plasma volume:

Ten minute plasma activity: 2860 cts./sec.

$$\text{Plasma volume} = \frac{2{,}894{,}742}{2860 \times 18} = 56.2 \text{ ml./kg.}$$

Intravascular albumin pool:

Serum albumin concentration: 3.11 gm.%
Intravascular albumin pool = 56.2 x 0.0311 = 1.75 gm./kg.

Total body albumin pool:

Position of intercept 'c_1' on extrapolated plasma disappearance curve (Fig. 5.1.): 0.50

$$\text{Total albumin pool} = \frac{1.75}{0.5} = 3.50 \text{ gm./kg.}$$

Extravascular albumin pool:

Extravascular albumin pool = 3.50 - 1.75 = 1.75 gm./kg.
Thus: $\frac{EA}{CA} = 1.00$

Apparent plasma half-life:

Apparent plasma half-life = 262 hrs. = 10.92 days. (Fig. 5.1.).

Fractional catabolic rate (Matthews method):

Position of second intercept ('c_2' - Fig. 5.1.). 0.32
Apparent half-life of second curve: 10 hrs. = 0.42 days.

$$\text{Gradient of first exponential curve} = \frac{0.693}{10.92} = 0.0634$$

$$\text{Gradient of second curve} = \frac{0.693}{0.42} = 1.7$$

$$\text{Fractional metabolic rate} = \frac{1}{\frac{0.50}{0.0634} + \frac{0.32}{1.7}} = 0.12$$

APPENDIX 5 (Continued)

Table 6 - The mathematic treatment of data collected from Pig 1233 (continued from Appendix 5, Table 5) - Campbell's method

Day	Urine Counts	Faeces Counts	U + F	Plasma Counts	QpXVp	$\frac{U+F}{Qp \times Vp}$	$\frac{U+F}{Qp \times Vp} \times CA$
	U	F		Qp		K	absolute degradation rate
0	90,000	680	90,680	2,860	2,894,320	0.031	0.05
1	144,430	17,000	161,430	1,487	1,504,844	0.11	0.19
2	137,000	14,604	151,604	1,287	1,302,444	0.12	0.21
3	86,806	7,901	94,707	1,173	1,187,076	0.08	0.14
4	70,679	7,684	78,363	1,115	1,128,380	0.07	0.12
5	60,037	6,780	66,817	1,030	1,042,360	0.06	0.11
6	82,372	2,782	851,154	972	983,664	0.09	0.16
7	54,267	6,862	61,129	915	935,980	0.07	0.12
8	58,072	6,171	64,243	858	868,296	0.07	0.12
9	46,297	6,025	52,322	801	810,612	0.07	0.12
10	53,470	4,866	58,336	744	752,928	0.08	0.14
11	47,277	7,432	54,709	715	723,580	0.08	0.14
12	56,982	8,299	65,281	656	663,872	0.10	0.18
13	50,000	5,000	55,000	615	622,380	0.09	0.16
14	47,500	3,924	51,424	586	593,032	0.09	0.16
15	43,822	4,943	48,765	543	549,516	0.09	0.16
16	51,442	7,325	58,767	515	521,180	0.11	0.19
17	49,823	7,325	57,148	486	491,832	0.12	0.21
18	35,737	6,048	41,785	458	463,496	0.09	0.16
19	46,563	3,247	49,810	429	434,148	0.12	0.21
20	47,527	3,690	51,217	400	404,800	0.13	0.23
Mean						0.09	0.16 gm./kg./day

APPENDIX 5 (Continued)

Table 7 - An example of the mathematical treatment of the data obtained in Experiments 5.3. and 5.4. (the fate of ^{131}I-polyvinylpyrrolidone in pigs infected with Oesophagostomum or Hyostrongylus)

Pig 1871 (control)

Total injected activity:

Weight of syringe containing PVP solution: 8.9618 gm.
Weight of syringe after solution injected into pig: 6.2479 gm.
Weight of solution injected into pig: = 2.7139 gm.

Weight of solution used to make standard: 0.8335 gm.
Made up to 250 ml., 1 ml. removed and made up to 5 ml.

Activity of standard: 1074 cts./sec.
Quantity of isotope solution in standard = $\frac{0.8335}{250}$ = .003334 gm.

Number of counts from 1 gm. isotope solution = $\frac{1074}{.003334}$ = 322,136 cts./sec.

Total injected dose = 2.7139 x 322,136 = 874,245 cts./sec.

Faecal plasma clearance:

Day	Plasma activity counts	Total faecal activity counts	Total urine activity counts	Apparent plasma clearance ml.
1	158	875	168,432	-
2	148	2,366	242,627	15.0
3	114	1,808	5,899	12.2
4	89	1,586	4,890	13.9
5	70	935	3,354	10.5
6	54	1,133	3,172	16.2
Total		8,706	428,374	67.8

% total injected activity excreted in faeces in six days: 0.996%
% total injected activity excreted in urine in six days: 48.999%

APPENDIX 5 (Continued)

Table 8 - Serum protein values from a pig (P173) infected with 30,000 Oesophagostomum larvae on 18/2/66

Date	Total serum protein g. per cent	A/G ratio	Serum albumin concentration g. per cent	Serum globulin concentration g. per cent
29/12/65	7.29	0.92	3.49	3.81
8/1/66	5.94	0.64	2.30	2.64
15/1	6.22	1.01	3.13	3.09
21/1	6.48	0.67	2.60	3.88
29/1	7.22	0.68	2.91	4.32
5/2	6.63	0.38	1.82	4.81
12/2	7.03	0.94	3.03	4.00
19/2	-	-	-	-
26/2	7.73	0.47	2.44	5.29
5/3	7.33	0.62	2.79	4.54
12/3	-	-	-	-
19/3	6.09	0.52	2.09	4.00
26/3	7.29	0.66	2.89	4.40
2/4	7.10	0.63	2.74	4.36
9/4	-	-	-	-
15/4	6.91	0.71	2.90	4.01
23/4	7.21	0.93	3.00	4.21
1/5	7.34	0.85	3.36	3.98
7/5	6.32	0.64	2.49	3.83

APPENDIX 5 (Continued)

Table 9 - Serum protein values from a pig (P174) infected with 30,000 Oesophagostomum larvae on 18/2/66

Date	Total serum protein g. per cent	A/G ratio	Serum albumin concentration g. per cent	Serum globulin concentration g. per cent
29/12/65	7.41	0.77	3.20	4.21
8/1/66	6.70	0.62	2.55	4.15
15/1	-	-	-	-
21/1	6.42	0.92	3.02	3.40
29/1	6.60	0.89	3.10	3.50
5/2	5.79	1.01	2.90	2.89
12/2	6.75	1.15	3.61	3.14
19/2	-	-	-	-
26/2	5.65	0.60	2.09	3.56
5/3	5.49	0.61	2.09	3.40
12/3	6.60	0.56	2.38	4.22
19/3	6.07	0.83	2.12	3.95
26/3	6.47	0.83	2.90	3.57
2/4	5.98	0.54	2.09	3.89
9/4	6.44	0.64	2.51	3.93
15/4	6.62	0.93	3.18	3.44
23/4	-	-	-	-
1/5	6.08	0.57	2.22	3.86
7/5	-	-	-	-

APPENDIX 5 (Continued)

Table 10 - Serum protein values from a pig (P175) infected with 30,000 Oesophagostomum larvae on 18/2/66

Date	Total serum protein g. per cent	A/G ratio	Serum albumin concentration g. per cent	Serum globulin concentration g. per cent
29/12/65	5.49	0.97	2.70	2.79
8/1/66	8.00	0.73	3.37	4.63
15/1	-	-	-	-
21/1	7.18	0.48	2.31	4.87
29/1	6.32	0.78	2.77	3.55
5/2	-	-	-	-
12/2	5.98	0.56	2.14	3.84
19/2	-	-	-	-
26/2	6.42	0.80	2.82	3.60
5/3	6.97	0.40	1.97	5.00
12/3	5.60	0.42	1.66	4.11
19/3	5.65	0.37	1.54	4.20
26/3	6.74	0.55	2.40	4.34
2/4	6.46	0.60	2.42	4.04
9/4	7.03	0.73	2.96	4.97
15/4	6.51	0.59	2.42	4.09
23/4	-	-	-	-
1/5	6.05	0.70	2.49	3.56
7/5	-	-	-	-

APPENDIX 5 (Continued)

Table 11 - Serum protein values from a pig (P176) infected with 30,000 Oesophagostomum larvae on 18/2/66

Date	Total serum protein g. per cent	A/G ratio	Serum albumin concentration g. per cent	Serum globulin concentration g. per cent
29/12/65	6.50	0.69	2.66	3.84
8/1/66	5.79	0.79	2.55	3.24
15/1	6.80	0.71	2.83	3.97
21/1	6.06	0.94	3.15	2.91
29/1	6.20	0.71	2.54	3.66
5/2	6.75	1.06	3.44	3.31
12/2	-	-	-	2.32
19/2	6.12	0.51	2.02	4.10
26/2	6.90	0.72	2.90	4.00
5/3	-	-	-	-
12/3	-	-	-	-
19/3	7.12	0.70	2.92	4.20
26/3	6.02	0.69	2.45	3.57
2/4	5.70	0.67	2.28	3.42
9/4	-	-	-	-
15/4	6.06	0.75	2.60	3.46
23/4	6.07	1.25	3.38	2.68
1/5	6.34	0.75	2.72	3.62
7/5	6.63	0.88	3.12	3.51

APPENDIX 5 (Continued)

Table 12 - Taffs (1966) records the clinical histories of the four pigs used in Experiment 3.1.

These may be summarized as follows:

Nature of faeces:

Pig No.	Days after infection	
P173	4	loose
	5 - 9	diarrhoeic
	10 - 16	diarrhoeic with spots of blood and mucus
	17 - 22	loose
P174	10, 15	loose
	16	diarrhoeic with spots of blood and mucus
	17	loose
P175	5 - 9	diarrhoeic
	10 - 17	diarrhoeic with spots of blood and mucus
	18 - 22	diarrhoeic
	23	diarrhoeic with spots of blood and mucus
	24	diarrheoic
	25 - 27	loose
	29	loose
P176	6, 8, 9	loose

Appetite:

P173 was anorexic from the 5th to the 13th day

P175 was anorexic from the 6th to the 16th
19th to the 21st
and 24th

P176 was anorexic from the 6th to the 8th

Growth-rate:

P174 and P175 lost weight soon after infection

Number of adult worms recovered at autopsy:

P173 -	3,206	P175 -	6,344
P174 -	1,942	P176 -	4,642

APPENDIX 6

Table 1 - Individual weight changes of guinea-pigs infected with 10,000 Oesophagostomum larvae (Group 1). Animals weighed when infected (Day 0) and again at slaughter

Killed day	Guinea-pig No. GP.67	Weight change gms.	Killed day	Guinea-pig No. GP.67	Weight change gms.
1	A.410	-8	15	A.408	-21
	F.286	-152		D.418	58
	F.287	0		E.421	58
	C.CLF	-1			
	J.B1	3			
	J.B8	-7			
3	B.P01	25	17	B.OY2	93
	B.OP1	15		D.416	76
	E.420	-5		D.417	37
	G.B2	5		G.B3	120
	K.B11	-6		G.B4	111
5	A.409	11	23	B.G02	91
	D.414	23		B.OG2	78
	D.415	11		C.CW	129
7	A.407	-50	24	G.B5	107
	A.412	-113		G.B6	90
	D.413	28			
	B.G01	46			
	B.OG1	15			
	J.B10	24			
9	A.411	-39	29	C.CB	135
	B.OY1	47		C.CP	169
	B.Y01	46		C.CG	201
11	B.P02	76	39	C.DF	97
	B.OP2	61		Z.4	163
	F.285	25		Z.5	147
				Z.6	101
13	B.Y02	-48	48	J.B13	79
	E.419	57		J.B14	65
	E.422	22		Z.2	132
	E.423	62		Z.3	255
	J.B12	47			

APPENDIX 6 (Continued)

Table 2 - Individual weight changes of normal (Group 2) guinea-pigs Weighed thrice weekly during experiment

Day	Guinea-pig No.					
	I.1	I.2	I.3	I.4	I.5	I.6
	Weight gain (gms.)					
2	3	6	16	1	17	-1
5	32	42	46	19	34	20
7	55	52	57	44	57	46
9	55	61	65	35	61	46
12	80	81	84	48	87	70
14	87	93	96	70	100	82
16	86	111	111	94	109	97
19	101	127	128	112	128	119
21	102	136	135	122	138	128
23	124	153	154	137	162	157
26	123	164	156	140	166	159
28	138	173	176	161	182	185
30	142	170	173	166	189	195
33	158	194	191	193	204	223
35	169	207	210	205	217	244
37	178	213	217	202	230	247
40	-	231	222	202	228	256
42	-	244	234	200	254	285

Weighed Day 0 and again at slaughter

Guinea-pig No.	Killed day	Weight gain gms.
A.402	1	3
A.405	5	28
A.403	7	27
A.401	9	54
A.404	9	36
A.406	15	54

APPENDIX 6 (Continued)

Table 3 - Individual weight changes of guinea-pigs (Group 3) given portions of the liquid in which the larvae for the Group 1 infection were suspended. Animal weighed Day 0 and again at slaughter

Guinea-pig No. GP.67	Day killed	Weight gain gms.
L.1	7	38
L.2	7	45
L.3	7	35
M.1	13	104
M.2	13	74
C.YY	22	105
C.GG	22	110
C.BB	31	225
C.FF	31	196
C.WW	31	237

APPENDIX 6 (Continued)

Table 4 - Lengths of individual larvae (μ) recovered from the intestinal mucosa of guinea-pigs infected with 10,000 larvae

	Σl	d	d^2
Day 1	535	27	729
	535	27	729
	485	23	529
	560	52	2,704
	510	2	4
	530	22	484
	510	2	4
	480	28	784
	490	18	324
	510	2	4
	470	38	1,444
	485	23	529
	485	23	529
	500	8	64
	480	28	748
	580	72	5,184
	485	23	529
	480	28	784
	535	27	729
	485	23	529
Day 3	495	13	169
	470	38	1,444
	505	3	9
	500	8	64
	480	28	784
	500	8	64
	500	8	64
	490	18	324
	480	28	784
	515	7	49
	525	17	289
	495	13	169
	540	32	1,024
	510	2	4
	480	28	784
	540	32	1,024
	495	13	169
	540	32	1,024
	515	7	49
	515	7	49

$\Sigma l = 20,720$

$n = 41$

$\bar{x} = 505$

$d^2 = 25,804$

$SD = \sqrt{629}$
$= \pm 25$

$SE = \frac{25}{6.4} = 3.9$

	Σl	d	d^2
Day 13	520	12	144
	490	18	324
	515	7	49
	555	45	203
	515	7	49
	495	13	169
Day 17	535	27	729
	480	28	784
	520	12	144
	480	28	748
	445	63	3,969
	500	8	64
	515	7	49
	480	28	784
	485	23	529
	480	28	784
	445	63	3,969
	540	48	230
	525	17	289
	470	38	1,444
	495	13	169
Day 24	465	43	185
	515	7	49
	495	13	169
	530	22	484
	445	63	3,969
	470	38	1,444

$\Sigma l = 13,405$

$n = 27$

$\bar{x} = 496$

$d^2 = 21,959$

$SD = \sqrt{813}$
$= \pm 29$

$SE = \frac{29}{5.2} = 5.6$

APPENDIX 6 (Continued)

Table 5 - Number and distribution of larvae and nodules in the large intestine of individual guinea-pigs infected with 10,000 Oesophagostomum larvae

Day	Caecum	Colon		
		I	II	III
Larvae				
1	176/253	130/152	21/7	78/116
3	28/148	2/156	7/95	19/88
7	12/110	3/24	8/3	4/18
13	2/233	9/52	13/88	12/18
17	15/16	11/12	30/10	40/78
24	15/29	14/1	0/40	17/11
48	10/11	0/4	5/13	2/30
Nodules				
1	0/0	0/0	0/0	0/0
3	0/0	0/0	0/0	0/0
7	1/0	19/1	10/0	0/0
13	2/26	18/15	23/0	3/1
17	0/0	16/13	5/2	16/13
24	19/12	5/53	2/12	7/16
48	4/+	8/+	5/4	4/16

+ confluent - impossible to count.