STRONGYLUS VULGARIS INFECTION IN THE HORSE

A Thesis

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by

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GENERAL INTRODUCTION

Although the fact that horses harbour intestinal parasites has been known since Greek and Roman times it was only at the beginning of this century that the first attempt was made to differentiate equine parasites into their various genera and species (Loos, 1901). Of all of these, the genera belonging to the families Strongylidae and Trichonematidae are now clearly established as the most pathogenic (Marotel, 1931; Olt, 1932). These parasites inhabit the large intestine and have been variously termed "Sclerostomes", "Palisade worms" and "Red worms". Although the morphological characters of most of these adult parasites are now well known and have allowed their classification into a large number of different species, they are customarily and conveniently referred to under the general term "Horse Strongyles". An arbitrary division is made according to size and generally those parasites over 1½ cm. in length are termed the "large strongyles" whereas those under 1½ cm. in length are referred to as "small strongyles".

All grazing horses carry a mixed burden of various species and parasitic disease in the horse, other than that associated with the migratory larvae of the large strongyles, is generally considered to be due to the combined pathogenic effect of the adults of these species. This, however, may not be the case as similar views were held with regard to parasitic gastro-enteritis in cattle and sheep but within the last decade it has become clear that this syndrome, i.e. parasitic gastro-enteritis, is in fact divisible into several disease entities each caused by a single nematode species, e.g. Ostertagiasis in young cattle and Nematodiriasis and Haemonchiasis in sheep. Recognition of

these separate disease entities subsequently stimulated a closer study of several aspects of each, such as the pathogenesis, epidemiology and immunity.

Over the past 10 - 15 years there has been a great increase in the numbers of horses and ponies kept for sporting and recreational purposes. Despite this increase in interest in the horse, research into various equine disease problems has been relatively slow. This is, perhaps, due mainly to the fact that the horse is a difficult and expensive animal to use under experimental conditions. Also the horse population in Britain does not lend itself easily to studies comparable with those on sheep and cattle due to the large variety of breeds, the various purposes for which they are kept, and the different methods of husbandry employed; one only has to compare the situation of the Shetland pony in its native habitat and that of the English Thoroughbred on a stud farm to realise the gross differences which exist between individual members of the horse population.

The object of the work presented in this thesis was to gain more information on various aspects of infection with the best known and the most pathogenic of the equine helminths, Strongylus vulgaris. This is a large strongyle with a well-developed buccal capsule which inhabits the large intestine of horses and feeds by withdrawing and digesting plugs of intestinal mucosa. The significance of these feeding habits is, however, not fully understood and the notoriety of the nematode depends largely on the numerous reports on the damage caused by the extensive migration of the parasitic larval stages in naturally infected animals.

Before undertaking an extensive study of Strongylus vulgaris infection two major pre-requisites were apparent. First, it was necessary to have a regular supply of worm-free foals for experimental infection. Secondly, it was essential to have a reliable method of obtaining a pure culture of S. vulgaris infective larvae in sufficient numbers to infect groups of animals. Once these objectives had been achieved the following aspects of the disease were studied:

1. The life cycle of S. vulgaris
2. The pathogenesis of experimental infection
3. Immunity in relation to S. vulgaris infection.

During the course of the experimental work the epidemiology of mixed strongyle infection was also studied under field conditions.

A full account of available knowledge of these separate aspects is discussed in the introduction of the appropriate section before presenting and discussing the results of experimental work carried out under these headings.

MATERIALS AND METHODS

A. EXPERIMENTAL ANIMALS

(1) The rearing and maintenance of parasite-free animals

Cross bred pony mares were brought into clean loose boxes immediately after foaling. The mares were dosed on arrival and subsequently weekly with a broad spectrum anthelmintic (Thiabendazole* or Pyrantel tartrate+). In the first foaling season Thiabendazole was used at a rate of 88 mg/Kg. body weight and subsequently Pyrantel tartrate was administered at a rate of 12.5 mg/Kg. body weight. Both anthelmintics were given in the concentrate ration and although both were acceptable Pyrantel tartrate appeared more palatable than Thiabendazole. All faeces were removed daily and the boxes were thoroughly scrubbed out twice weekly. The mares were removed when the foals reached 3 months of age and were eating a ration of hay and concentrates. Regular examination of both mare and foal faeces for nematode eggs gave consistently negative results.

(2) Naturally infected animals

Certain experiments described in this thesis required the use of ponies which had a naturally acquired mixed infection of various helminths. These animals were either brought indoors and maintained on hay and concentrates during the experimental period or kept at

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* Thibenzole: Merck Sharp & Dohme Ltd., Hoddesdon, Herts.

\+ Strongid: Pfizer Ltd., Sandwich, Kent.

pasture in the case of those used in epidemiological studies. Faecal samples were examined regularly to enable the strongyle egg count to be established in each case.

B. BLOOD ANALYSIS

(1) Collection and storage of samples

Each blood sample was obtained from the jugular vein and collected in 3 Vacutainers* which had been prepared in different ways. Firstly for haematological estimations 2 ml. of blood was withdrawn into a vacutainer containing a few crystals of the anti-coagulant ethylenediaminetetracetate (E.D.T.A.) and the tube gently shaken to dissolve the crystals. Haematological examinations were made within a few hours of collecting the samples.

Secondly, for estimations requiring plasma, 7 ml. of blood was withdrawn into a vacutainer containing a few drops of a 1:1,000 solution of heparin. The heparinised sample was thoroughly mixed then centrifuged at room temperature for 20 minutes at 2,000 r.p.m. in a M.S.E. centrifuge (Measuring Scientific Equipment, London, England). The plasma was then transferred, by means of a pipette, into plastic tubes (Metal Box Co., Portslade, Sussex, England), immediately frozen and stored at -5°C.

Thirdly, for estimations involving serum, 7 ml. of blood was withdrawn into a vacutainer containing no anticoagulant. This sample was left standing overnight at room temperature and the serum

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* Vacutainers: Becton-Dickinson, Rutherford, New Jersey, U.S.A.

which had separated was recovered and stored as described above for plasma.

(2) Packed cell volume (P.C.V.)

The packed cell volume percentage was determined by the micro-haematocrit method. Capillary tubes containing the blood sample were sealed by heat at one end and centrifuged for 5 minutes in a microhaematocrit centrifuge (Hawksley & Sons Ltd., London, England). The percentage P.C.V. was determined from the scale on a Hawksley Microhaematocrit Reader.

(3) Haemoglobin concentration (Hb)

Haemoglobin concentration expressed as grams per 100 ml. was estimated by the oxyhaemoglobin method of Dacie and Lewis (1966). A 1 in 200 dilution of blood was prepared in 0.04 per cent solution of ammonium hydroxide and, after thorough mixing, the resulting solution of oxyhaemoglobin was read in a colorimeter (Evans Electro-selenium Ltd., Harlow, England) using a yellow green filter (Ilford No. 625). The colorimeter was calibrated using a cyanmethaemoglobin standard solution (cyanmethaemoglobin standard solution - C. Davis Keeler Ltd., London, England).

(4) Total red and white blood cell counts (R.B.C.'s)(W.B.C.'s)

Total red blood cell counts (x 10^6/cu. mm.) and total white blood cell counts (x 10^3/cu. mm.) were determined by an electronic particle counter (Coulter Model "D", Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.).

(5) Differential white cell counts

Differential white cell counts were made using the technique described by Dacie and Lewis (1966). Blood smears were made and stained with a 1 in 10 solution of Giemsa's Stain R 66 (G.T. Gurr, London, England) for 20 - 25 minutes. The counts were performed by selecting a thin strip of cells between the centre and the edge of the smear and counting all white cells along this line until 200 cells had been counted. The numbers of neutrophils, lymphocytes and eosinophils counted in this manner were then expressed as a percentage of the total number of cells counted.

(6) Total serum protein concentration

Total serum protein concentration was estimated by the biuret method of Weichselbaum (1946).

(7) Serum protein fractionation

Separation of serum protein fractions was carried out by electrophoresis. Cellulose acetate strips (Oxoid Ltd., London, England) were saturated with barbitone buffer (pH 8.6), lightly blotted to remove excess buffer, and laid across the supports of an electrophoresis tank (Shandon Scientific Co., London, England). Serum (0.003 ml.) was applied to the strip, about 4 cms. from the cathode end, using a micro-pipette. A constant voltage of 150 volts was applied for 1 hour from a Vokam power pack (Shandon Scientific Co.). The strips were removed, dried in a hot air oven at 80 to 100^{o}C for 10 minutes to 'fix' the proteins, and

developed by staining with 0.2% Ponceau S (G.T. Gurr Ltd., London, England) in 3% trichloracetic acid for 5 minutes. After staining the strips were washed in 5% acetic acid until the background was white. The strips were evaluated automatically as described by Neill (1963), using a Chromoscan recording densitometer (Joyce Loebl & Co. Ltd., Gateshead, England). The results were expressed as grams of albumin, total globulin, alpha, beta and gamma globulin per 100 ml. of serum.

C. NECROPSY PROCEDURE

(1) Details of slaughter

Young animals were killed by an intravenous injection of Pentobarbitone sodium,* while larger ponies and adults were shot using a captive bolt pistol. The animals were then bled out, the thorax and abdomen opened and the viscera removed, care being taken not to damage the aorta. A gross pathological examination of the rest of the carcass was then made.

(2) Gross examination of viscera

Any lesions on the serosal surfaces of the stomach and intestines were noted and the lungs and liver examined for any pathological change before proceeding to separate the various organs. Histological blocks were prepared from visible lesions of the lungs and liver.

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* Euthatal: May & Baker Ltd., Dagenham, England.

(3) Heart, aorta and mesenteric arteries

The heart, aorta and kidneys were removed together with the anterior mesenteric artery and its branches. The mesenteric branches of the anterior mesenteric artery were separated from the small intestine close to the intestinal wall; similarly the ileal, medial and lateral caecal, ventral, middle and dorsal colic arteries were all stripped from the serosal surface of the various organs. The heart, aorta, anterior mesenteric artery and the above-mentioned intestinal arteries were then removed intact and taken to the laboratory for examination and dissection. After noting any gross pathological change in this arterial preparation the left ventricle was opened and, using scissors, the arteries were dissected proceeding from the aorta down to the smaller intestinal branches of the anterior mesenteric artery. Histological blocks were prepared from arterial lesions.

(4) Intestinal tract

The stomach, small intestine, caecum and colon were separated from each other, care being taken not to lose any of the contents.

(a) Stomach

The stomach was opened along the greater curvature and examined by the naked eye for the presence of parasites. Histological blocks were prepared from any visible lesions.

(b) Small intestine

After examining the serosal surface for lesions and removing blocks for histological examination, the small intestine was opened, washed under running water and inspected.

(c) Caecum and colon

After dividing the colon at the pelvic flexure into ventral and dorsal components, the contents of the caecum, ventral colon and dorsal colon were emptied into separate containers. Any large strongyles attached to the mucosal surface were removed and placed in 5% formalin before gently washing the viscera and adding these washings to the appropriate gut contents. Water was then added to the washings and contents until a thorough mixture was achieved and a 10% sample of the final volume was removed for subsequent microscopic enumeration of the worm population; formalin was added as a preservative.

(5) Parietal peritoneum

Since the parietal peritoneum of the right flank is the predilection site for the late larval stages of Strongylus edentatus this was examined for the presence of lesions containing fifth stage larvae.

D. PARASITOLOGICAL TECHNIQUES

(1) Culture and harvesting of S. vulgaris infective larvae

A technique was developed for the isolation and maintenance of a monospecific infection of S. vulgaris based on the introduction of adult worms into the caecum of a worm-free foal. Details of this technique are given in Chapter 1.

(2) Preparation and administration of larval inoculum

The doses of S. vulgaris infective larvae used for experimental infection were generally in the range 750 - 2,000 L_3. As these are comparatively small doses the majority were obtained by removing and counting larvae by means of a pipette from freshly harvested stock cultures. The individual doses, after checking larval viability, were admiuistered using a polythene stomach tube* which was then flushed through with water. By blowing into the end of the stomach tube while slowly withdrawing it, any leakage or reflux of larval suspension was avoided.

(3) Faecal egg counting techniques

Faecal samples collected either from the rectum or from freshly voided faeces were examined by a modified McMaster technique (Gordon & Whitlock, 1939). In this method 3 gms. of faeces were mixed with 42 ml. of water and passed through a sieve (60 meshes per inch);

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* Portex Ltd., Hythe, Kent.

two 15 ml. samples of the filtrate were centrifuged in flat bottomed test tubes for 2 minutes at 2,000 r.p.m. and the supernatant poured off. The sediment of one of these tubes was resuspended in saturated salt (NaCl) solution, the test tube inverted several times, then, using a pipette, both chambers (volume 0.15 ml.) of a McMaster Worm Egg Counting Slide (Hawksley & Sons, London, England) were filled with the suspension. The number of eggs in both chambers was multiplied by 50 to give the numbers of eggs per gram of faeces.

If the sample proved negative for strongyle eggs using the above method, the second test tube containing sediment was filled with saturated salt solution and thoroughly mixed. More saturated salt solution was then added until the meniscus was above the rim of the tube. The sample was allowed to stand for a few minutes before using a platinum loop to remove the upper layer of the fluid and smear it on a clean glass slide. A microscopical examination then confirmed the absence of strongyle eggs or their presence in small numbers.

(4) Faecal culture technique

Samples of faeces were set up for culture and identification of various strongyle infective larvae. Moist faeces were loosely packed in 1 lb. honey jars and the lid lightly screwed down. The jars were then stored in an incubator at 28°C for 10 to 14 days.

Infective third stage larvae were recovered by a method essentially similar to that of Roberts and O'Sullivan (1950). The jars were filled with lukewarm tap water and allowed to stand without lids for several hours in diffuse light. The fluid was then poured through a sieve (60 meshes per inch) to remove coarse material before being poured on to a double layer of gauze strengthened milk filter mediums (Cloverleaf No. 9, Johnson & Johnson, Slough, Buckinghamshire, England) placed on top of a Buchner funnel. The larvae were trapped on the milk filter pads which were placed, without being inverted, in a Baermann apparatus. The larvae migrated through the pads and were collected 1 - 2 hours later, motile and free from foreign material, at the neck of the filter funnel. These larval collections were then examined microscopically in order to differentiate the third stage larvae into 3 categories:

(a) Trichonema species (8 - 12 intestinal cells)

(b) Strongylus vulgaris (28 - 32 intestinal cells)

(c) Others. Any larvae which did not fall into the above 2 categories.

(5) Recovery of S. vulgaris larvae from arterial lesions

Thrombus material present in the anterior mesenteric artery and its branches was scraped off using a scalpel blade and put into a honey jar containing a pepsin-hydrochloric acid mixture. The jar and contents were incubated for a period of 4 - 6 hours at 42°C and then formalinised. Subsequently the digest was examined

microscopically and all S. vulgaris larvae removed and counted. The pepsin-HCl mixture was adapted from that described by Herlich (1956) i.e. 10 gm. of 1:2500 pepsin powder (British Drug Houses, Poole, Dorset, England) were dissolved in 600 ml. of water and acidified with 30 ml. concentrated HCl.

(6) Worm counting and identification

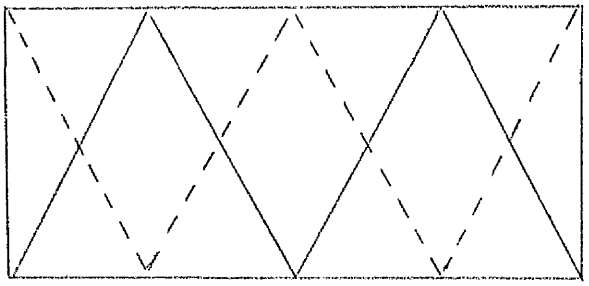
The 10 per cent samples of washings and contents of the caecum, ventral colon and dorsal colon collected at necropsy were each treated as follows: after thorough mixing, 100 ml. samples were removed and using a 10 ml. straight pipette sawn off at the 8 ml. mark, 5 ml. aliquots were withdrawn and pipetted into Petri dishes. These were stained for a few minutes with a few drops of a 45 per cent iodine solution (to 720 gm. potassium iodide in 500 ml. of warm distilled water, 450 gm. iodine crystals were added and made up to 1 litre with distilled water), then decolourised with a 5 per cent sodium thiosulphate solution and the worms counted under a Wild dissection microscope (Model M.5, Wild, Heerbrugg, Switzerland). This facilitates counting in that after decolourising with sodium thiosulphate, the worms retained the iodine stain and were readily visible (Whitlock, 1948). A number of 100 ml. samples were examined in this manner until at least 200 worms had been recovered. When very low numbers of worms were present, samples were examined until 100 worms were recovered. The average number of worms per 5 ml. aliquots was calculated and multiplied by the appropriate dilution factor to give the total number of worms

present in the caecal and colonic contents.

Adult and immature Oxyuris equi were present in varying numbers in the majority of colon samples but these were not included in the calculation of the total strongyle worm burdens.

(7) Technique for the collection and examination of herbage for strongyle larvae

Pasture larval counts were carried out using a modification of the technique described by Parfitt (1955). By crossing the paddock repeatedly, in both directions, as shown in the diagram, 100 samples were collected along both route A and route B.



These samples were taken from 4 areas around the feet by removing the amount of grass which could conveniently be pulled out using the thumb and forefinger.

The total sample of grass was weighed, placed in a large bucket of water and allowed to stand for a minimum of 2 hours. The grass was then removed, squeezed dry over the bucket and discarded. After allowing the contents of the bucket to sediment overnight, the supernatant was siphoned off and the sediment poured through a double

layer of milk filters in a Buchner funnel. These milk filters were then placed in a Baermann apparatus and left for 24 hours before collecting the larvae from the narrow neck of the filter funnel. The larval suspension was then made up to a volume of 10 ml., well mixed, and 0.025 ml. samples removed, stained with iodine and counted under a dissecting microscope. Differentiation of the larvae into 3 categories, i.e. Trichonema sp., S. vulgaris and Others, was carried out and the total numbers of larvae in each of these categories were then expressed as larvae per kilogram of herbage after correction for the total weight of grass collected.

E. PATHOPHYSIOLOGICAL TECHNIQUES

(1) Preparation of ^{125}I-labelled Albumin

Albumin was separated from horse serum by molecular sieve chromatography on columns of Sephadex G100 (Pharmacia Fine Chemicals, Uppsala, Sweden) and trace-labelled with ^{125}I according to the method of McFarlane (1958).

The labelled preparation was transferred to a dialysis sac containing "carrier" protein (bovine serum albumin) to reduce the specific activity of the preparation to less than 5 µCi/mg. The labelled protein was then dialysed for 48 hours at 5°C against two 20 litre changes of 0.9% NaCl, removed from the dialysis sac and prepared for intravenous injection.

(2) Preparation of ^{51}Cr-labelled erythrocytes

Erythrocytes were labelled with ^{51}Cr by the method of Gray and Sterling (1950). Incubation of red cells with ^{51}Cr as anionic hexavalent sodium chromate results in the attachment of the label to the globin of the haemoglobin molecule. In their original studies Gray and Sterling (1950) demonstrated that cationic trivalent chromic chloride ($^{51}Cr\ Cl_3$), which does not label intact erythrocytes, is more efficiently incorporated into haemoglobin than the anionic hexavalent form. It is therefore considered that anionic hexavalent ^{51}Cr diffuses through the red cell membrane and, upon reduction to the cationic trivalent state within the red cell, becomes bound to the haemoglobin.

Procedure

Heparinised samples of blood were centrifuged at 1500 r.p.m. for 10 minutes. The plasma was removed and retained and the cells resuspended in 0.85% NaCl solution. A measured volume of isotonic saline containing $Na_2{}^{51}CrO_4$ was added with gentle mixing, the amount of activity added being adjusted to yield a net activity of 100 µCi per ml. of packed red cells, assuming 50% incorporation of the label. The cells were incubated at 37°C for 1 hour, washed 3 times, and finally reconstituted with plasma for injection. Each animal received its own red cells and plasma.

(3) Radioactivity measurements

Radioactivity measurements were carried out in an automatic well-type gamma scintillation spectrometer (Nuclear Chicago, High Wycombe, Bucks., England). One ml. samples of blood and plasma were pipetted into counting bottles and made up to a volume of 15 ml. with 0.01 N NaOH. Five random samples (20 gm.) of each total daily faecal collection were packed in counting bottles to a volume of 15 ml. Suitable aliquots of standard solutions of each isotope were assayed at regular intervals, corrections for radioactive decay being based on the activities of these solutions. Count rates of less than 3 times background were considered to be beyond the lower limit for accurate determination.

F. HISTOLOGICAL METHODS

At necropsy various tissues were taken for histological examination and fixed in mercuric chloride-formaldehyde and in Carnoy's fixative (absolute alcohol, chloroform and glacial acetic acid). After dehydration and embedding in paraffin wax, sections were cut and stained with haematoxylin and eosin. In addition selected sections were stained with carbolchromatrope, picro-Mallory, phosphotungstic acid haematoxylin and Verhoff van Giesen.

G. IRRADIATION PROCEDURE

Irradiation of larvae was carried out in a gamma ^{60}Co irradiation unit (Gamma Chamber Mark IV B - Nuclear Engineering Ltd., Southampton St., Reading, England) calibrated by Fricke dosimetry.

The Fricke reaction (Weiss, 1952; Miller, 1953) is based on the oxidation of ferrous to ferric ions when a solution containing ferrous ammonium sulphate, sulphuric acid and potassium chloride is exposed to ionising radiations. The ferric ion yield measured in a U.V. spectrophotometer at 304 mμ is then used to calculate the dose output of the unit.

Two ml. samples of a freshly harvested larval suspension were pipetted into each of 10 perspex test tubes held in a cylindrical perspex rack; the rack was placed in the central shaft of the ^{60}Co source and lowered into the irradiation position. After each total dose, e.g. 60 kr., was delivered to the larvae, the rack was raised from the irradiation chamber and an appropriate number of tubes were removed and replaced by "blanks" containing 2 ml. saline, thus maintaining the geometry of the chamber. The remaining tubes were then irradiated further until the required dose, e.g. 80 kr., had been achieved. Following irradiation each larval preparation was diluted and the required doses prepared for administration.

H. METEOROLOGICAL DATA

The meteorological information which is presented in the chapter on epidemiology was obtained by courtesy of the Weather Centre at the nearby Glasgow (Abbotsinch) Airport.

CHAPTER 1

THE DEVELOPMENT OF A TECHNIQUE FOR THE ISOLATION AND MAINTENANCE OF A MONOSPECIFIC INFECTION OF *STRONGYLUS VULGARIS* IN THE HORSE

INTRODUCTION

Before attempting to initiate a study of experimental *S. vulgaris* infection in the horse it was necessary to develop a reliable method of obtaining a pure culture of infective larvae uncontaminated by larvae of the many other strongyle species parasitic in this host. In the past infective larvae have been obtained using a variety of techniques. One of these necessitated the collection of adult female parasites from the large intestine of naturally infected horses (Round, 1969). These worms were then ground up to release eggs from their uteri; subsequently infective larvae were obtained by incubating these eggs, mixed with sterile horse faeces, at 28°C for approximately 1 week. If sufficient larvae were recovered by the Baermann technique, they were used for experimental infections but often only enough larvae were obtained to infect a single worm-free foal. A period of 6 - 6½ months, *i.e.* the prepatent period of *S. vulgaris*, then elapsed before infective larvae could be cultured in quantity from the faeces of this animal.

Another method of obtaining infective larvae of *S. vulgaris* is simply to pick out the larvae of this species from mixed larval cultures. This is, of course, a very difficult and time-consuming technique and again usually only sufficient larvae are obtained to infect a culture pony. However, a development of this technique was reported by Drudge, Lyons and Szanto in 1966. They selected naturally infected horses whose faeces on culture contained a high

proportion of S. vulgaris larvae. These horses were then treated repeatedly with piperazine at 44 mg. base per kg. body weight, which selectively removed a large proportion of Trichonema species from the large intestine. The faeces of these animals were then collected, cultured and examined. All strongyloid larvae, other than S. vulgaris, could then be removed manually using a fine pipette or needle. Although this technique was probably the best of those available at the start of our studies, it had the disadvantage that the culture still required manual removal of contaminating larvae.

A number of the above techniques were used initially by me with varying degrees of success and as an extended research project was to be carried out on S. vulgaris infection, a more consistent method for the isolation and maintenance of a monospecific culture of infective larvae was the first objective. The result was the development of a culture technique which greatly facilitated the experimental work described in this thesis.

CULTURE TECHNIQUE

Adult S. vulgaris were carefully detached from the caecal mucosa of horses immediately after their slaughter at the abattoir. These worms were placed in warm saline and transferred to the Veterinary School, where a worm-free yearling pony had been anaesthetised and prepared for abdominal surgery. After counting,

sexing and checking the viability of these worms, they were introduced into the caecum using a sterile filter funnel held in position through a small incision in the caecal wall. The funnel was then removed and the wound in the caecal wall closed by means of a purse-string suture of chromic catgut, making sure that the edges of the incision were inverted. Closure of the abdominal musculature and skin were effected by sutures of chromic catgut and monofilament nylon respectively. After completion of surgery, the pony was allowed to recover in a well-bedded box. Starting the following day, faecal samples were taken and examined by the McMaster and direct salt flotation techniques. Faecal cultures were also set up daily and maintained at 28°C for 10 - 14 days.

This technique was carried out on 2 animals with the following results. In the first instance 30 adult S. vulgaris (18 females, 12 males) obtained from abattoir horses were implanted into the caecum of a yearling worm-free pony and the results of faecal egg counts and faecal cultures are shown in Figure 1.

The second implant was carried out with 75 adult S. vulgaris (48 females, 27 males) and the results of the faecal egg counts and faecal cultures of this animal are shown in Figure 2.

Although this technique provided sufficient infective larvae for our initial experimental work, one disadvantage was that each implant of adult worms required the use of one worm-free pony recipient. This, of course, was expensive and at certain times when adult worms were plentiful, suitable worm-free recipients were not always available.

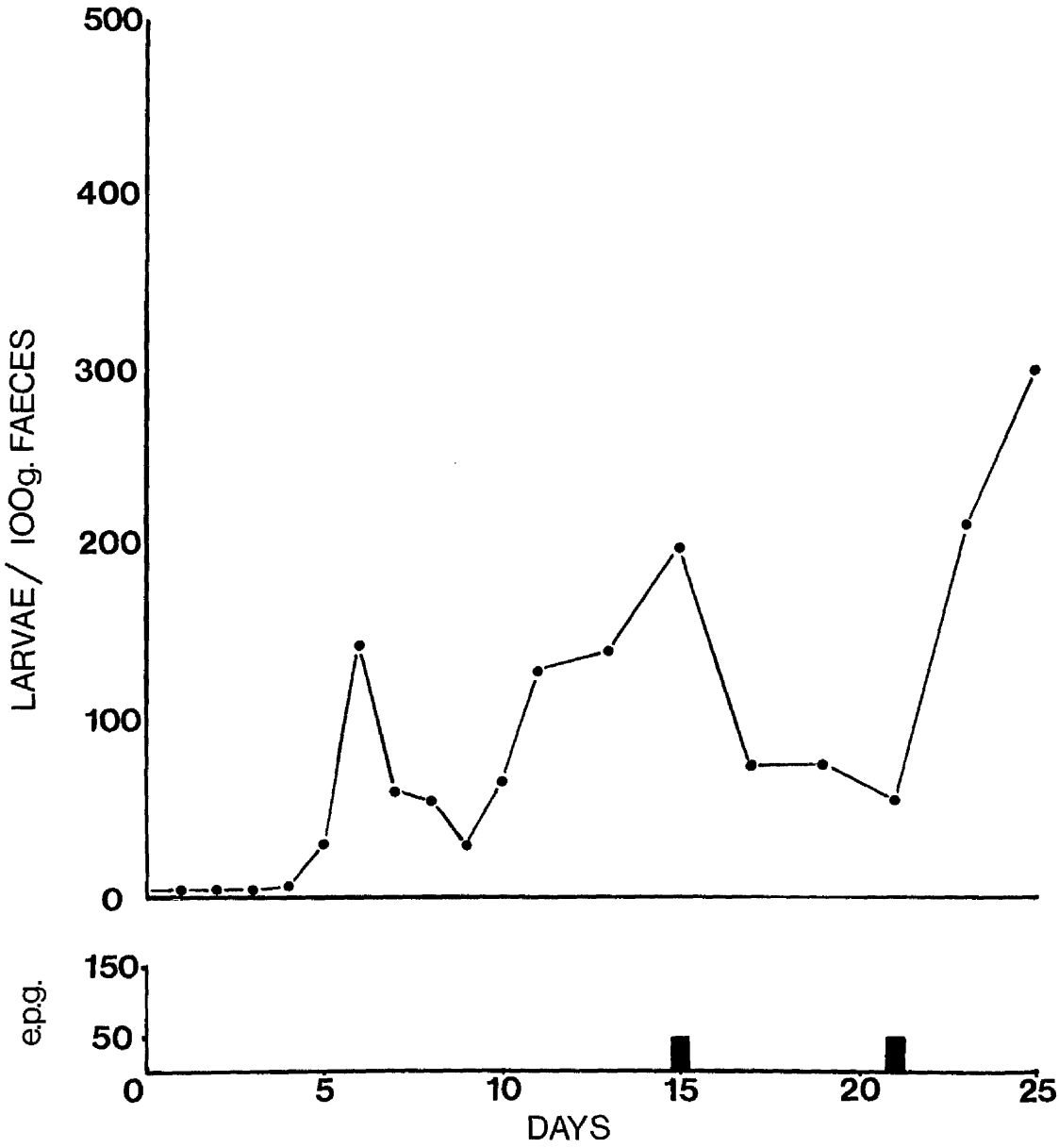


FIG. 1 Faecal egg counts and larval output after implantation with 30 adult S. vulgaris.

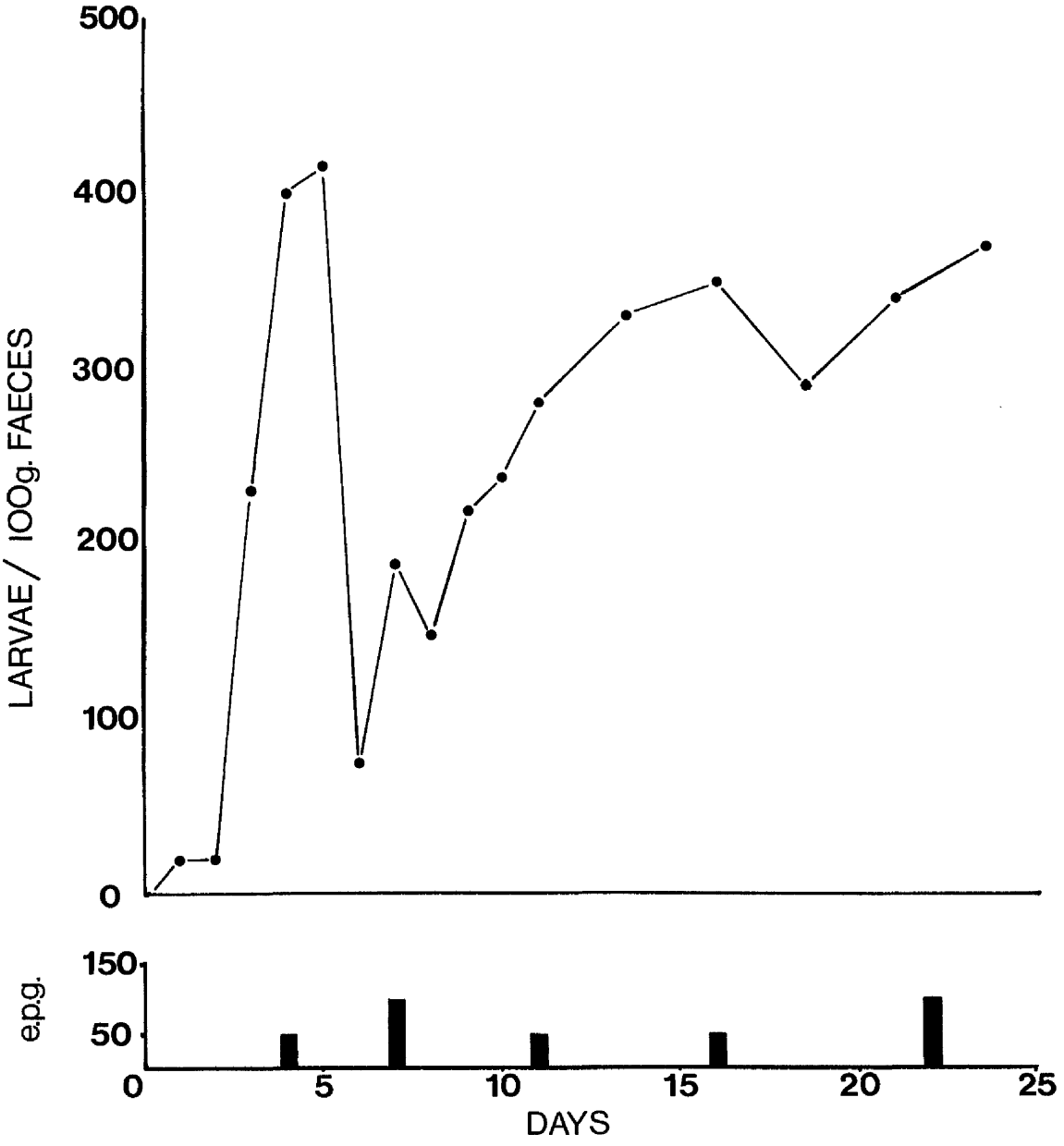


FIG. 2 Faecal egg counts and larval output after implantation with 75 adult S. vulgaris.

From various reports in the literature on studies of digestion in the horse requiring long-term fistulation of the large intestine (Alexander, 1970; Lowe, Hintz & Schryver, 1970) we conceived the possibility of adapting the surgical techniques used in these studies in order to facilitate the re-introduction of adult S. vulgaris into the caecum of a single recipient. Whereas the work on digestion required permanent fistulae for easy and frequent access to intestinal contents, our requirements were simply easy access to the caecal lumen whenever adult worms became available for implantation. We therefore decided when carrying out our next implant to fix the caecum of the worm-free pony to the right flank where subsequently adult parasites could be introduced directly in the caecal lumen by means of a trochar and cannula using local anaesthesia. A laparotomy was performed as described previously; the caecum was identified and using chromic catgut and continuous sutures a circular area of the caecum approximately 6 cm. in diameter, just proximal to the base, was attached to the body wall all round the laparotomy wound. The caecum was punctured and 75 adult S. vulgaris (51 females, 24 males) introduced and the organ closed with a purse-string suture of chromic catgut. Muscle layers were closed over the area using continuous sutures of chromic catgut and the skin incision was repaired using interrupted sutures of monofilament nylon.

Eight months later it was decided to carry out a second implantation of mature worms. Although it had been assumed that

identification of the area of attachment of the caecum to the body wall would not be difficult, this did not prove to be the case. However, by digital palpation of the caecal adhesion through a small incision made anterior to the expected site, the area was identified. Direct penetration of the caecum was then performed by means of a trochar and 28 adult S. vulgaris (16 females, 12 males) were implanted by flushing the worms through a polythene tube introduced through the cannula. Closure of the trochar wound required only a nylon purse-string suture and the exploration was repaired with an interrupted suture of nylon inserted through the skin and superficial muscle layers.

Six months then elapsed before a further implantation with 234 adult worms (138 females, 96 males) was carried out in exactly the same manner. Throughout this period the pony was stabled under conditions which precluded infection with other strongyle larvae.

The results of faecal egg counts and faecal cultures from this animal over the period of the 3 implants are shown in Figure 3.

DISCUSSION

The differences in numbers of larvae recovered from the various numbers of adult worms implanted is probably due to a number of factors. A possible explanation is the variation in the age and

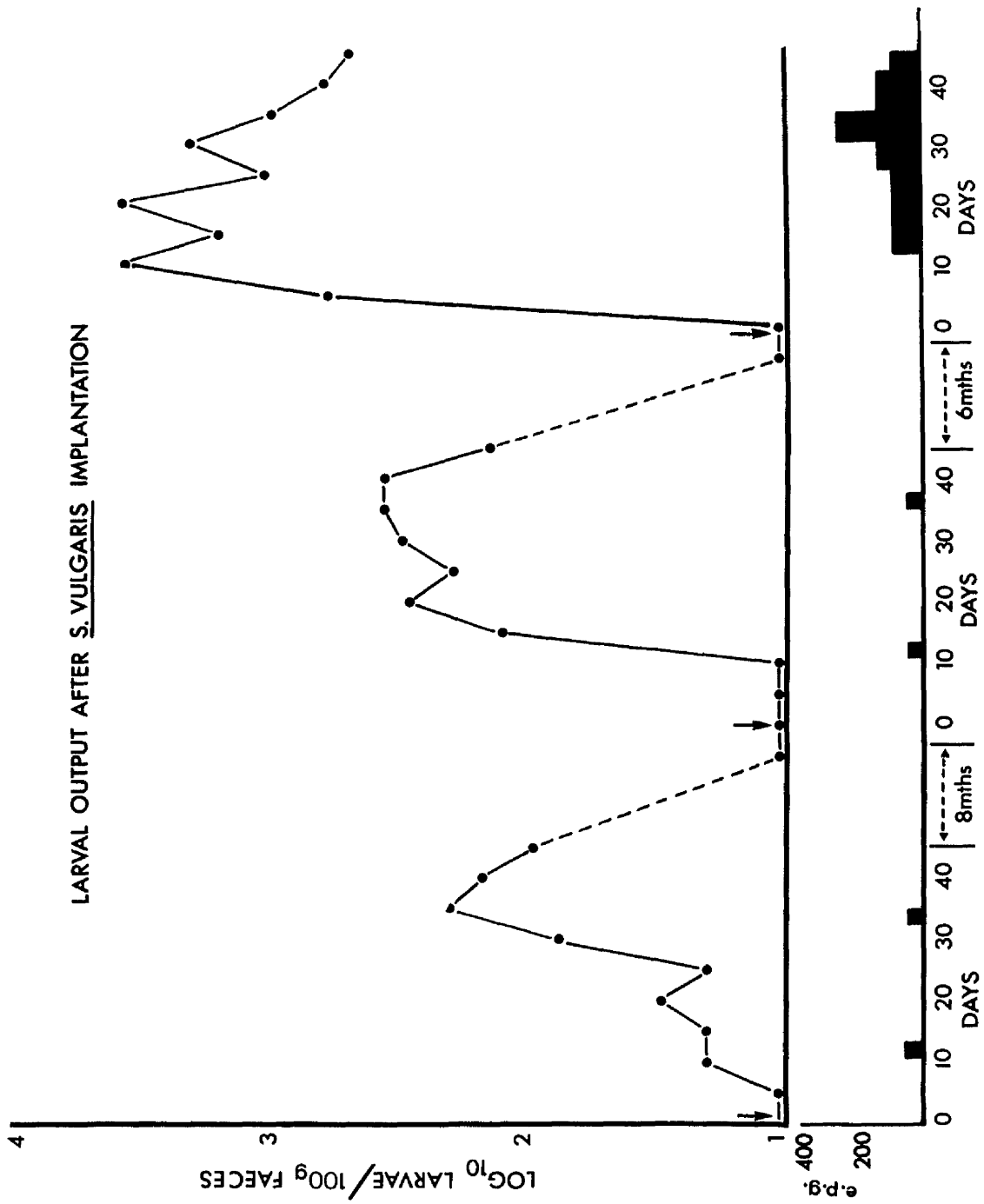


FIG. 3 Faecal egg counts and larval output after 3 successive implants (↓) with 75, 28 and 234 adult S. vulgaris.

development of the implanted population. There is no known method of ageing adult parasites collected from the caecum although it is considered by some that well-developed adult females which contain few or no eggs may have ceased reproduction. This, however, may not be the case and in this series of experiments no attempt was made to differentiate parasites on this basis as it was considered more important to transfer and implant all the adult worms into the caecum of the recipient pony with the minimum delay possible. Ershov (1949) suggested, as a result of a survey of arterial lesions in horses killed at different times of the year, that larger numbers of S. vulgaris larvae are found in arterial lesions between October and January and that they reached the adult stage early in the spring. It may be that if such a young population were used for an implant in the spring higher faecal egg counts and larval recoveries, which would be maintained over a longer period, might result. In the 5 implants described here, adult parasites were obtained at various times of the year and each proved successful, but it is difficult with such a small number of animals to assess the significance of Ershov's observation. One interesting point arose from the repeated re-implantation of adult parasites into the caecum of the single worm-free recipient. This was the fact that although this pony had previously experienced intestinal infection with adult S. vulgaris there was little apparent effect on the subsequent establishment in the caecum of fairly large numbers of adult worms.

In conclusion, it is apparent that the technique of intra-caecal implantation of adult worms provides an ideal solution to the problem of maintaining a monospecific culture of S. vulgaris. In particular the success of the modified technique eliminated the expense involved in rearing a succession of worm-free foals for use as recipients and also greatly reduced the amount of surgery involved.

SUMMARY

In order to facilitate experimental work on S. vulgaris infection in horses it was considered desirable to develop a reliable technique for the production of large numbers of infective S. vulgaris larvae in pure culture since previous procedures, evolved by other workers, all had certain disadvantages.

A satisfactory technique was developed in which pure cultures of third-stage S. vulgaris larvae were obtained by transferring adult worms, collected from horses immediately after slaughter, directly into the caeca of worm-free yearling ponies via a laparotomy. Worms transferred in this fashion live for a period of months during which they produce large numbers of viable eggs.

A further adaptation of the technique was the successful use of a single recipient for repeated implantations, thus reducing the number of worm-free recipients required.

CHAPTER 2

THE LIFE CYCLE OF *STRONGYLUS VULGARIS* IN THE HORSE

INTRODUCTION

Although the association between S. vulgaris larvae and "aneurysms" of the anterior mesenteric artery has been recognised for many years, the precise route taken by the migrating parasitic larval stages has remained the subject of considerable controversy. From a review of the literature it is apparent that 4 separate theories have been proposed.

Olt (1932) was interested in the relationship between aneurysms of the anterior mesenteric artery and the occurrence of colic in the horse. As a result of his work he proposed a migratory route for S. vulgaris larvae which was similar to that of Ascaris suum. In Olt's opinion third stage larvae, after ingestion, exsheathed in the intestine and after penetrating the intestinal wall travelled via the portal system to the liver; the larvae travelled from the liver via the posterior vena cava to the right heart and then via the pulmonary arteries to the lungs. In the lungs the larvae gained access to the air spaces, migrated via the bronchi and trachea to the pharynx, were swallowed and thus reached their final site in the large intestine. All larvae found in the anterior mesenteric site were considered by Olt to be aberrant. He believed that these erratic larvae penetrated the intestinal wall and migrated between the layers of the mesentery until they reached the root of the mesentery where the anterior mesenteric artery originates from the aorta. The larvae then penetrated the wall of this vessel to gain access to the lumen and thus initiated a typical aneurysm.

Wetzel and Enigk (1938) experimentally infected a group of foals and found no evidence of migration of S. vulgaris larvae in the trachea or the oesophagus. Subsequent work by Enigk (1950, 1951) using experimental infections of worm-free pony foals suggested that the migratory route was almost entirely intra-arterial. He reported that in his opinion infective larvae exsheathed in the intestine and penetrated the mucous membrane of the caecum and colon; these larvae moulted in the sub-mucosa of these organs before penetrating small, sub-mucosal arteries. Fourth stage larvae then migrated within the intima of these vessels until they reached larger intestinal arteries and eventually the anterior mesenteric artery. This migration was completed between 11 and 15 days after infection and the fourth stage larvae remained and developed in this situation until 45 days after infection. From this time onwards, fourth stage larvae returned to the intestinal wall via the blood stream. The fourth moult occurred at 90 days at the earliest and a proportion of larvae completed this moult at the arterial site before they returned to the intestine.

Ershov (1949) as a result of a series of post-mortem examinations of naturally infected adult horses and foals originally suggested that ingested larvae after penetrating the mucous membrane of the large intestine migrated between the layers of mesentery and that these larvae reached the lumen of the anterior mesenteric artery by penetrating the wall of this vessel. Larvae then developed in the arterial lesion before

returning to the gut via the branches of the anterior mesenteric arteries. Further observations by Ershov (1956) supported Enigk's views in that he suggested an intra-arterial migration but Ershov was of the opinion that only a proportion of the larvae penetrated sub-mucosal arteries while of the remainder some penetrated veins and lymphatics and others migrated between the layers of the mesentery. Ershov believed that only those larvae which reached the lumina of sub-mucosal arteries underwent further development by migrating to the anterior mesenteric site where they developed for a period of 6 months. Fifth stage larvae then returned to the large intestine via the caecal and colic arteries and thus completed their migration.

The fourth and final proposals on the migratory route of S. vulgaris were advanced by Farelly (1954) and subsequently by Poynter (1960). Farelly based his proposals on the finding of S. vulgaris in thrombi at the origin of the aorta in a number of naturally infected yearling ponies. Since there was little evidence of larvae having arrived at that site by a forward migration from the anterior mesenteric artery he considered that these larvae must have travelled via the posterior vena cava to the right heart; the larvae were then carried into the pulmonary circulation but returned to the left heart via the pulmonary veins (cf. Olt's theory) and thus reached the aorta. He explained the occurrence of lesions in the arterial system on the basis of haemodynamics together with a proposal that the majority of larvae

complete their systemic migration while still in the fourth stage of development: Farelly considered the mature fourth and fifth stage larvae frequently found in arterial lesions to be those which for some reason had been held up in their previous migratory phase. Subsequently Poynter (1960) carried out post-mortem examinations on 43 horses naturally infected with S. vulgaris and produced valuable information on the distribution, nature and development of the arterial lesion. He concluded as a result of this work that it was likely that S. vulgaris larvae travelled through the heart and returned via the intestinal arteries to the gut.

In retrospect, most of the controversy concerning the migration of S. vulgaris larvae has been due to the fact that only Wetzel and Enigk (1938) and Enigk (1950, 1951) used experimental infections in worm-free animals; other workers based their conclusions on material obtained at post-mortem examination of naturally infected animals.

It is interesting that although experimental work on the pathogenesis of S. vulgaris infection by Drudge, Lyons and Szanto (1966) lent support to the migratory pathway described by Enigk, the subject remained controversial and was again discussed by most of these workers at the Second International Conference on Equine Infectious Diseases in Paris in 1969.

The object of the experimental work described in this chapter was to attempt to elucidate the migratory cycle of S. vulgaris by making serial observations on worm-free foals given monospecific experimental infections. These foals were killed at intervals over a period of 9 months, thus encompassing the entire pre-patent period and approximately 3 months of patency.

MATERIALS AND METHODS

Experimental Animals and Design

Nine worm-free pony foals aged 3 - 4 months were infected with 750 freshly harvested third stage larvae administered through a stomach tube. This small dose was selected as a previous pilot experiment had shown larger doses to be lethal during the early pre-patent period. Post-mortem examinations were carried out on single animals on days 2, 7, 9, 10, 14, 25, 60, 120 and 270 of the experiment in an attempt to locate the position of the migrating larvae at these times.

Parasitology

Infective larvae used in this experiment were obtained as described in the previous chapter.

Up to and including day 14, the larval stages could not be quantitatively collected and were only detectable on stained

histological sections. At 25 days after infection fourth stage larvae were recovered by scraping and digestion of the thrombus material from the affected arteries. At later stages of infection larvae were grossly visible and could be recovered without difficulty.

Patency was established on day 178 of the experiment.

Pathology

At each post-mortem examination tissues were taken for histological examination from the intestine, the intestinal arteries and any other organ which showed signs of pathological change. These tissues were fixed, embedded in wax, cut and stained with haemotoxylin and eosin. In addition, special staining techniques were employed for selected sections.

RESULTS

In order to show the course of larval migration in the tissues of the host, descriptions of the lesions and parasitic stages of *S. vulgaris* found at the various stages after infection beginning at the second day and terminating after patency, are presented serially.

DAY 2

The gross lesions consisted of haemorrhagic spots in the mucosa of the intestine. These varied from 2 - 10 mm. in diameter and were seen mainly in the caecum though they were also present in the ileum and the ventral colon. There was oedema and dilation of lymphatics around the ileo-caeco-colic area.

Microscopic examination of the intestinal lesions revealed oedema, haemorrhage and lymphatic dilatation in the sub-mucosa with infiltration of polymorphonuclear leucocytes and mononuclear cells.

DAY 7

Again haemorrhagic nodules were present on the mucosal surface of the small intestine, caecum and colon with a few larger areas of congestion and haemorrhage on the serosa of the bowel wall.

Microscopically, arteritis and thrombosis of small sub-mucosal arteries could be seen (Fig. 4) and fourth stage larvae were found for the first time present in the lumina of these vessels (Fig. 5).

DAY 9

Yellowish, rough raised lesions, up to 2 cm. in diameter, surrounded by an area of congestion were evident on the serosal surface of the small intestine, caecum and, to a lesser extent, the colon and there was a small area, approximately 4 cm. in diameter, of peritonitis with adhesions between the body of the caecum near the apex and the right flank. On stretching and viewing the caecal

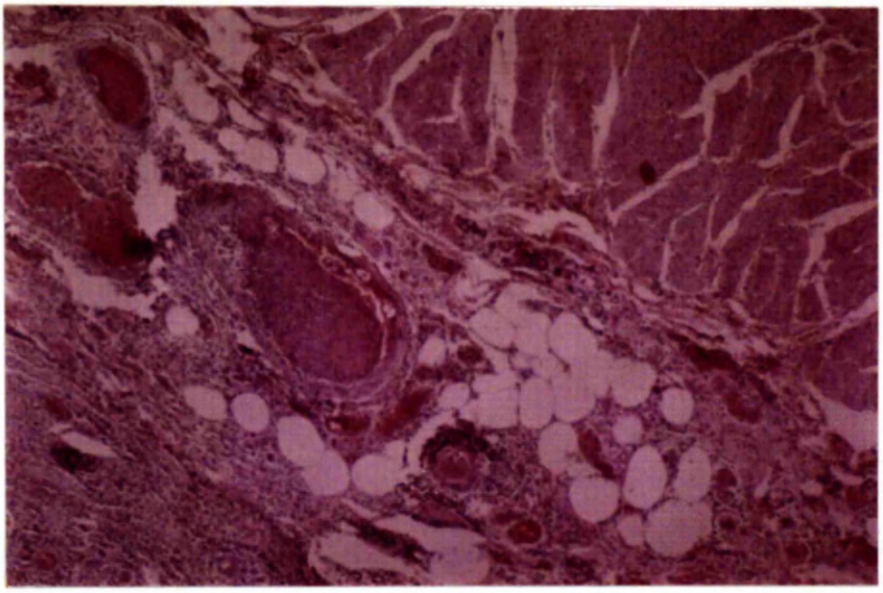


Fig. 4. Caecal wall at 7 days illustrating sub-mucosal arteritis and thrombosis. H & E x 60.

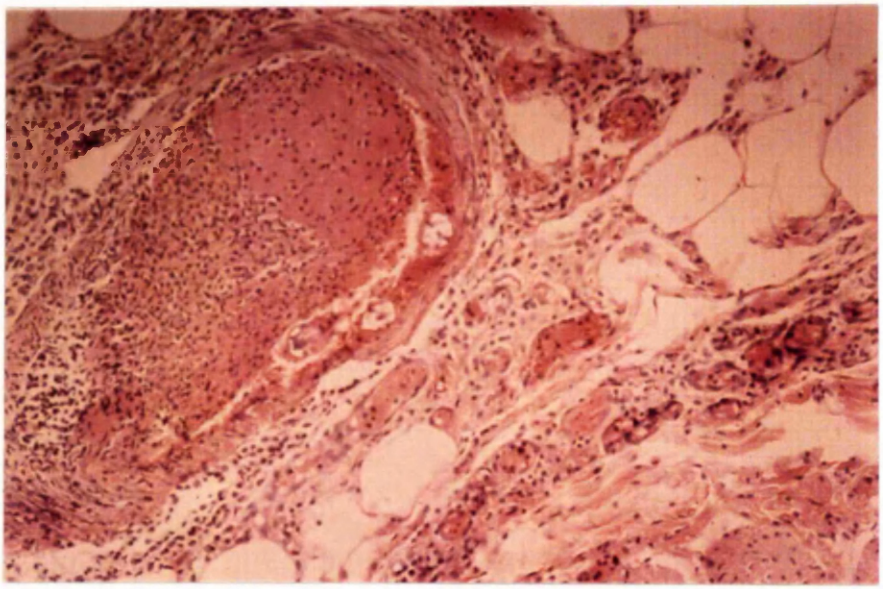


Fig. 5. Sub-mucosal artery of caecum examined at 7 days with larvae situated between thrombus material and the arterial wall. H & E x 240.

wall over a strong light source the small oedematous and haemorrhagic nodules could be seen to be closely associated with the small blood vessels in the area. Moderate diffuse haemorrhage was visible along the course of both medial and lateral caecal vessels.

There were no gross lesions visible on dissection of the aorta, anterior mesenteric artery and the intestinal arteries.

Histologically arteritis and thrombosis could be seen affecting the arteries in the sub-mucosa, muscularis and serosa (Fig. 6); fourth stage larvae of S. vulgaris were again seen in the lumina of a number of the affected sub-mucosal arteries (Fig. 7).

DAY 10

The post-mortem findings in this animal were essentially similar to those of the foal killed at 9 days. Haemorrhagic lesions were scattered all along the length of the small intestine and the apex of the caecum had an area of haemorrhage similar to those found in earlier cases. Histologically the sub-mucosal and serosal arteries of the small and large intestine showed marked arteritis and thrombosis with, in a few sub-mucosal arteries, necrosis of the arterial walls. Larvae were again found in the lumina of some of the affected arteries.

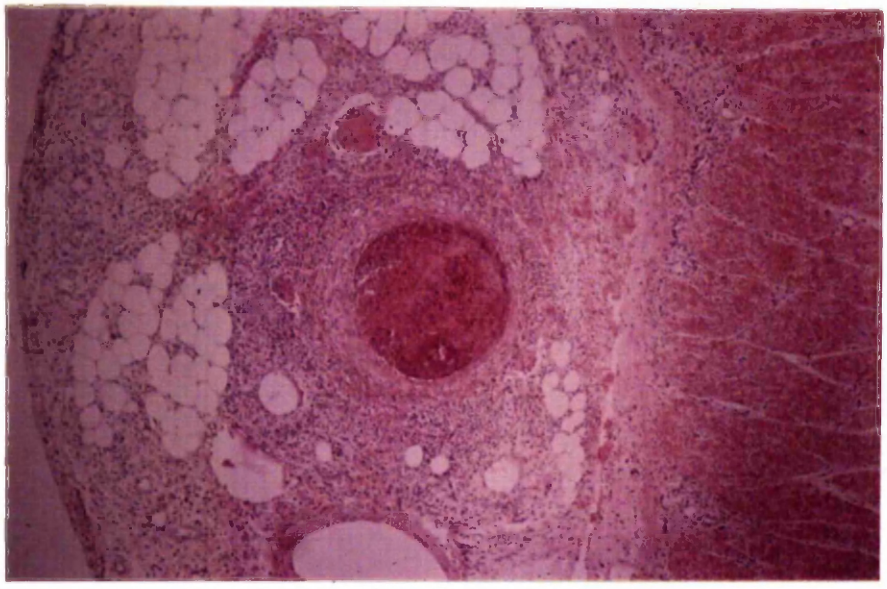


Fig. 6. An artery in the serosa of the intestine with arteritis and thrombosis at 9 days. H & E x 60.

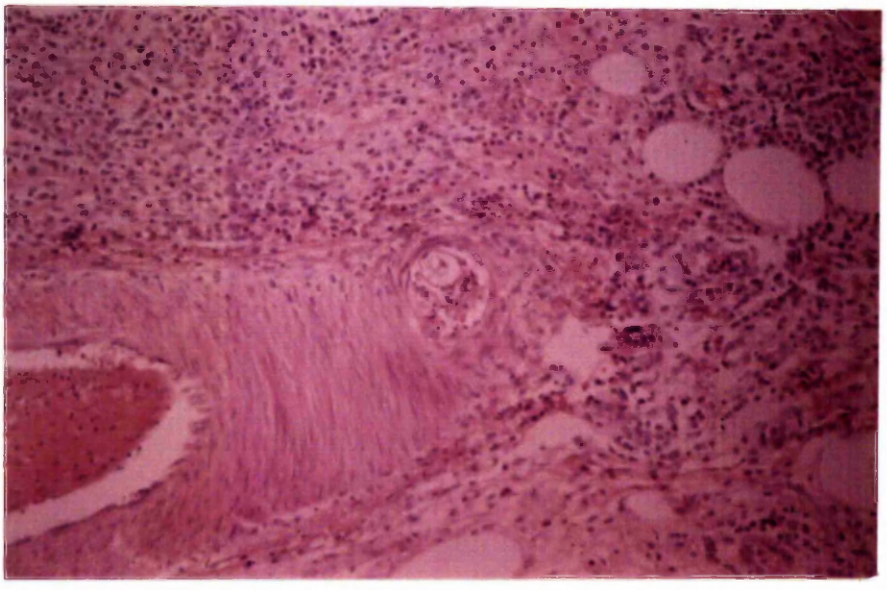


Fig. 7. Tangential section of a sub-mucosal artery at 9 days. The artery contains a larva within its lumen. H & E x 120

DAY 14

Raised nodules were again seen on the serosal surface of the small and large intestine but these had pale white centres with a surrounding area of haemorrhage. There was congestion again at the apex of the caecum and a large infarct in the ventral colon. Small thread-like tracts could be seen on the endothelium of the dorsal colic and caecal arteries. Microscopically larvae were detected in several thrombi in the caecal artery mid-way between its origin from the anterior mesenteric trunk and the caecal wall (Fig. 8).

DAY 25

Other than a small area of congestion at the apex of the caecum and pale circular lesions in the intestinal wall nothing of significance was noted in this animal until the arterial tree was dissected. In the aorta raised thread-like tracts were visible radiating from the origin of the anterior mesenteric artery and although marked thrombosis was evident in both the anterior and right branches of this artery no parasites were visible macroscopically (Fig. 9). After scraping and digesting the bulk of thrombus material from the arteries 104 fourth stage larvae were recovered. These ranged from 1.0 to 2.0 mm. in length. In sections prepared from unscraped arterial lesions more larvae were seen situated superficially in the thrombi (Fig. 10).

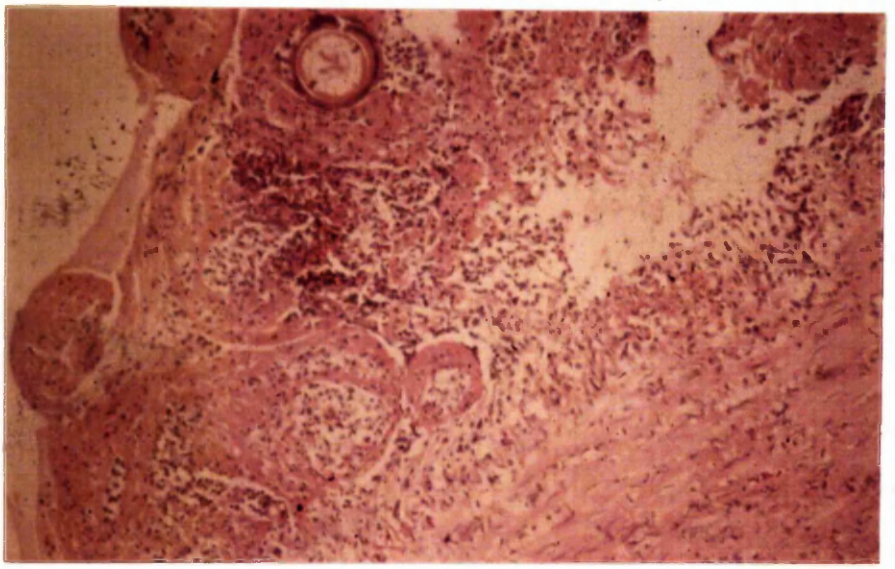


Fig. 8. A larva within a large mural thrombus of the lateral caecal artery at 14 days. H & E x 150.

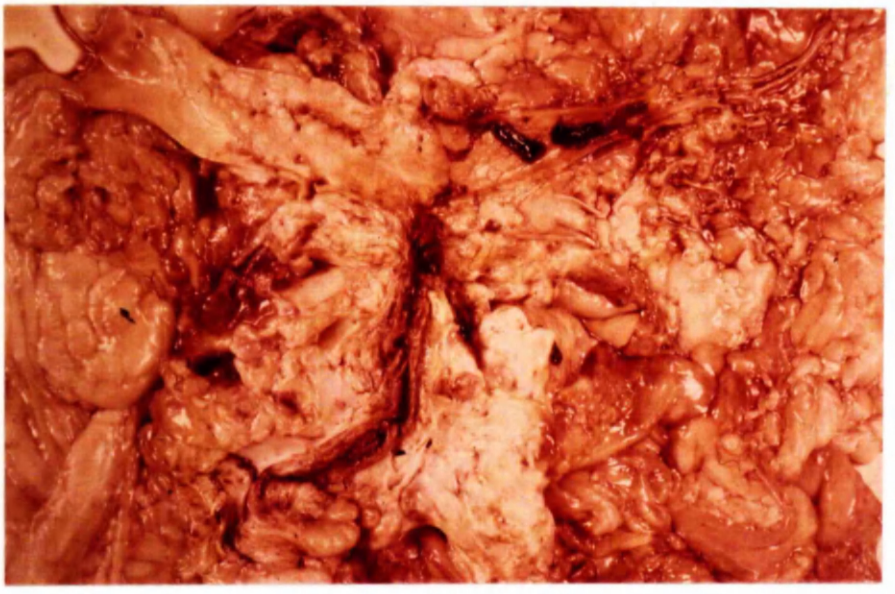


Fig. 9. Dissection of the aorta and the intestinal arteries at 25 days with thrombosis of the anterior mesenteric and ileo-caeco-colic arteries. Numerous S. vulgaris larvae were recovered from this site.

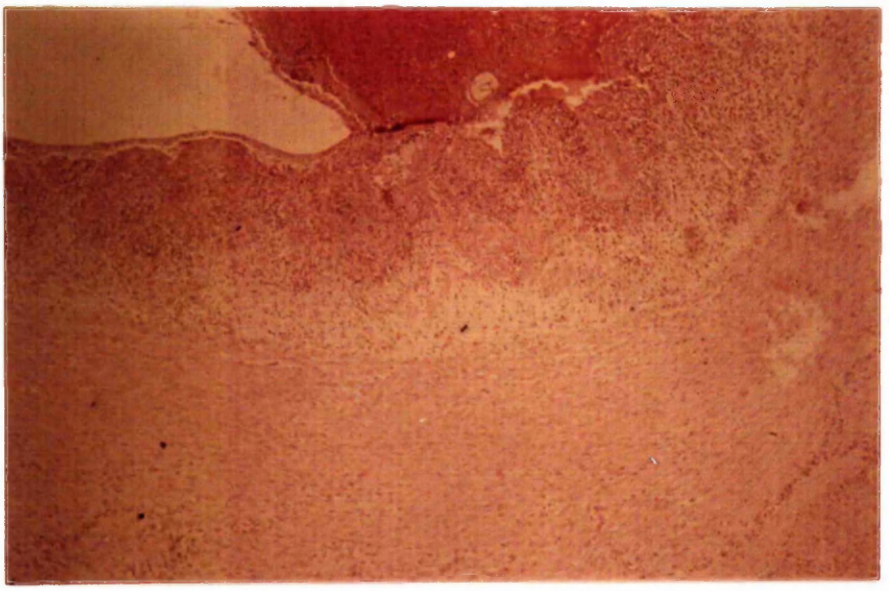


Fig. 10. The ileo-caeco-colic artery at 25 days with an inflammatory reaction in the thickened intima and a larva in a mural thrombus. H & E x 60.

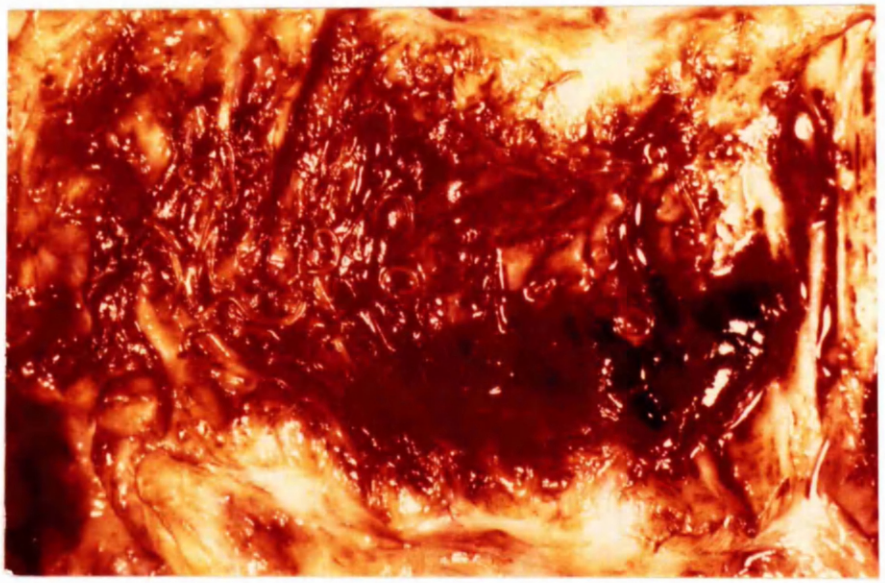


Fig. 11. The anterior mesenteric artery opened to demonstrate numerous well-developed S. vulgaris larvae at 60 days.

DAY 60

By this time the only significant lesions appeared in the anterior mesenteric artery and its main branches. Their walls were markedly thickened, difficult to cut and, when opened, many mature fourth stage larvae, from 5.0 to 13.0 mm. long, were grossly visible (Fig. 11). A total of 207 larvae were recovered from the surface of the thrombus material.

DAY 120

A number of interesting features appeared at post-mortem examination of this animal. On dissection of the arteries a total of 111 larvae, from 10.0 - 18.0 mm. long, were found lying on the granular and roughened endothelial surface of the aorta, coeliac and anterior mesenteric vessels (Fig. 12). Most of the larvae at this arterial site were fifth stage, but still retained the sheath of the fourth stage; a few immature fourth stage larvae were also found.

Several fifth stage larvae were found in the lumina of the distal branches of the coeliac and anterior mesenteric arteries (Figs. 13 & 14).

Along the length of the intestine there were numerous pea-sized nodules in the wall close to the arterial blood supply. These nodules were most abundant in the terminal part of the small intestine, and close to the caecal and colic arteries; several were also found along the course of mesenteric blood vessels.

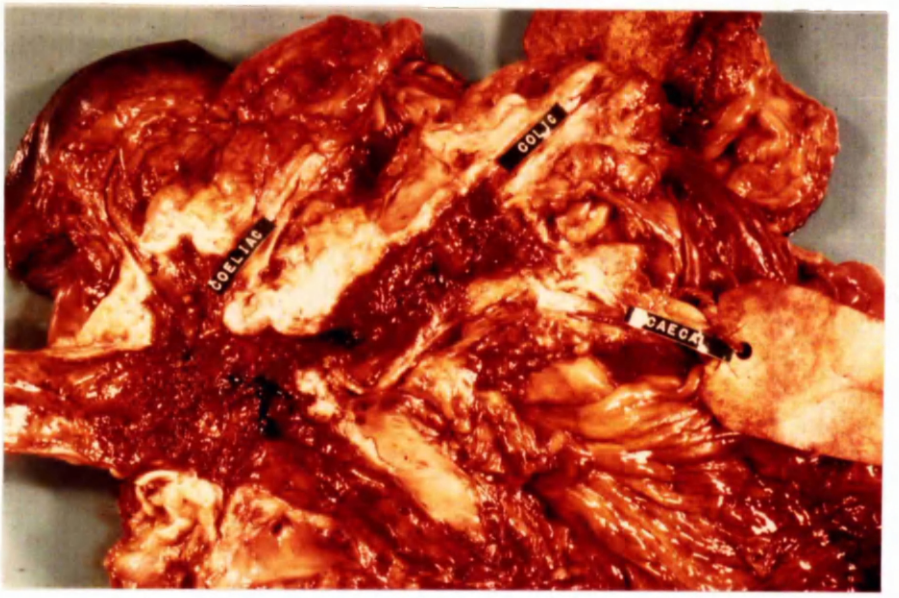


Fig. 12. Dissection of the arteries with numerous larvae at 120 days.

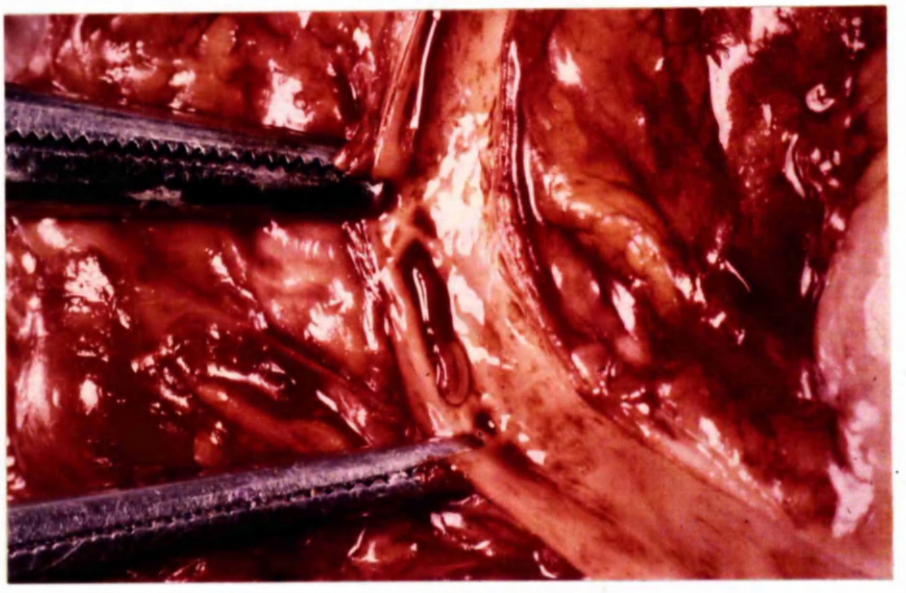


Fig. 13. A larva descending within a branch of the anterior mesenteric artery at 120 days.

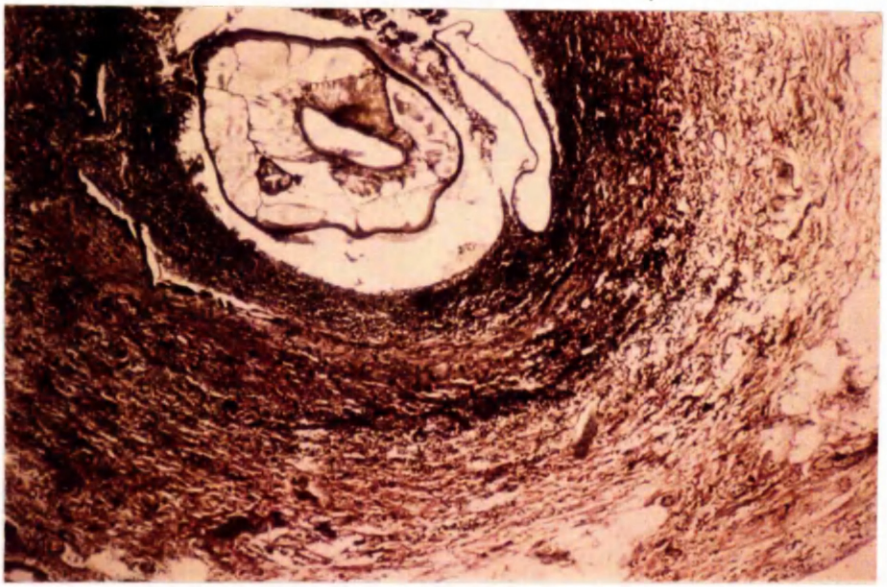


Fig. 14. A well-developed larva migrating down a branch of the anterior mesenteric artery at 120 days.
Verhoff van Giesen x 50.

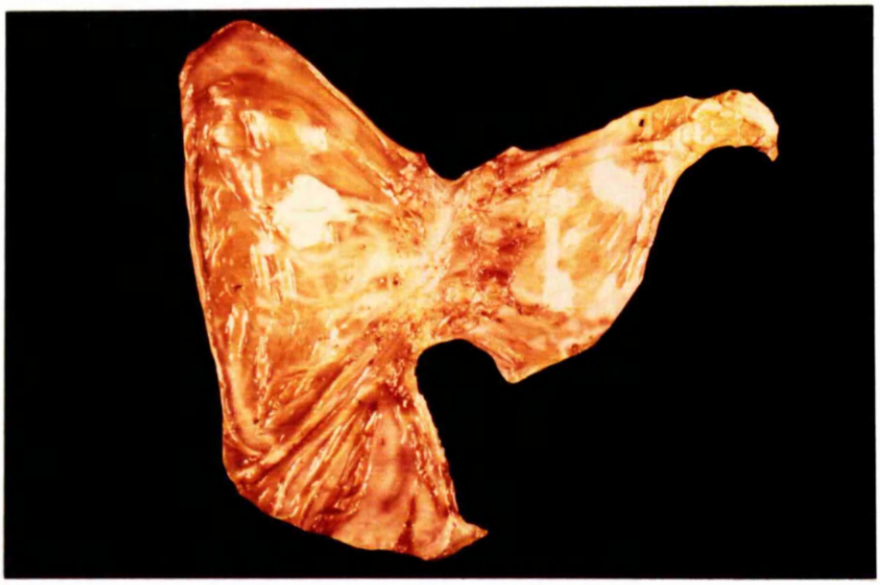


Fig. 15. A portion of the wall of the caecum with an opened nodule containing a fifth stage larva at 120 days.

When the nodules were opened some were found to contain young adult parasites in yellowish liquid pus (Fig. 15) whereas others contained yellow dry inspissated material with no parasite present. Twenty-one fifth stage S. vulgaris were recovered from the nodules (Fig. 16) and 8 young adults were found attached to the mucosa of the caecum. A total of 140 parasites were recovered from these various sites.

DAY 270

There were no gross lesions at post-mortem examination of this animal. Dissection of the arterial tree revealed no parasites but there was evidence of previous damage to the common ileo-caeco-colic arterial wall resulting in fibrosis and structural alterations (Fig. 17). A raised plaque 10 mm. x 5 mm. was seen on the endothelium of the aorta between the last 2 pairs of intercostal arteries and histologically a degenerating parasite was seen in this lesion. There was no abnormality of the endothelium surrounding this plaque. On opening the caecum a total of 75 mature adult S. vulgaris were recovered by detaching them from the mucosa.

Fig. 16. Fifth stage larvae recovered from intestinal nodules at 120 days.

Fig. 17. At 270 days dissection at the arterial site illustrated the absence of both larvae and thrombosis.

DISCUSSION

The results of this experiment seem to provide sufficient information to indicate the migratory route of the developing stages of S. vulgaris.

From the gross and microscopic lesions observed in the caecum, colon and small intestine as early as 2 days after infection it is apparent that infective larvae have exsheathed and penetrated the mucosa of these organs by this time. Their presence in the sub-mucosa results in oedema and a marked dilatation of small arteries, veins and capillaries accompanied by local haemorrhage. In view of the bulk of the intestinal tissue, the small infective dose and the relative size of the larvae, it was not possible to demonstrate larvae in the lesions at this time.

Approximately 5 days later, however, severe sub-mucosal arteritis is apparent histologically and a number of fourth stage larvae were found free within the lumina of the affected arteries. Subsequently there is a progressive migration up the arterial tree and many larvae have reached the predilection site, the anterior mesenteric artery and its main branches, by 3 weeks. During this phase larvae migrate on the endothelium and are incorporated in mural thrombi. The larvae remain in the predilection site for 2 - 4 months during which they mature and ultimately moult to fifth stage, their presence being associated with the development of arteritis, thrombosis and gross thickening of the arterial walls.

At the end of this period the young adults migrate down in the lumina of the arteries to the small and large intestine. After they have reached the serosal surface of the intestine, still within the arteries, nodules form around the affected vessels and their subsequent rupture releases young adults into the lumen of the intestine. The formation of nodules apparently occurs when the lumen of an affected artery becomes so narrow that further progress of the larva is impeded. Thus a number of nodules containing fifth stage larvae were found in the mid-zone of the mesentery associated with arteries in which the diameter was relatively narrow. These young adults then required another 6 - 8 weeks in the intestinal lumen before reaching sexual maturity giving a pre-patent period of 6 - 7 months.

These results raise several points which are at variance with conclusions in previous published work.

Since several field surveys have shown an incidence of parasitic arterial lesions of over 90% (Ottoway & Bingham, 1946; Poynter, 1960), and since in this experiment larval recoveries from the arterial lesions were in the region of 15 - 30% of the infective dose, it seems highly unlikely that larvae in arterial lesions are aberrant as suggested by Olt (1932). Rather it seems more reasonable to assume that the anterior mesenteric site is an integral part of the normal migratory pathway.

Despite histological examination of numerous arteries, larvae have never been found other than free in the lumen or in thrombi on the endothelium; there was, therefore, no evidence to support the

theory of Olt (1932) and Ershov (1949), that larvae penetrated the wall of the anterior mesenteric artery to reach the lumen. Also the presence of larvae in sub-mucosal arteries on Days 7 and 9 suggests that the route is entirely intra-arterial soon after penetration of the mucosa.

In the present experiment no tracts were observed in the aorta or anterior mesenteric artery until Day 14, but larvae could be demonstrated in the arteries closer to the gut wall in animals killed before that time. This appears to refute the theory that larvae travel from the heart to the anterior mesenteric artery. The basis for this theory had been the finding by Poynter (1960) of fibrin tracts in the aorta before he could demonstrate lesions in the anterior mesenteric artery. This discrepancy is probably due to the fact that in the present experiments foals with a single experimental infection were used whereas Poynter's conclusions were based on examination of material from horses which had been exposed to natural infection during life.

From our results it seems clear that fourth stage larvae develop in the arterial site until they are about to moult to the fifth stage, as all of the larvae found moving down the arteries and in the intestinal nodules of the foal killed on Day 120 were fully moulted young adults. There is therefore no evidence to support the proposal by Farelly (1954) that S. vulgaris larvae complete their migration while still in the fourth stage, and that mature parasites which are found in the arterial lesions are necessarily those which have been held up in their development.

A simple explanation for Farelly's finding of larvae at the origin of the aorta and other parts of the circulation is that S. vulgaris larvae seem to have the ability to migrate and establish themselves on the endothelium of arteries at some distance from the origin of the anterior mesenteric artery. This is borne out by the finding of mature fourth stage larvae in the aorta and coeliac artery of the animal examined 120 days after infection. Also in the animal killed at 270 days a degenerating parasite was found in an endothelial plaque in the region of the last pair of intercostal arteries. The fact that there was no endothelial lesion around this plaque was probably due to a rapid healing of the thread-like fibrin tracts which were produced by the migrations of the early fourth stage larvae.

Finally, in the animals killed before the tenth day it was evident from histological examination of the sub-mucosal arteries of the small and large intestine that the parasites were free within the lumina of these vessels and only when they reach larger arteries, by Day 14, are they found in mural thrombi on the endothelium. These facts are at variance with the views of Enigk (1950) that larvae penetrate the mucous membrane of the caecum and ventral colon and that their subsequent migration up the intestinal arteries is entirely in the intima.

From the work described here we have concluded that the life cycle of S. vulgaris is as follows:

(i) Third stage larvae exsheath and penetrate the small and large intestine within a few days of infection; these larvae migrate in the sub-mucosa of the intestine, moult, and have penetrated small arteries by 7 days.

(ii) Fourth stage larvae migrate up the intestinal arteries reaching the anterior mesenteric site by approximately 14 days; development takes place in this site for a period of 3 - 4 months before larvae moult to fifth stage and migrate down the arteries towards the intestine.

(iii) Nodules are formed in the intestinal wall with subsequent release of young adults into the lumen. The pre-patent period is 6 - 7 months.

Early in the pre-patent phase the foals showed varying degrees of pyrexia, anorexia and colic and this will be discussed as part of a separate chapter on the pathogenesis of S. vulgaris infection.

SUMMARY

In an attempt to elucidate the migratory route of S. vulgaris larvae 9 worm-free pony foals were each infected with a pure culture of 750 infective larvae and killed at intervals over a period of 9 months. The results obtained show that infective larvae exsheath and penetrate the intestine within a few days of infection. These larvae then moult in the sub-mucosa, penetrate small arteries and have migrated within the lumina of intestinal arteries to the anterior mesenteric site by 14 days. In the predilection site larvae develop from early to late fourth stage and after a period of 3 to 4 months the fourth moult is completed and young adults return to the intestine again via the lumina of the arteries. Nodules are formed with the subsequent release of young adults into the intestinal lumen. The migration is complete by 6 to 7 months after infection.

CHAPTER 3

THE PATHOGENESIS OF EXPERIMENTAL *STRONGYLUS VULGARIS* INFECTION IN THE HORSE

INTRODUCTION

In the assessment of the total pathogenic effect of S. vulgaris infection, two factors must be taken into account. First, the effect of migrating larvae within the arteries; secondly, that of the adult parasites in the caecum and colon of the horse.

It is generally accepted that the principal pathological effects of S. vulgaris are almost entirely due to migration of the developing larval stages within the host and pathological changes associated with these, in naturally infected horses, have been described by numerous authors (Ottoway & Bingham, 1941, 1946; Farelly, 1954; Poynter, 1960; Mathieson, 1964).

The major lesion produced is an inflammation of the anterior mesenteric artery and its major branches and is frequently referred to as a "verminous aneurysm". Although true aneurysms with dilation of the artery and thinning of the arterial wall do occur, Mathieson found that the most common lesion is one of arteritis and thrombosis with marked thickening of the arterial walls. The clinical significance of these lesions depends on their magnitude and location and whether or not there is an associated thrombo-embolism. Thrombosis and embolism of branches of the ileo-caeco-colic artery may lead to inflammation and infarction of the intestine (Enigk, 1951a).

Other lesions have been reported apparently associated with migration of larvae within the arterial system beyond the predilection site. Thus Cronin and Leader (1952) reported a case of

sudden death in a Thoroughbred horse as a result of thrombosis in the right coronary artery caused by S. vulgaris larvae, and Mahaffey and Adam (1963) described a clinical case of infarction of the kidneys with larvae and adult stages of S. vulgaris.

There are comparatively few descriptions of the pathogenesis of single experimental infections in worm-free animals, the first comprehensive account being by Enigk (1950). Using single doses of 800 - 8,000 infective larvae administered to 5 foals aged between 3 - 6 weeks, he produced a severe clinical syndrome which proved fatal within 1 - 3 weeks of infection. The cause of death was infarction of the small or large intestinal wall which was invariably preceded by pyrexia, anorexia and severe colic.

Subsequently Drudge, Lyons and Szanto (1966) infected a group of 11 worm-free foals with doses of 2,500 - 5,000 larvae; nine of these animals developed acute reactions and died or were sacrificed in extremis within 14 - 22 days after infection. The clinical syndrome and gross post-mortem findings produced in these experiments were basically similar to those reported by Enigk. They also reported a moderate normocytic normochromic anaemia and a polymorphonuclear leucocytosis. In the ponies which survived the initial acute phase they observed a progressive increase in the total serum protein due mainly to increases in the beta-globulins. Round (1970) demonstrated similar changes in the serum proteins of ponies experimentally infected with 700 - 1,500 S. vulgaris larvae.

Information is lacking on the second aspect of the pathogenesis of S. vulgaris, i.e. the effect of the adult parasites in the lumen of the intestine. All observations up to the present time have been on naturally infected animals carrying a mixed burden of the various strongyle species parasitic in the horse. Adult strongyles are inhabitants of the large intestine and feed by attaching to the glandular epithelium and drawing a plug of mucosa into the buccal capsule. The damage thus caused results in the formation of crater-like ulcers, which in the case of the large strongyles may extend into the gut wall. This damage is believed to be the cause of the anaemia, unthriftiness and poor performance commonly associated with helminth infections in the horse, but there have been few detailed investigations similar to those carried out on parasitic infections of cattle and sheep.

The main object of this section is to describe the clinical signs, pathology and clinical pathology associated with a single experimental infection of S. vulgaris in worm-free ponies and to report the results of an initial study using radio-isotopic tracer techniques to assess the pathogenic effect of the adult parasites in the large intestine.

The results of these studies will be discussed under 2 separate headings.

Experiment 1 - the pathogenesis of migrating S. vulgaris larvae.

Experiment 2 - the pathogenic effect of adult parasites in the large intestine.

MATERIALS AND METHODS

EXPERIMENT 1

Experimental Animals

These were the 9 infected foals used in the experiment designed to elucidate the life cycle of S. vulgaris (Chapter 2). In addition, 2 worm-free foals acted as normal controls in the evaluation of the clinical, haematological and biochemical changes associated with single experimental infections with 750 third stage larvae. The clinical signs and pathology produced in several other foals given different doses of larvae are also included in this report.

Clinical Examinations

Each animal was under daily observation throughout the period of the experiment and rectal temperatures were recorded daily.

Blood Analysis

Routine haematological and serum protein estimations were carried out on blood samples collected twice weekly from all foals.

Pathology

At necropsy various tissues showing pathological changes were removed and processed for histological examination. Sections were routinely stained with haemotoxylin and eosin and in addition selected sections were subjected to various special staining techniques.

EXPERIMENT 2

Experimental Animals and Design

Using ^{51}Cr-labelled red blood cells and ^{125}I-labelled albumin red blood cell turnover and plasma protein metabolism were investigated in 3 groups of animals.

Group 1	Group 2	Group 3
Two worm-free foals	Two experimentally infected foals each with a worm burden of approximately 100 adult S. vulgaris.*	Two naturally infected ponies with mixed strongyle faecal egg counts of 100 - 300 e.p.g.

Each animal was injected intravenously with a ^{51}Cr-labelled preparation of its own red blood cells and ^{125}I-labelled albumin. To ensure the rapid excretion of ^{125}I from degraded albumin each animal was dosed orally with 20 ml. 0.75% KI beginning 4 days prior to the labelled albumin injection and thereafter daily throughout the investigation. In this way liberated ^{125}I was not taken up and stored by the thyroid gland.

Labelling of albumin and red cells

Labelling of horse albumin with ^{125}I and autologous red cells with ^{51}Cr was carried out as described earlier.

- - - - -

* Based on percentage 'take' in other foals infected on the same occasion and on necropsy findings of one of the foals in Group 2.

Injection of labelled materials, blood and faecal sampling

Labelled albumin and red cell suspensions were injected via a jugular catheter. Heparinised blood samples (5 ml.) were collected 10 minutes later from the opposite vein. Further samples were withdrawn twice daily during the following 6 days and thereafter at regular 24-hour intervals for a further 8 days. One ml. samples of whole blood and plasma and 20 gm. samples of faeces were prepared and assayed for radioactivity.

Calculations and expression of results

Plasma and red cell volume estimations

The plasma and red cell volume of each animal was estimated from the radioactivity of the 10-minute samples of plasma, and of whole blood corrected for venous haematocrit, respectively, by the isotope dilution principle. Blood volume was calculated as the sum of the plasma and red cell volumes.

Construction of disappearance curves

The count rate of each plasma and blood sample, corrected for radioactive decay was expressed as a percentage of the 10-minute post-infection sample and a semi-log plot made of activity against time. By use of the venous haematocrit determination on each sample, the radioactivity per ml. of red cells was calculated.

Albumin turnover calculations

The plasma volume together with the serum albumin concentration enabled the intravascular pool (CA) to be calculated. The determination of the extravascular pool (EA) was based on the extrapolation procedure described by Sterling (1951).

The catabolic rate of albumin was assessed in 2 ways. First from the "apparent half-life" ($T\frac{1}{2}$) of the final exponential of the plasma activity curve (Sterling, 1951); secondly, by calculating the fractional catabolic rate (K), i.e. the fraction of the intravascular pool degraded each 24 hours. K was calculated by analysis of the plasma activity curve by the method of Matthews (1957).

Analysis of faecal excretion of isotopes

The total ^{125}I radioactivity in each 24-hour collection of faeces was divided by the activity per ml. of plasma taken at the beginning of the collection period to give a daily faecal "clearance" of plasma. "Clearances" of whole blood and red cells were calculated in a similar manner by relating blood and red cell ^{51}Cr activity to that in the faeces. These faecal "clearance" figures represent the amounts of plasma, blood and red cells, or their breakdown products, which have to appear in the gastro-intestinal tract to account for the radioactivity in the faeces. Since unpublished observations (Duncan & Dargie) have shown that ^{51}Cr is excreted quantitatively in the faeces when administered orally to horses, it is assumed that red cell "clearance" figures provide a valid estimate of intestinal haemorrhage. Faecal

"clearances" of plasma (i.e. ^{125}I) on the other hand, seriously underestimate protein loss into the alimentary tract because of the substantial breakdown and reabsorption of the label which is known to occur with radio-iodinated plasma proteins. Nonetheless elevated values, if found, provide good qualitative evidence of pathological enteric protein leak.

RESULTS

EXPERIMENT 1

Clinical Observations

The most significant clinical signs in the group of foals which received doses of 750 infective larvae occurred during the first 3 - 4 weeks after infection. During this period a constant finding was an increase in the body temperature. This did not occur at the same time in all foals but the general pattern is shown in Figure 18 where the rectal temperatures of infected foals are compared with uninfected controls. Figure 19 shows the individual temperature curves of 2 infected foals during this period. Pyrexia in the individual animals was accompanied in most cases by varying degrees of dullness and anorexia with the foals spending a great deal of time in sternal recumbency. During these periods of recumbency there was a reluctance to rise and move around together with a degree of apprehension and discomfort on abdominal palpation which was considered to be due to low grade abdominal pain. Intermittent colic occurred in 3 of

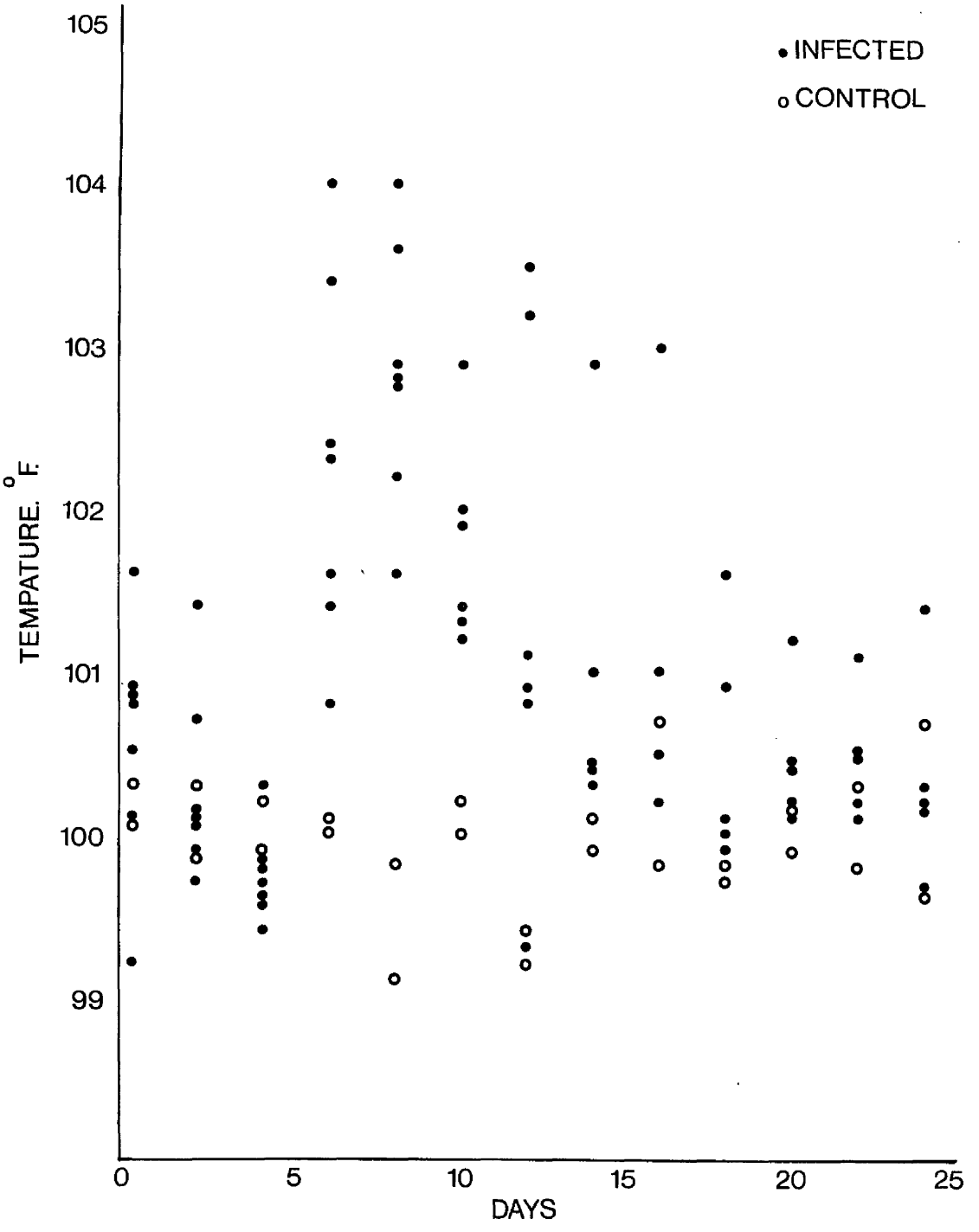


FIG. 18 Individual rectal temperatures of foals following infection with 750 S. vulgaris larvae.

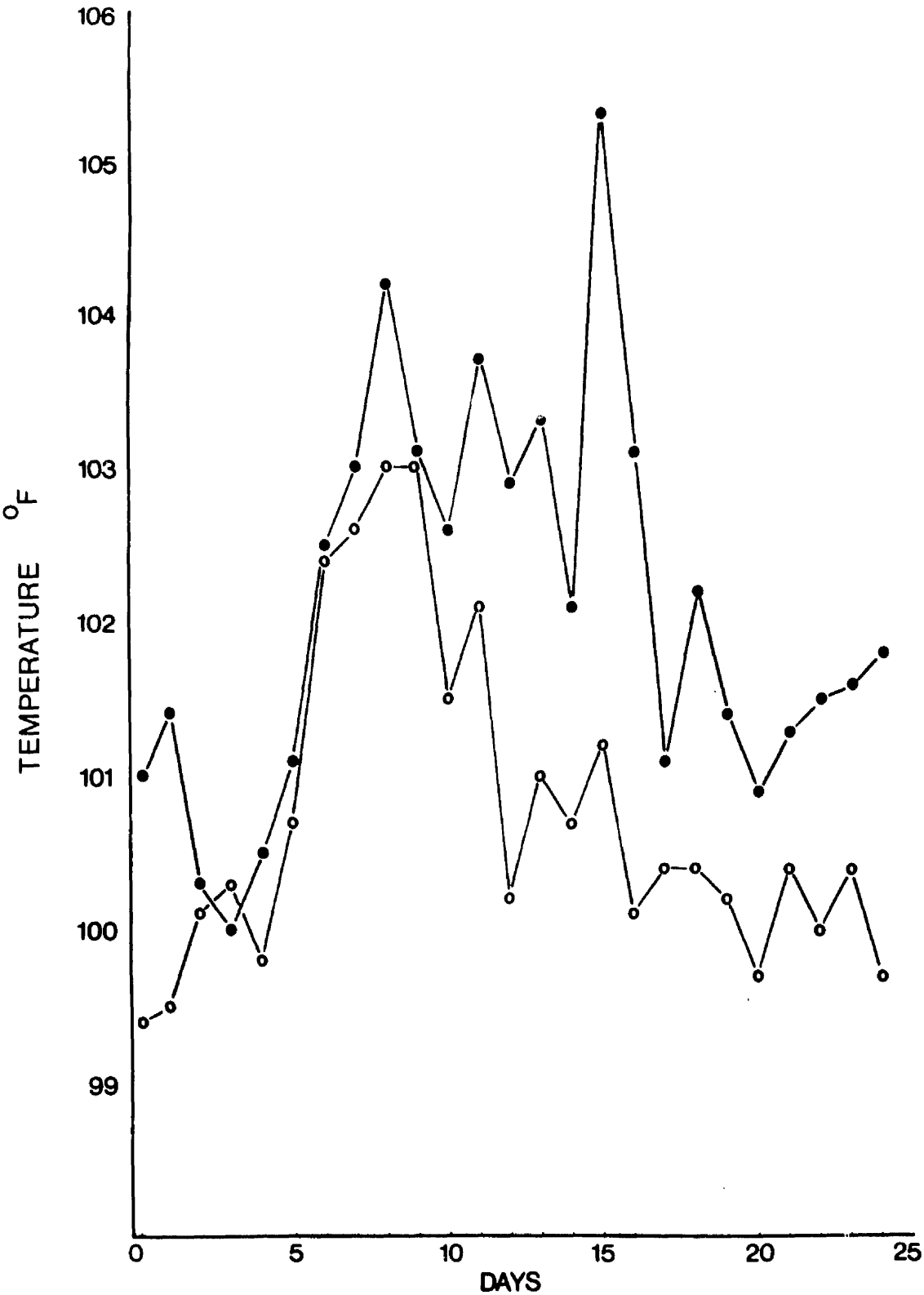


FIG. 19 Temperature curves of 2 foals following infection with 750 S. vulgaris larvae.

the infected foals between the 13th and 17th day after infection. The affected animals showed varying degrees of abdominal pain evidenced by turning round and looking at the flank, discomfort on lying down, kicking at the abdomen, sweating and rolling. On auscultation of the abdomen during these bouts of colic, there was generally increased intestinal motility with frequent fluid gas borborygmi. The pulse rates and respiratory rates of the infected animals increased during these bouts of colic and the mucous membranes were markedly injected. The faeces of the infected animals were generally scant and dry during the temperature reaction but several animals developed soft faeces during and after bouts of colic. Two animals used in pilot experiments and given 1,400 and 2,500 larvae died on Day 14 after showing signs of severe colic.

After the initial 3-week period the animals infected with 750 larvae showed little clinical abnormality other than an occasional moderate temperature rise for a few days together with a general unthriftiness.

Pathology

The pathological changes associated with a single experimental infection of *S. vulgaris* are presented sequentially by describing the lesions associated with 3 distinct stages of infection.

Stage 1

Lesions occurring within the first 3 weeks after infection involving initially the intestine and the subsequent progressive development of the lesions in the mesenteric arteries.

Stage 2

Arterial lesions associated with maturation of fourth stage larvae at the predilection site over a period of 1 - 4 months after infection.

Stage 3

The pathological changes present in the arteries after 4 months together with lesions associated with the return of mature S. vulgaris larvae to the intestine.

Stage 1

Within 2 days of infection grossly visible haemorrhagic spots appear in the mucosa of the ileum, caecum and colon as a result of penetration by third stage larvae. On histological examination the sub-mucosal haemorrhage and oedema is seen to be associated with infiltration of neutrophils, leucocytes and eosinophils (Fig. 20). Dilated lymphatics containing many neutrophils are obvious and occasional lymphocytes and fibrin strands are visible in some lymphatics. Similar lesions are visible in the lamina propria but these are less conspicuous than the sub-mucosal lesions.

By 1 week after infection a severe inflammatory reaction with vascular engorgement and local cellular infiltration is present in the sub-mucosa. Thrombosis of small sub-mucosal arteries is evident (Fig. 21) and in some of these vessels fourth stage larvae are visible. Focal necrotising lesions can be seen around thrombosed vessels together with necrotic tracts in the sub-mucosa. There is widespread sub-mucosal arteritis with damage to arterial walls and

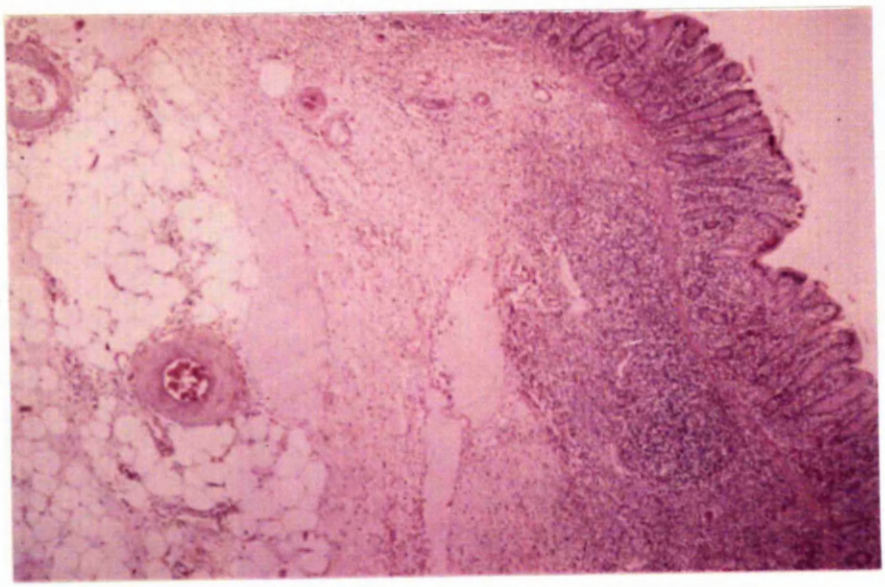


FIG. 20 Sub-mucosal oedema and haemorrhage at 2 days after infection H & E x 50

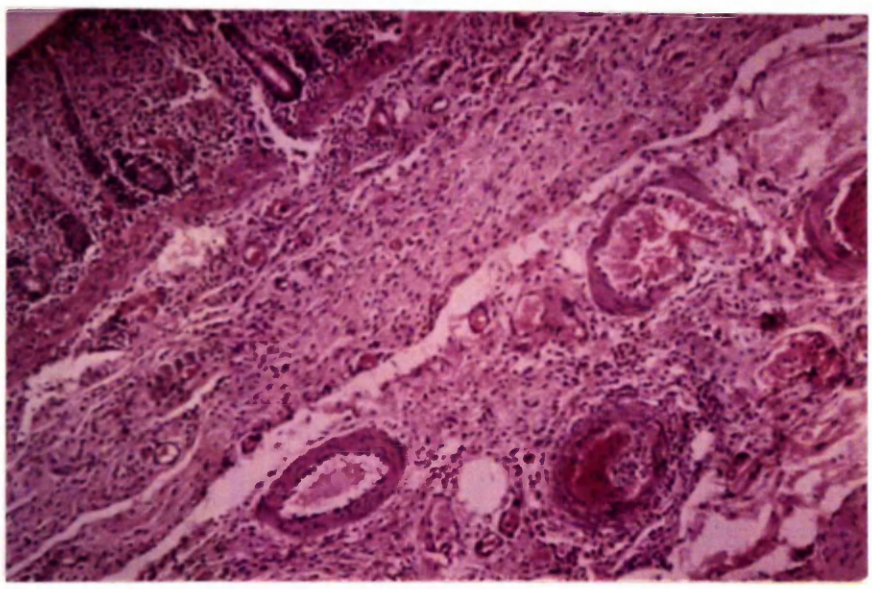


FIG. 21 Thrombosis of sub-mucosal arteries at 7 days after infection H & E x 110

infiltration by neutrophils together with fibrin plugs and masses of inflammatory cells in the sub-mucosal lymphatics (Fig. 22). Nodular thickening caused by marked haemorrhage and cellular infiltration is apparent in parts of the sub-mucosa. This is also seen to affect the inner layer of muscularis adjacent to these nodular lesions. From these lesions many congested capillaries extend through the muscularis surrounded by lymphocytes, macrophages and eosinophils.

By 9 - 10 days after infection, changes similar to those seen at 7 days are evident but these are seen to extend through the muscularis to the serosa with the development of a sub-serosal arteritis. Separation of smooth muscle cells in the muscularis occurs due to marked infiltration of neutrophils and macrophages (Fig. 23). Serosal plaques are prominent at this stage consisting of congested blood vessels with fibroblasts and scattered macrophages and lymphocytes (Fig. 24).

Approximately 2 weeks after infection residual lesions are still visible on the serosal surface of the intestine. These are discrete, pale, red nodules which on section show fibrous thickening, focal congestion and haemorrhage, involving primarily the sub-mucosa. Groups of cells, mainly lymphocytes, are visible around some arteries and focal areas of necrotic eosinophilic debris are found scattered in the sub-mucosa. It is at this stage that haemorrhagic infarction of the colon occurred in the 2 animals given 1,400 and 2,500 larvae respectively (Fig. 25). In several branches of the anterior mesenteric artery fourth stage larvae are found in association with mural thrombi.

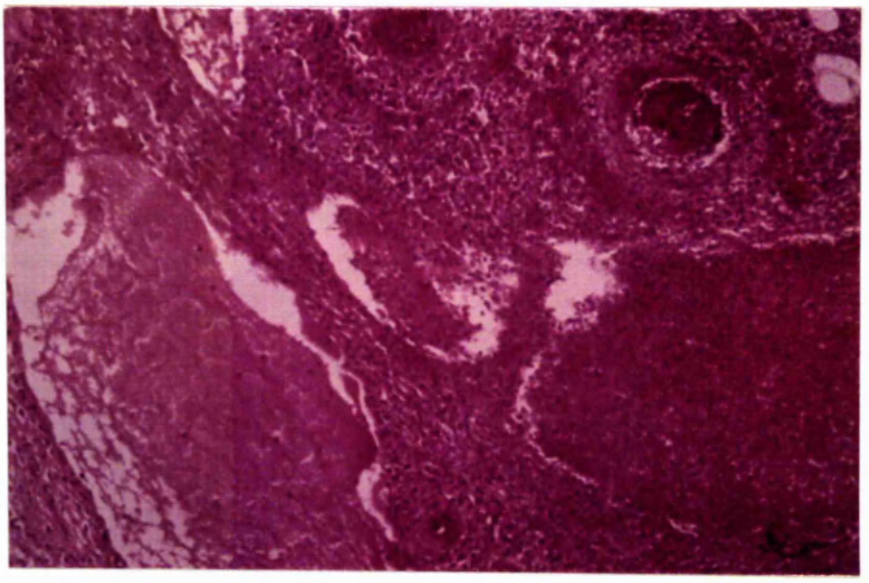


FIG. 22 Widespread sub-mucosal arteritis with infiltration of inflammatory cells H & E x 110

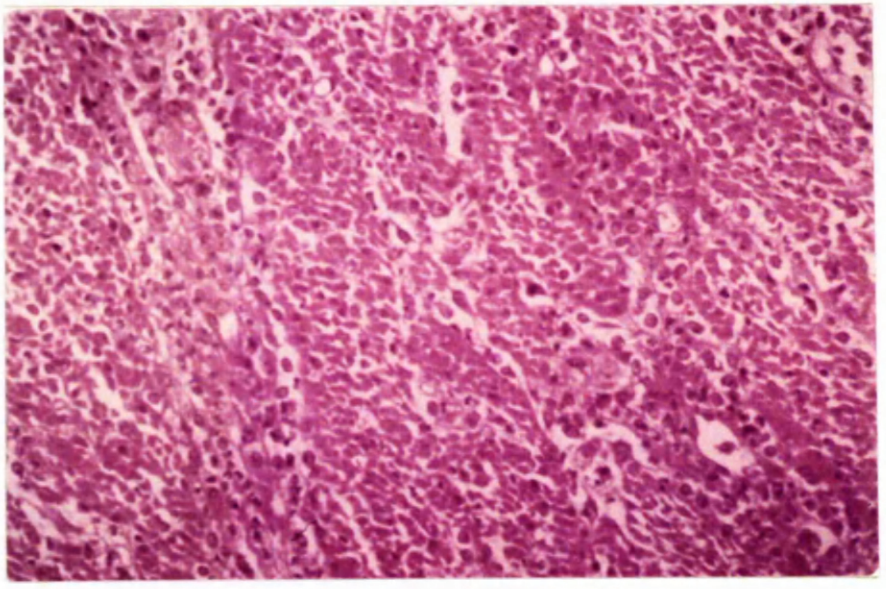


FIG. 23 Separation of smooth muscle cells in muscularis due to infiltration with neutrophils and macrophages 9 days after infection H & E x 250

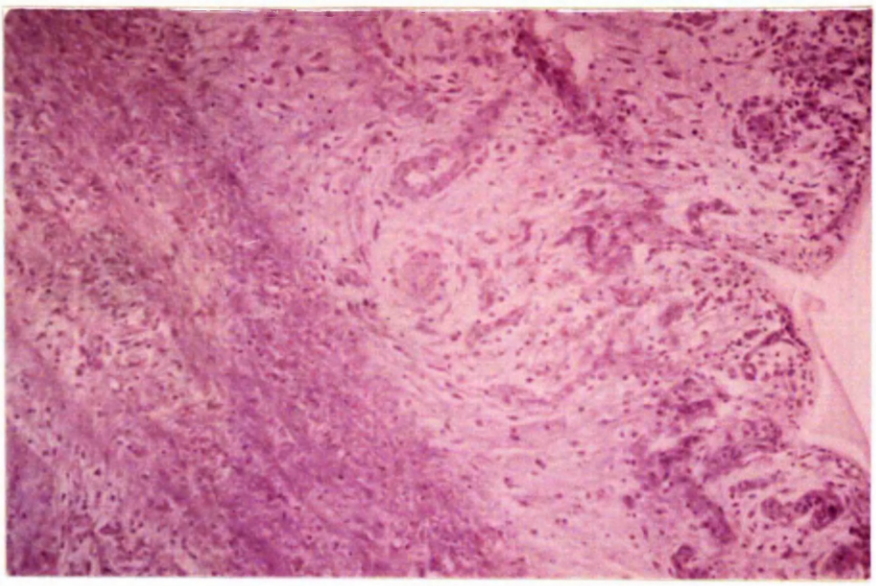


FIG. 24 Serosal plaque at 9 days after infection
H & E x 110

FIG. 25 Haemorrhagic infarction of the colon at 14 days after infection

The major lesion 3 weeks after infection is gross thrombosis of the anterior mesenteric artery and its major branches associated with the presence of fourth stage larvae, although the intestinal lesions due to the earlier migration of these larvae are still evident. On section the intima of these affected arteries is seen to be considerably thickened and appears to be thrown into folds and to be infiltrated by plasma cells, lymphocytes, macrophages and some eosinophils (Fig. 26). Branches of these vessels which are seen in the adventitia are thrombosed with masses of eosinophils scattered in the adventitia of the main vessel.

Stage 2

Between 1 - 4 months after infection the gross lesions are predominantly in the anterior mesenteric artery; fibrous thickening and thrombosis is more prominent and is associated with the presence of developing fourth stage larvae. On histological examination intimal thickening with oedema and some plasma cell infiltration is evident at 2 months after infection; this is particularly so deep in the lesion where there is also marked infiltration of the media by plasma cells and lymphocytes. On the surface of the intima there is marked thrombus formation containing many neutrophils and some eosinophils (Fig. 27). Some organisation of the thrombus material has occurred at this stage and the adjacent intima is thrown into folds with many plasma cells and some macrophages

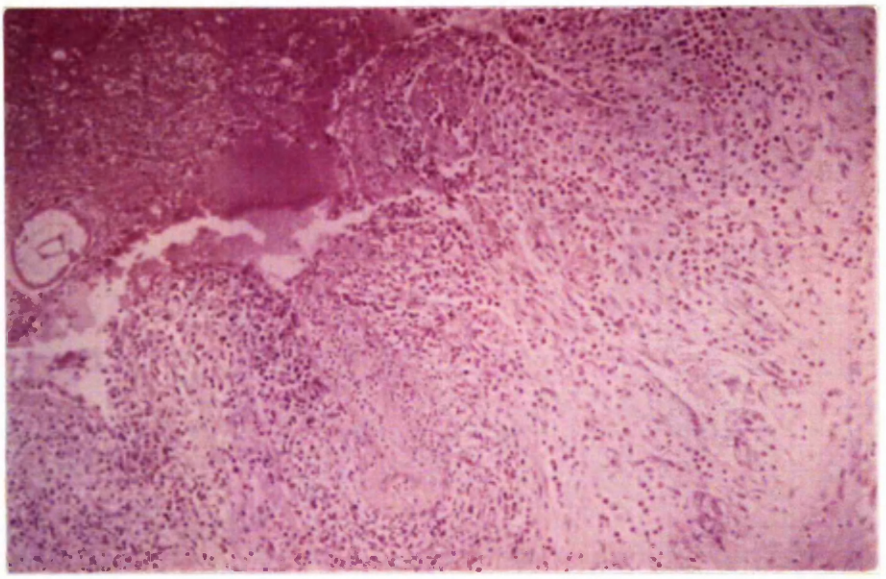


FIG. 26 Section of the intima of the anterior mesenteric artery at 25 days after infection H & E x 110

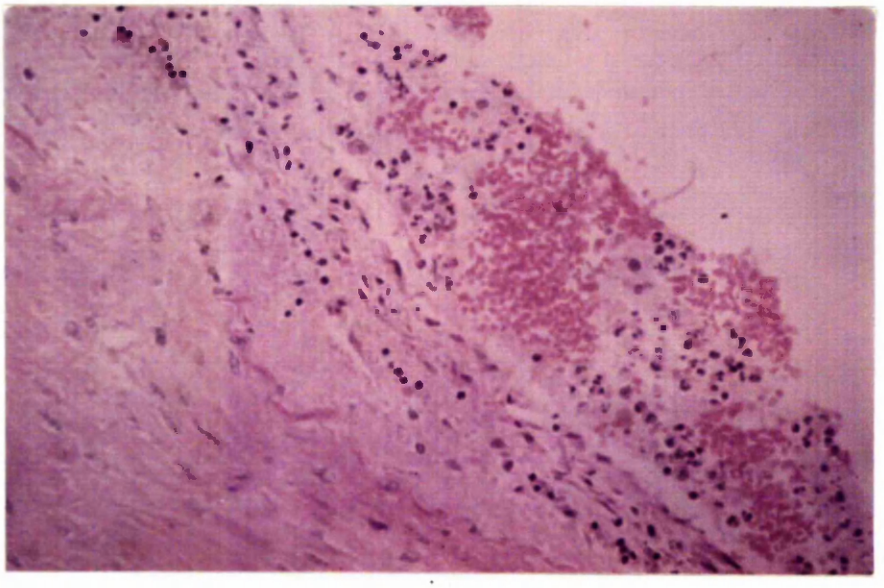


FIG. 27 Superficial thrombus formation on the intima at 2 months after infection H & E x 250

containing haemosiderin. Near the major sites of thrombus formation there is fibrous tissue proliferation which has damaged the media of the vessel (Fig. 28). In the aorta there are small intimal accumulations of eosinophils and lymphocytes forming raised lesions (Fig. 29), which are associated with the migration of fourth stage larvae beyond the predilection site, i.e. the anterior mesenteric artery. In addition, some superficial thrombus formation with thickening of the intima and the local accumulation of lymphocytes is apparent and associated with these lesions are focal accumulations of dead neutrophils.

Stage 3

At 4 months after infection the arterial lesion is still prominent: mature larvae are still present but a proportion have already returned to the intestine. Microscopically fibrosis of the inner part of the wall of the anterior mesenteric artery with disruption of the intima is seen to be widespread (Fig. 30). There are scattered collections of macrophages with haemosiderin in the arterial wall together with fibrosis and thickening of the walls of the vasa vasorum (Fig. 31). Organised thrombi containing macrophages and eosinophils are apparent in these small vessels and result in the obliteration of their lumina.

The typical intestinal lesions caused by larvae returning to the intestine consist of nodule formation in close proximity to a thrombosed artery (Fig. 32). The thrombosed vessel can often be

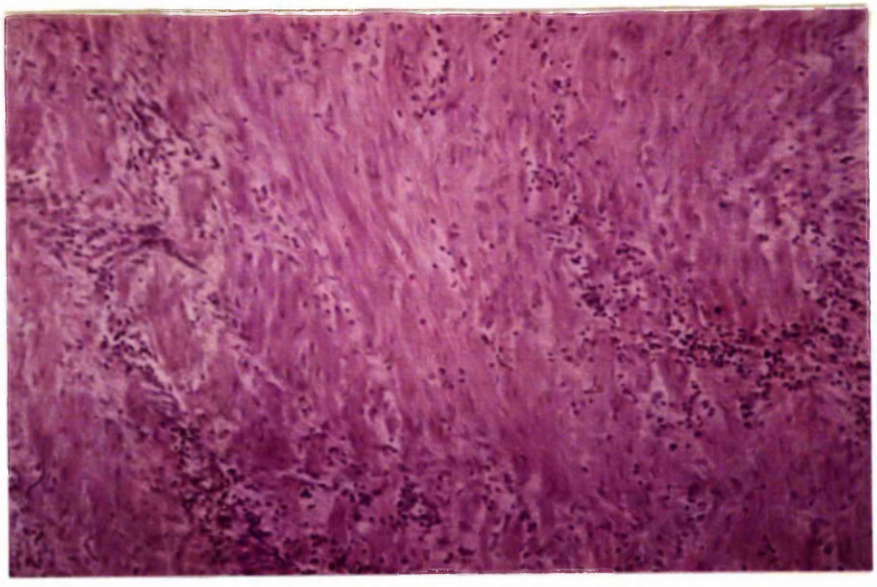


FIG. 28 Fibrous tissue proliferation in media of affected artery 2 months after infection H & E x 110

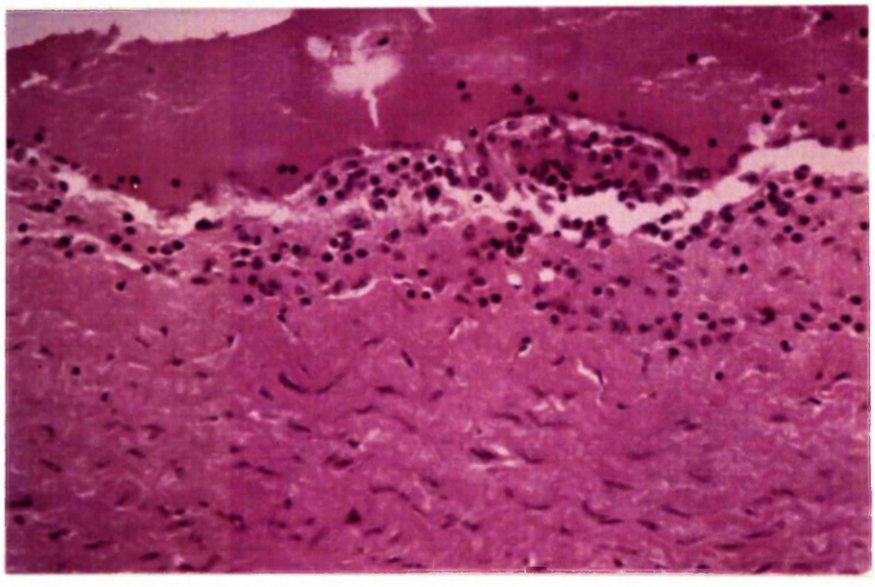


FIG. 29 Section of aorta showing raised intimal lesion 2 months after infection H & E x 250

FIG. 30 Fibrosis of anterior mesenteric artery 4 months after infection H & E x 110

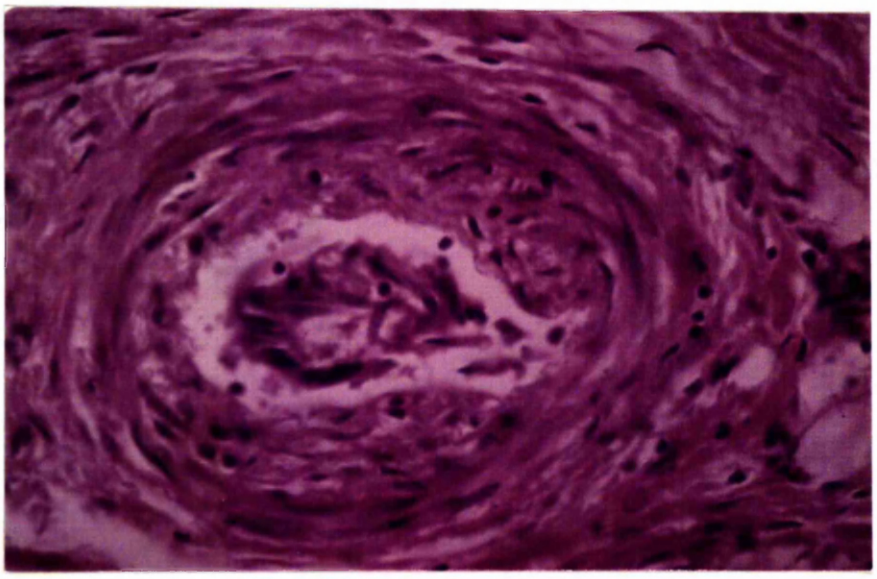


FIG. 31 Example of fibrosis and thickening of wall in one of the vasa vasorum H & E x 400

seen entering the muscularis from the serosa, the serosal end of it being full of neutrophils. In the sub-mucosa there is oedema and dense accumulations of neutrophils with some eosinophils at the periphery. At a later stage sections of larvae are seen in the intestinal wall surrounded by neutrophils, necrotic debris and some eosinophils (Fig. 33). Surrounding this there is congestion with macrophages but rarely much fibrous tissue.

Over a period of several months larvae leave the arteries and return to the intestine and by 9 months after infection the only significant gross finding is a healed arterial lesion. Histologically there is fibrosis of the intima with large groups of macrophages containing haemosiderin in the inner part of the wall and to a lesser extent in the media. Macrophages and scattered foci of lymphocytes are also apparent in the adventitia (Fig. 34).

Clinical Pathology

Haematology

Routine haematology was carried out on samples from both infected and control foals throughout the prepatent period. Nine worm-free foals were infected at the start of the experiment but due to serial killing to elucidate the life cycle, this number was reduced to 2 animals by the end of the experimental period.

The results of these estimations are presented in Figure 35. There were no significant differences in the mean red cell indices

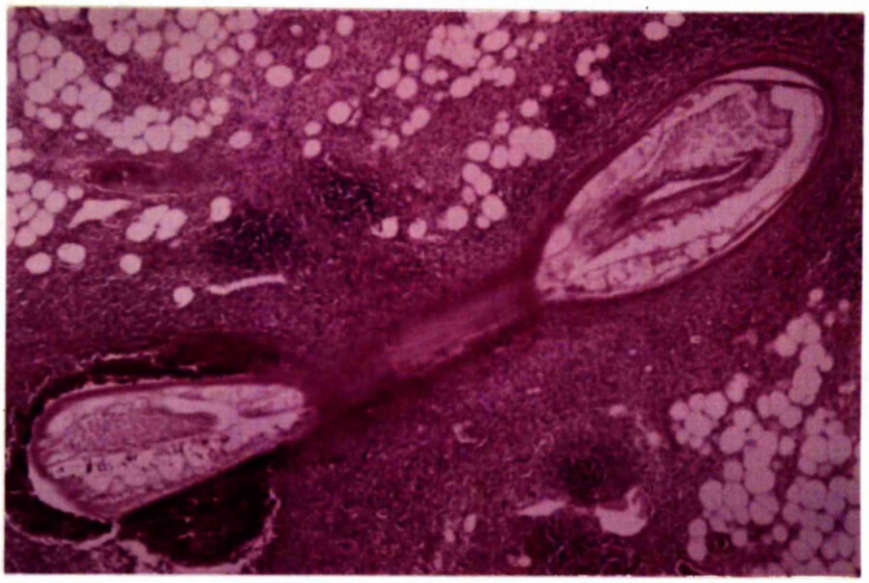


FIG. 32 Nodule formation at termination of intestinal artery at 4 months after infection H & E x 50

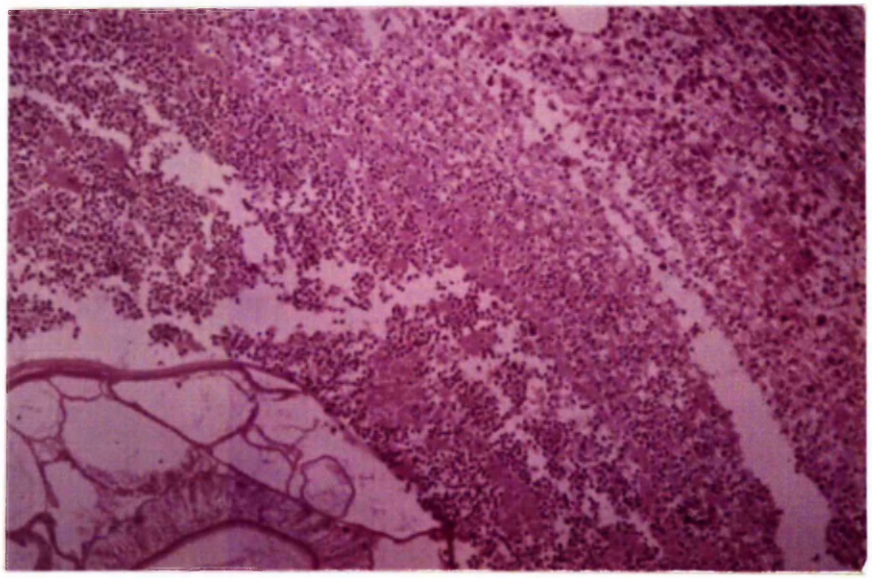


FIG. 33 Section of mature larva in the intestinal wall H & E x 110

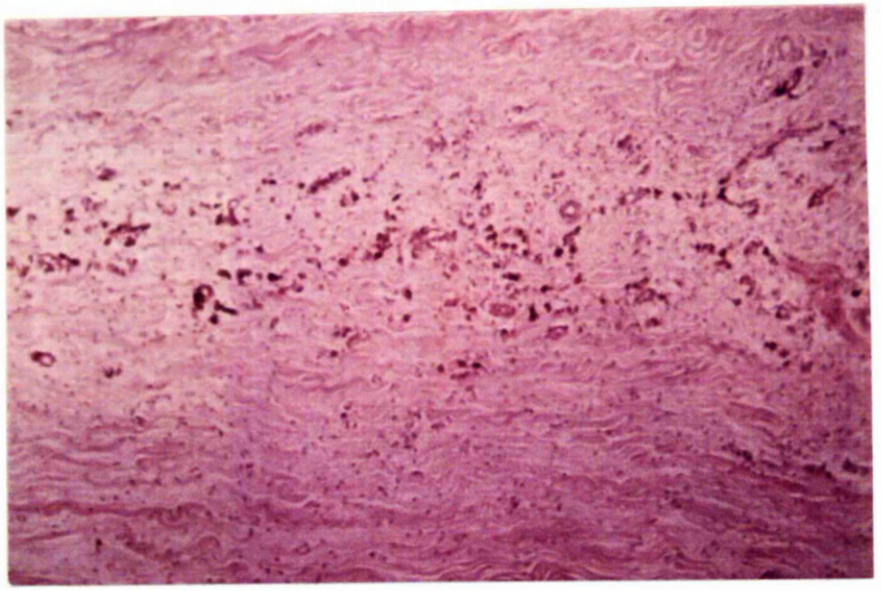


FIG. 34 At 9 months after infection lymphocytes and macrophages containing haemosiderin in arterial wall H & E x 110

MEAN RED CELL INDICES FOLLOWING INFECTION WITH S. vulgaris

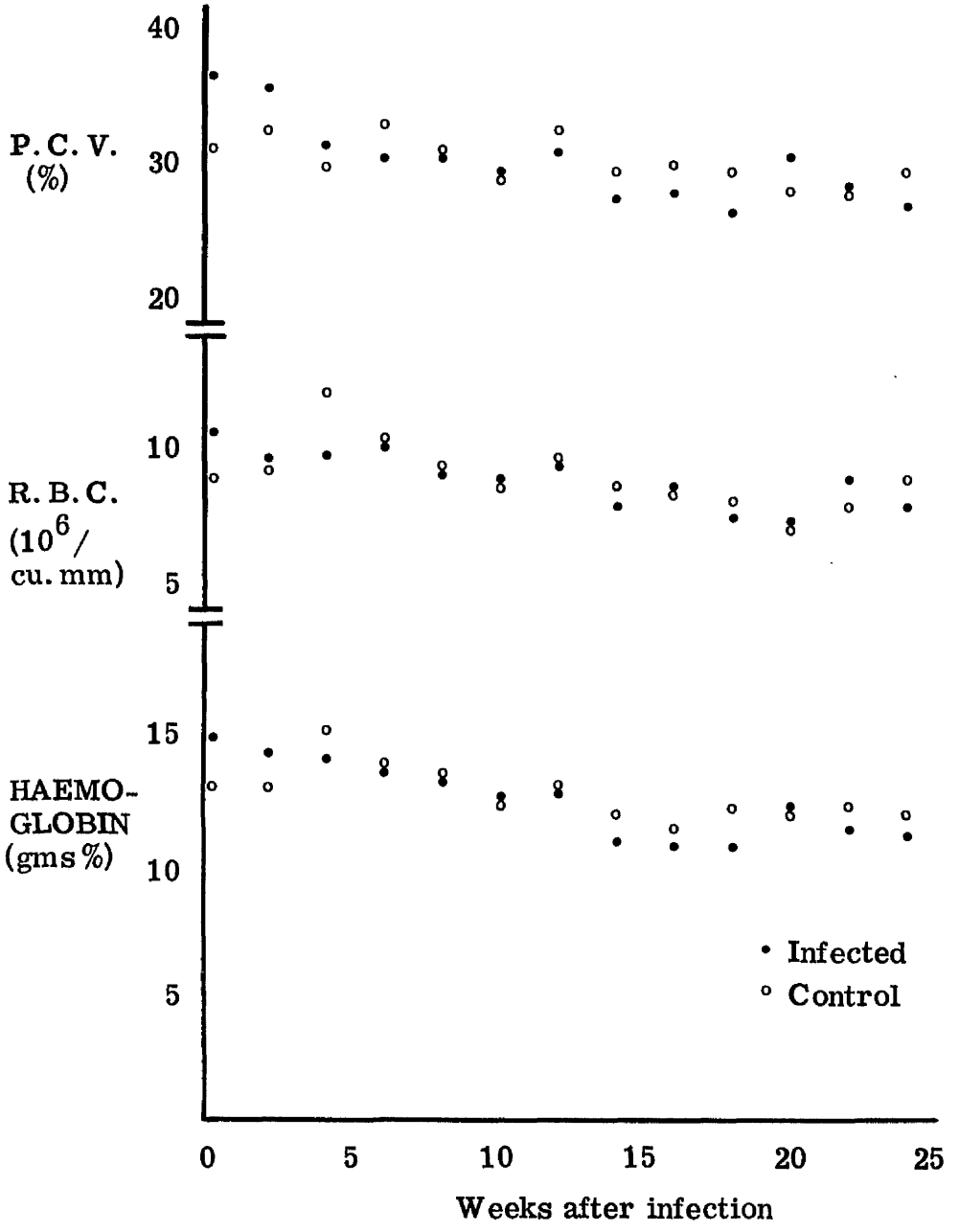


FIG. 35

of the infected and control groups during the period of observation. Marked changes did occur however in the total and differential white blood cell counts of the infected foals when compared to the uninfected controls (Fig. 36).

Initially there was a sharp rise in total white blood cell counts of the infected animals, i.e. from a mean of 10,000/cu. mm. to a mean of 17,000/cu. mm., and these counts remained high throughout the prepatent period. Also marked in the infected group was an increase in the neutrophil/lymphocyte ratio and a rise in the number of circulating eosinophils.

Serum Proteins

The results of serum protein estimations in the 2 groups of foals are shown in Figure 37. Marked progressive increases in the total serum protein of the infected foals are evident and these reached a peak approximately 120 days after infection.

Albumin levels of the 2 groups remained the same throughout the period of observation and the increase in total serum protein was entirely due to increases in the serum globulins. On cellulose acetate electrophoresis the major increase was seen to occur in the β-globulin fraction. Table 1 illustrates the serum protein values of 2 infected and 2 control foals at 3 points during the course of this experiment.

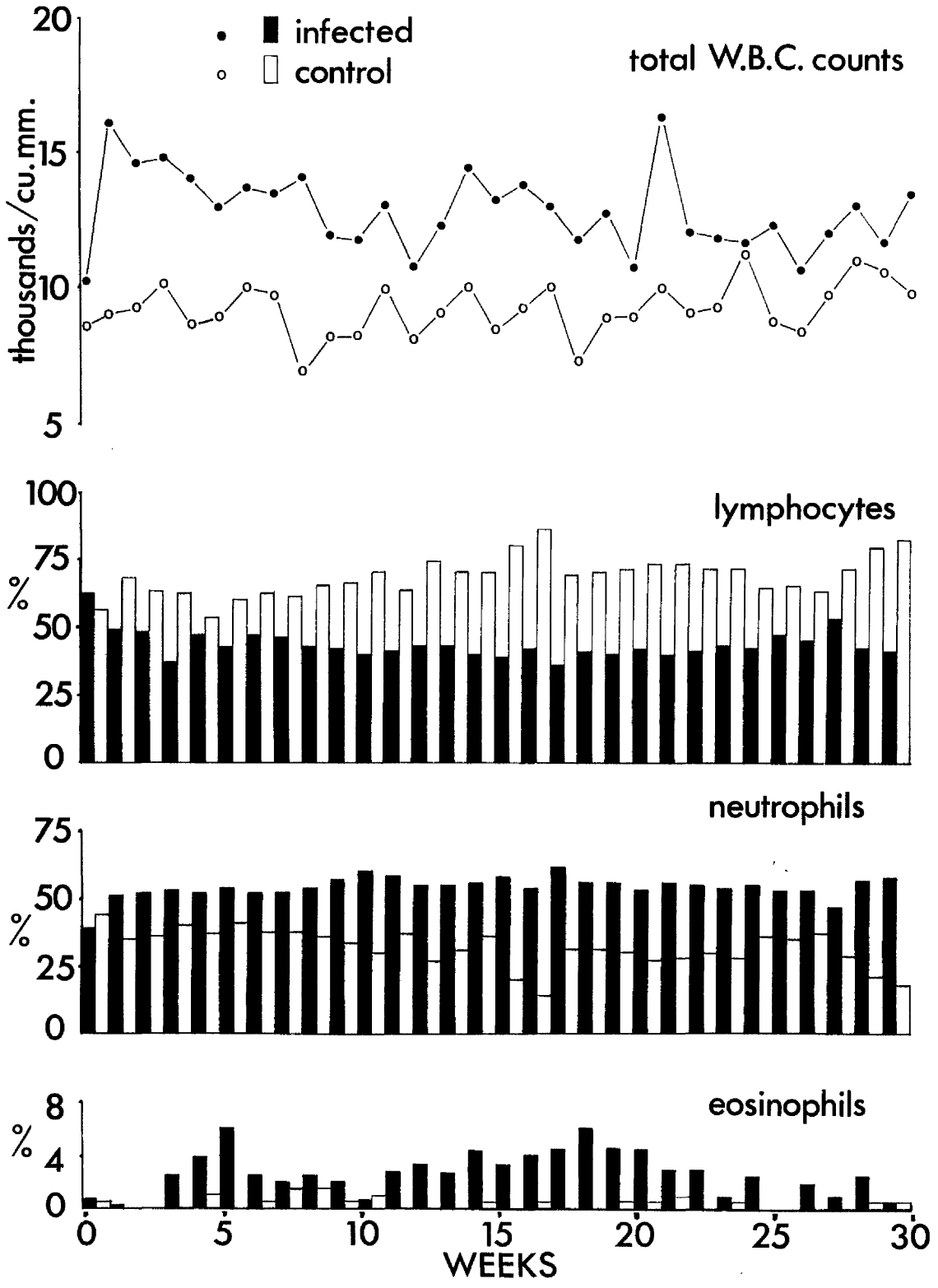


FIG. 36 Mean white blood cell changes following infection with 750 S. vulgaris larvae.

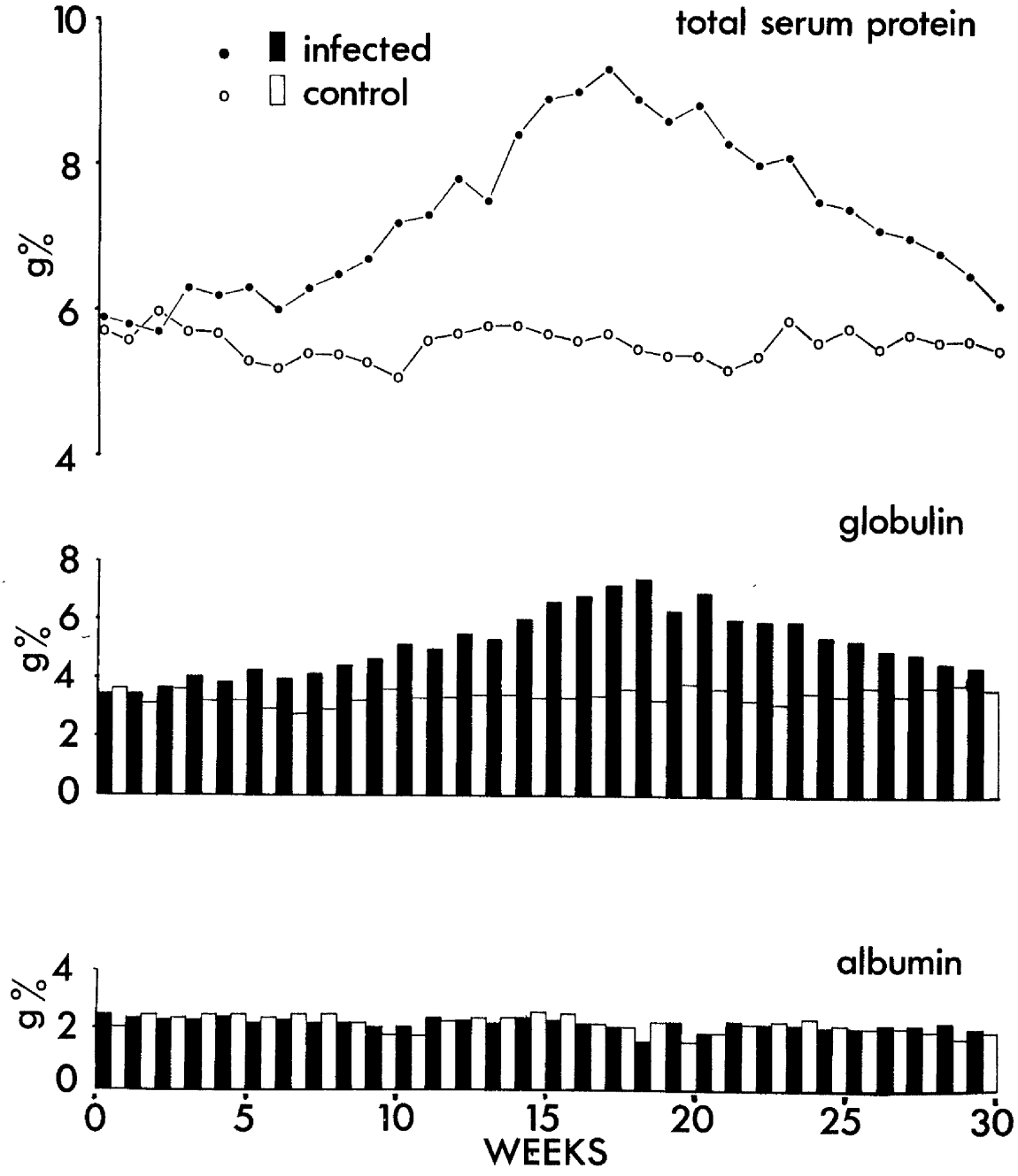


FIG. 37 Mean serum protein changes following infection with 750 S. vulgaris larvae.

TABLE 1

SERUM PROTEIN VALUES OF 2 FOALS INFECTED WITH 750 *S. VULGARIS* LARVAE AND 2 WORM-FREE CONTROLS

	INFECTED				CONTROLS			
	Alb.	∝	β	γ	Alb.	∝	β	γ
Day 0	2.5	1.1	0.9	1.6	2.1	1.1	1.6	0.4
	2.1	1.1	1.4	1.4	2.1	1.0	0.9	1.9
Day 120	1.8	2.3	3.7	1.2	2.1	0.9	1.5	1.0
	2.0	1.4	4.7	1.3	1.8	1.0	1.6	1.2
Day 210	2.1	1.1	2.1	0.9	2.0	1.1	1.2	0.8
	1.9	1.2	1.9	1.2	1.8	1.1	1.3	0.9

EXPERIMENT 2

Values obtained for each parameter of albumin and red cell metabolism investigated are presented in Tables 2 and 3 respectively. In essence, 2 features distinguished infected and worm-free horses. First, although serum concentrations and body pools of albumin were similar in all animals, the rate of albumin catabolism was higher in the infected horses (Table 2). This is shown by the shortened "apparent half-life", and also by the elevated values for the fractional and absolute catabolic rates, particularly in the naturally-infected group. That this hypercatabolism was due to increased movement of albumin into the alimentary tract is indicated by the higher faecal "clearance" recorded for each of the parasitised animals (Table 2).

TABLE 2

ALBUMIN TURNOVER STUDIES IN INFECTED AND WORM-FREE HORSES

^{125}I-Albumin Results

Group	Animal No.	Serum Albumin	Plasma Volume (ml./kg.)	Albumin Distribution			Albumin Catabolism			Faecal "Clearance" of plasma (ml./day)
				CA (g./kg.)	EA (g./kg.)	TA	Apparent half-life (days)	K	Absolute Amount Catabolised	
1	1	2.0	42.4	0.85	1.22	2.07	20.9	0.08	0.07	10.3
	2	1.8	51.5	0.93	1.45	2.38	20.9	0.08	0.08	11.4
	Mean	1.9	47.0	0.89	1.34	2.23	20.9	0.08	0.075	10.9
2	1	2.1	47.2	0.99	1.34	2.33	17.9	0.09	0.09	25.1
	2	1.9	49.0	0.93	1.19	2.12	15.7	0.10	0.09	17.7
	Mean	2.0	48.1	0.96	1.27	2.23	16.8	0.095	0.09	21.4
3	1	2.1	38.2	0.80	0.94	1.74	12.5	0.12	0.09	15.7
	2	1.8	38.6	0.69	1.79	2.48	11.4	0.20	0.14	20.0
	Mean	1.95	38.4	0.75	1.37	2.12	12.0	0.16	0.115	17.9

Second, in strongyle infected horses, red cell survival is reduced as a result of gastrointestinal haemorrhage. This is shown by the increased rate of removal of ^{51}Cr-labelled red cells from the circulation of those animals as compared with controls (Table 3) and by the high faecal excretion of isotope expressed as a faecal "clearance" of whole blood and red cells. Despite these intestinal red cell losses of up to 30 ml. daily, anaemia was not clinically detectable.

DISCUSSION

The results of this experimental work have provided additional information on the pathogenesis and pathology of S. vulgaris infection. The most dramatic pathogenic effects of infection are clearly related to the migratory activities of developing parasitic larval stages in the mesenteric arteries of the horse. It is also evident, however, that the feeding habits of adult parasites may be responsible for a degree of blood and protein loss into the intestine.

Despite the relatively small numbers of larvae administered to the foals used in the pathogenesis experiment a distinct clinical syndrome was produced during the early pre-patent phase which was similar, but less severe, than that reported by Enigk (1950) and Drudge et al. (1966). Enigk produced a fatal syndrome in 5 foals aged approximately 2 - 6 weeks and the severity of signs observed in these foals appeared to be related to the dose of larvae administered. Thus one animal given 800 infective larvae survived

TABLE 3

^{51}Cr-LABELLED RED BLOOD CELL TURNOVER STUDIES IN INFECTED AND WORM-FREE HORSES

Group	Animal No.	P.C.V.	Circulating RBC Volume (ml./kg.)	Blood Volume (ml./kg.)	^{51}Cr RBC $T_{\frac{1}{2}}$ (hrs.)	Faecal Clearance (ml./day)	
						Whole Blood	RBC
1	1	28	13.9	56.3	314	14.9	4.1
	2	25	16.3	67.8	267	18.6	4.9
	Mean	26.5	15.1	62.1	291	16.8	4.5
2	1	27	16.9	64.1	226	34.8	10.0
	2	31	18.0	67.0	232	29.5	9.1
	Mean	29.0	17.5	65.6	229	32.3	9.6
3	1	28	17.0	55.2	215	37.9	10.7
	2	32	13.1	51.7	211	81.6	29.6
	Mean	30.0	15.1	53.5	213	59.8	20.2

for 19 days whereas a foal given 8,000 larvae succumbed 8 days after infection. Drudge et al. (1966) used foals aged 2 - 9 months and found that doses of 2,500 - 5,000 larvae were lethal within 14 - 22 days in 9 of the 11 foals infected. Basically the clinical syndrome is one of pyrexia, anorexia, colic and death and it appears that the severity of signs in any particular animal is related to the pathological changes induced by migrating larvae in the intestine and intestinal arteries. It is interesting to note that in Switzerland Gerber, Chuit and Pauli (1971) observed a similar clinical syndrome in horses of various ages suffering from infarction of the small intestine. The syndrome was generally one of intermittent colic, pyrexia and death within a few days and in a subsequent article Pauli, Gerber and Chuit (1971) suggest that this was due to the pathological changes associated with the migratory activities of S. vulgaris larvae.

From the histopathology observed during the early pre-patent period information is now available on the association of intestinal and arterial lesions with clinical signs and clinical pathology. The earliest lesion seen is an inflammatory reaction in the intestinal wall due to the penetration of third stage larvae and their subsequent migration in the sub-mucosa of the intestine. Following this the major lesions are confined to the intestinal arteries. Initially there is thrombosis and arteritis of sub-mucosal intestinal arteries and this together with the penetration lesions coincides with, and is probably responsible for, the primary

temperature reaction detected 5 - 7 days after infection. As the larvae migrate up the arterial tree with the production of mural thrombi in the larger intestinal arteries the temperature reaction generally subsides. Severe pyrexia and colic may then develop 2 - 3 weeks after infection when large numbers of larvae have reached the anterior mesenteric site. The development of these signs apparently depends on the situation and magnitude of the arterial lesions and whether or not there is an associated thrombo-embolism resulting in ischaemic necrosis and infarction of a part of the intestine. In primary infections, with 750 larvae, foals which survive this initial 3 week period generally show little obvious clinical abnormality during the remainder of the pre-patent period.

Microscopical examination of arterial preparations from foals killed at various stages during the pre-patent period has shown the progressive development of the arterial lesion. Within 1 week of infection larvae are seen free within the lumina of thrombosed and damaged sub-mucosal arteries. Two weeks after infection mural thrombi containing larvae are visible in the intestinal branches of the anterior mesenteric artery and by 3 weeks there is gross thrombosis in the anterior mesenteric site. The primary arterial lesion appears, therefore, to be thrombus formation with subsequent endothelial proliferation as larvae migrate from the intestine up the arterial tree. With the progressive concentration of larvae in the anterior mesenteric artery there is gross thrombosis and arterial thickening. The vasa vasorum of the affected artery are also thrombosed and together these changes cause secondary damage

to the arterial wall by interfering with its nutrition. The common lesion in the wall of the artery is one of fibrosis and thickening but in some cases extensive and long-standing damage to the media of the vessel may result in the development of a true aneurysm.

The most significant haematological findings after experimental S. vulgaris infection were a marked polymorphonuclear leucocytosis and an increase in the number of circulating eosinophils. These results are similar to those of Drudge et al. (1966) but whereas these authors observed a low-grade normocytic, normochromic anaemia during the early phase of infection with 2,500 - 5,000 larvae, this was not evident during the present experiment using 750 larvae. The marked increases in serum β globulins observed during this study confirmed the findings of Drudge et al. (1966) of the serum protein changes which occurred in their chronic infections, with the exception that the albumin values during the present study remained within the normal range. Round (1970) also reported marked increases in β-globulins during the course of primary and reinfection experiments with both S. vulgaris and Trichonema spp. These increases in β-globulins may be indicative of an antibody response but as yet no protective effect has been demonstrated.

Although the red cell indices and albumin values of all 6 animals used in Experiment 2 were within the normal range, it is evident that both groups of parasitised animals were losing both red blood cells and albumin into the intestine. This experimental work has shown the effect of relatively small numbers of adult

parasites in the large intestine and further experiments are planned to examine the situation in naturally infected, heavily parasitised horses.

SUMMARY

In this chapter there is a description of the clinical signs, pathology and clinical pathology associated with single experimental infections of 750 S. vulgaris larvae in 9 worm-free pony foals. The results of a study in which 6 ponies were injected with preparations of ^{51}Cr-labelled red blood cells and ^{125}I-labelled albumin to assess the pathogenic effect of adult strongyles in the large intestine are also presented.

The major clinical signs which became apparent in the infected foals during the first 3 weeks were pyrexia, anorexia, dullness and abdominal pain. Gross and histological examination of tissues taken from foals killed over a period of 9 months demonstrated the pathological changes associated with migrating S. vulgaris larvae. Within the first 2 weeks of infection lesions are confined to the intestine and terminal branches of the intestinal arteries and consist of mucosal, sub-mucosal and serosal haemorrhage together with arteritis of sub-mucosal and serosal arteries; there is also a marked inflammatory reaction with an infiltration of neutrophils in the sub-mucosa of the small and large intestine and dilated lymphatics containing many neutrophils and fibrin strands are visible. The main lesion seen 3 weeks after infection is gross thrombosis of the anterior mesenteric artery or one of its major branches. On section these affected arteries show marked intimal thickening with infiltration of plasma cells, lymphocytes and macrophages. Between 1 - 4 months

after infection the gross lesions are predominantly in the arteries and consist of fibrous thickening of the arterial wall and thrombosis associated with the presence of developing fourth stage larvae. At 4 months after infection the arterial lesions are still prominent and microscopically there is fibrosis of the wall of the affected artery with widespread disruption of the intima. In the adventitia organised thrombi are apparent in the vasa vasorum and result in the obliteration of their lumina. The typical lesion associated with the return of fifth stage larvae to the intestine is nodule formation in close proximity to thrombosed terminal intestinal arteries and sections of parasites are seen in the intestinal wall surrounded by neutrophils and necrotic debris. By 9 months after infection the arterial lesion has healed but histologically there is fibrosis of the intima and macrophages containing haemosiderin are seen in the arterial wall.

The most significant haematological findings during the experimental period were a marked polymorphonuclear leucocytosis and an increase in the number of circulating eosinophils in the infected animals. Also marked was an increase in the serum globulin levels of the infected foals and this appeared on electrophoresis to be due mainly to increases in the β-globulin fraction.

Using ^{125}I-labelled preparations of albumin and ^{51}Cr-labelled preparations of red blood cells in animals with patent infections, it was shown that the rate of albumin catabolism was higher and the red cell survival time was reduced in infected horses.

CHAPTER 4

FIELD STUDIES ON THE EPIDEMIOLOGY OF MIXED STRONGYLE INFECTION IN THE HORSE

INTRODUCTION

Studies on the specific epidemiology of S. vulgaris infection in the horse are desirable to provide information essential for the formulation of control measures for this parasite. However, since horses normally carry a large number of different species of strongyles with a variety of developmental cycles, epidemiological studies on mixed strongyle infection are most useful in the development of overall control programmes in the field.

A number of surveys (Russell, 1948; Poynter, 1970) carried out in Great Britain have provided information on the incidence and development of various helminths in the horse population and their probable pre-patent periods. The pre-patent periods of some horse nematodes have also been determined by experimental infection (Wetzel, 1942; Round, 1969).

Seasonal fluctuations in the mixed strongyle faecal egg counts of horses were described by Poynter (1954) who found that the lowest numbers of eggs were passed in the winter. In the spring the egg counts rose and reached a maximum during July, August and September before falling during the autumn and early winter. In Poynter's opinion the initial rise was related to an increased rate of egg production by existing parasites which was subsequently increased and maintained by additional parasites reaching maturity in the intestine. Ogbourne (1971) collected and examined 4 selected species of strongyles from horses killed at the abattoir and suggested that the initial increases in faecal

egg counts described by Poynter were, in fact, largely related to seasonal differences in the numbers of larvae ingested and to the length of parasitic development of the different species. For example, Ogbourne suggested that parasites with a long pre-patent period, such as S. vulgaris, reached maturity during the winter and were responsible for the rise in faecal egg counts in the spring, whereas parasites with a shorter pre-patent period may reach maturity during one grazing season and thus contribute to the high faecal egg counts observed during the summer months.

In America, Baker, Salisbury and Britton (1939) demonstrated that horse strongyle eggs may remain dormant on pastures during the winter and subsequently hatch and develop into viable infective larvae in the spring. Later Shumakovitch (1940) showed that third stage strongyle larvae can overwinter under climatic conditions prevalent in the European U.S.S.R. Recently in Britain Ogbourne (1972) studied the pre-parasitic development of a number of horse strongyles and found that eggs developed into infective larvae in faeces deposited on herbage plots during the period March - October but not during the remainder of the year. Apart from the work of these authors little is known of the epidemiology of naturally occurring strongyle infections in horses comparable to the mass of information now available in, for example, parasitic gastro-enteritis in sheep. In this species it is now apparent that two sources of infection are available to lambs in the spring. These are larvae which have overwintered on the pasture and eggs deposited by infected ewes during the spring. Boag and Thomas (1971) investigated the

relative importance of these 2 sources by observing the pattern of infection in ewes and lambs on clean and autumn contaminated pasture. Their studies indicated that the "spring-rise" in the ewes was largely responsible for high pasture larval levels in July resulting in a wave of infection in lambs in August and September. In contrast nematode eggs in faeces deposited on the pasture during the previous October failed to develop to the infective stage.

Gibson and Everett (1967) studied the development and survival of eggs and larvae of Trichostrongylus colubriformis on grass plots over a period of 3 years. They found that eggs deposited on pasture during the winter failed to develop but as conditions during spring and summer became more favourable, development gradually became more rapid and an increased proportion of eggs developed into infective larvae. The survival times of these larvae were extended and they did not die out on most plots until the following April. In a later study Gibson and Everett (1971) followed the seasonal fluctuations of the larval populations of 4 species of Trichostrongylid nematodes on pasture herbage and suggested that these fluctuations were responsible for the seasonal succession of species observed in lambs in the field.

Obviously epidemiological studies such as those described above provide valuable knowledge on which various control measures may be based, and similar studies were initiated in 1971 in an attempt to elucidate certain aspects of the epidemiology of

mixed strongyle infections in horses: these were carried out over a 2-year period using various anthelmintic dosing regimes in mares and foals grazing both infected and clean paddocks. In addition faecal samples were examined from a number of mares around the time of parturition in order to investigate the possible occurrence and significance of a periparturient strongyle egg rise.

EXPERIMENTAL DESIGN 1971 - 72

Two paddocks were available in the spring of 1971. One of these had been grazed in the previous summer and autumn by naturally infected ponies (Paddock A) whereas the second had not been grazed by horses within the past decade (Paddock B).

Three pony mares which had been dosed with pyrantel tartrate prior to foaling were introduced into Paddock A in May 1971 and allowed to graze with their foals until the end of October 1971. During this period the mares were dosed fortnightly with pyrantel tartrate mixed in a concentrate ration. At the same time 3 naturally infected mares and their foals were introduced into Paddock B and these mares remained undosed throughout the period of the experiment. Faecal samples were taken fortnightly from all 6 mares and foals and the strongyle egg counts per gram of faeces determined by the McMaster and salt flotation techniques.

Faecal cultures were also set up in order to differentiate some of the species present and pasture samples from both paddocks were examined fortnightly for third stage strongyle larvae.

In Paddock A infection of the foals could only take place from eggs deposited on the pasture from the previous summer/autumn, i.e. 1970, whereas in Paddock B infection could only occur from eggs in the faeces of the mares which were put out to grass in the spring of 1971.

It was hoped that this experiment would provide information on the following aspects:

1. The relative importance of contaminated pasture and infected mares in the propagation of infection in foals.
2. The numbers of infective larvae on pasture to which the grazing foal might be exposed under different conditions.
3. The subsequent adult burdens of these foals, under both situations.

EXPERIMENTAL DESIGN 1972 - 73

The mares had been removed from Paddock B in October 1971 but their foals remained in the paddock until March 1972, at which time they were also removed and taken indoors prior to necropsy. Thus Paddock B had been grazed continuously from May 1971 to February 1972

by infected animals and pasture larval levels continued to be estimated until May 1972 when 3 dosed mares and their foals were introduced into this paddock. In this experiment faecal samples from the mares were examined weekly and, unlike the 1971-72 experiment, they were dosed with pyrantel tartrate only on the evidence of a positive egg count.

Another clean paddock was available in the spring of 1972 (Paddock C) and 3 undosed mares and their foals were introduced into this paddock in April 1972. In this experiment the infected mares were removed from the paddock at the beginning of September 1972, having grazed for a period of approximately 4½ months. Faecal samples were taken fortnightly from all foals and the 3 infected mares together with fortnightly pasture samples from both paddocks. Faecal cultures were again set up in order to differentiate the various strongyle species present.

It was hoped that results of these experiments would supplement our 1971-72 epidemiological observations by providing information on the following:

1. The rate of development and levels of infective larvae on similar pastures over a period of 2 successive grazing seasons.
2. The effect of dosing mares on the basis of a positive egg count on the control of high levels of pasture contamination and therefore infection of susceptible foals.
3. The total worm burdens which developed in the foals under these experimental conditions; these could then be compared with the total worm burdens of the foals used in the 1971-72 studies.

The plans of the epidemiological studies on mixed strongyle infection carried out between 1971-73 are summarised in Table 4.

TABLE 4

1971 - 72

PADDOCK A	PADDOCK B
"Contaminated"	"Clean"
3 Mares & Foals	3 Mares & Foals
Mares dosed fortnightly	Mares undosed

Fortnightly pasture samples and faecal samples from all animals.

1972 - 73

PADDOCK B	PADDOCK C
"Contaminated"	"Clean"
3 Mares & Foals	3 Mares & Foals
Mares dosed on evidence of positive egg counts	Mares undosed

Fortnightly pasture samples and faecal samples from all foals and 3 infected mares in Paddock C. Mares in Paddock B sampled weekly.

PERIPARTURIENT EGG RISE

Although the "spring rise" in the faecal egg counts of ewes has been shown to be a significant component of the epidemiology of helminth diseases of sheep, and Poynter (1954) demonstrated a rise in the faecal egg counts of horses in the spring/summer, the relationship between the latter increase and parturition in the mare has not previously been studied.

In an attempt to find out if the physiological events accompanying parturition and lactation in mares have any effect on their faecal strongyle egg counts, 9 thoroughbred mares, due to foal between January and March 1972, had faecal samples examined at fortnightly intervals around the time of parturition.

RESULTS

WORM BURDENS AND FAECAL EGG COUNTS

The mean faecal egg counts of the mares and foals together with the pasture larval counts on Paddocks A and B during 1971-72 are presented in Figure 38. For reference purposes the results of individual faecal egg counts are detailed in Appendix 1.

In Paddock A the faecal egg counts and faecal cultures of the mares remained negative due to fortnightly treatments with anthelmintic and the foals were therefore exposed only to over-wintered infection. Evidence of patent infection in the foals

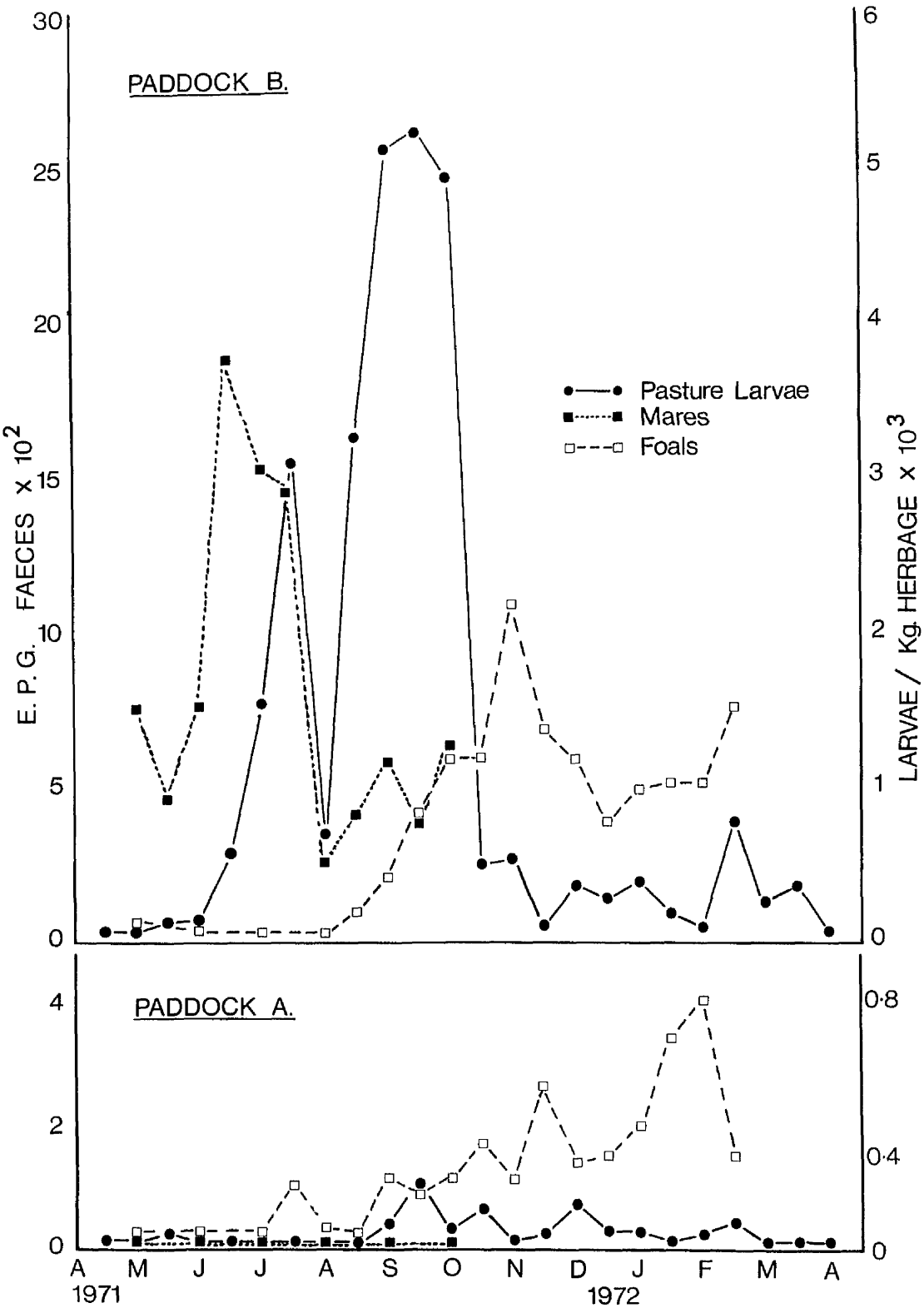


FIG. 38 Mean faecal egg counts of mares and foals and pasture larval counts in Paddocks A and B during 1971-72.

occurred approximately 10 weeks after their introduction into the paddock, i.e. mid-August, and thereafter there was a gradual increase in the mean faecal egg count until a peak of 400 e.p.g. was recorded near the end of the experimental period.

In Paddock B the egg counts of the mares rose during the summer months. This was followed closely by a marked rise in the levels of third stage strongyle larvae on the pasture and subsequently the foals showed evidence of a patent infection in September with a fairly rapid increase in the mean faecal egg count to over 1,000 e.p.g. by the end of November 1971.

One foal from each paddock was necropsied at 10 months, 11 months and 12 months of age and their total large intestinal strongyle burdens are presented in Table 5.

TABLE 5

MIXED STRONGYLE WORM BURDENS OF FOALS

	PADDOCK A			PADDOCK B		
	Caecum	Colon	Total	Caecum	Colon	Total
FOAL 1	20	2159	2179	1515	58488	60003
FOAL 2	28	3275	3303	7688	67709	75397
FOAL 3	83	5032	5115	3501	32830	36331
Total of three foals			10597			171731
Mean			3532			57277

Although the faecal egg counts of the 2 groups of foals suggested that more worms were present in the foals from Paddock B, this was much more marked than might have been anticipated. Over 16 times as many worms were recovered from the foals in Paddock B, i.e. a mean of 57,277 compared to a mean of 3,532 in the foals from Paddock A.

The results of the 1972-73 studies are presented in Figure 39 and show the mean faecal egg counts of the mares in Paddock C and the foals in both experimental paddocks together with pasture larval counts in Paddocks B and C. Individual faecal egg counts are detailed in Appendix 2.

Again in the paddock where the mares remained undosed (Paddock C) high pasture larval levels were recorded and the strongyle faecal egg counts of the foals became positive in July rising to a mean of approximately 2,500 e.p.g. by November 1972.

In Paddock B the mares were dosed before going out to grass on 8th May 1972 and subsequently when a positive egg count was recorded, i.e. on 1st June, 16th June, 13th July, 16th August. They were then removed from their foals in the first week of September. The 4 doses of anthelmintic given to the mares in Paddock B during their grazing period markedly reduced the pasture larval levels but was apparently less effective than routine fortnightly dosing in reducing the foal faecal egg counts, the latter rising to a mean of over 500 e.p.g. by November 1972. All 6 foals were necropsied during March 1973 and their total large intestinal strongyle burdens are shown in Table 6.

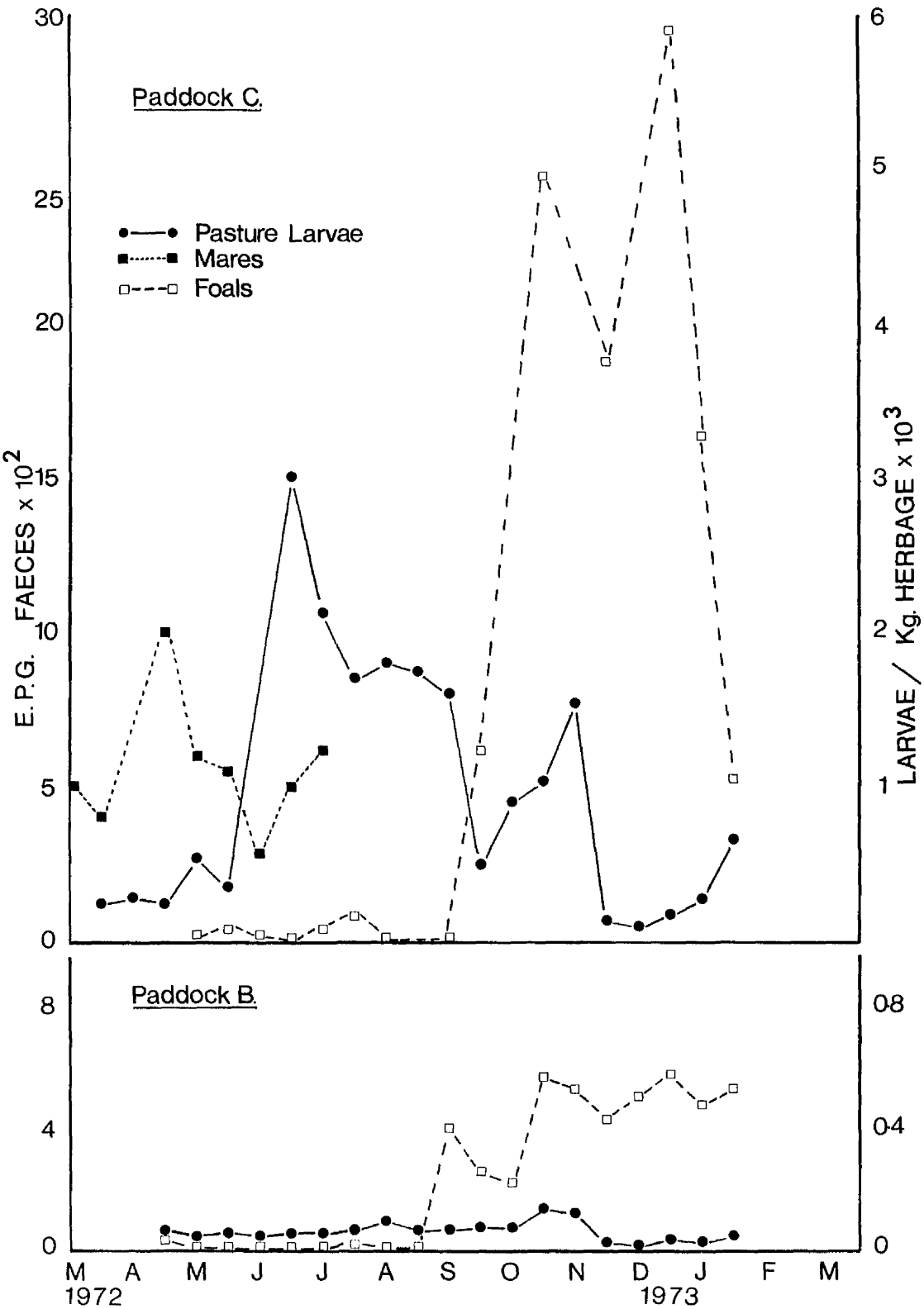


FIG. 39 Mean faecal egg counts of mares and foals and pasture larval counts in Paddocks B and C during 1972-73.

TABLE 6

MIXED STRONGYLE WORM BURDENS OF FOALS

	PADDOCK B			PADDOCK C		
	Caecum	Colon	Total	Caecum	Colon	Total
FOAL 1	591	8521	9112	1712	49022	50732
FOAL 2	299	6945	7244	347	21023	21370
FOAL 3	80	4012	4092	808	52783	53591
Total of three foals			20448			125695
Mean			6816			41898

Although the mean faecal egg counts of the foals in Paddock C had reached a level of 3,000 e.p.g. in January this had dropped to 1,000 e.p.g. at the beginning of March. A similar mean faecal egg count was recorded from the foals in Paddock B at this time but there were however marked differences in the total strongyle burdens recovered from the foals from both paddocks, i.e. a mean of 6,818 from the Paddock B foals compared to a mean of 41,898 from the Paddock C foals.

PASTURE LARVAL COUNTS

The results of pasture larval counts from all paddocks together with the relevant rainfall and temperature figures during the period of these experiments are presented in Figures 40 and 41.

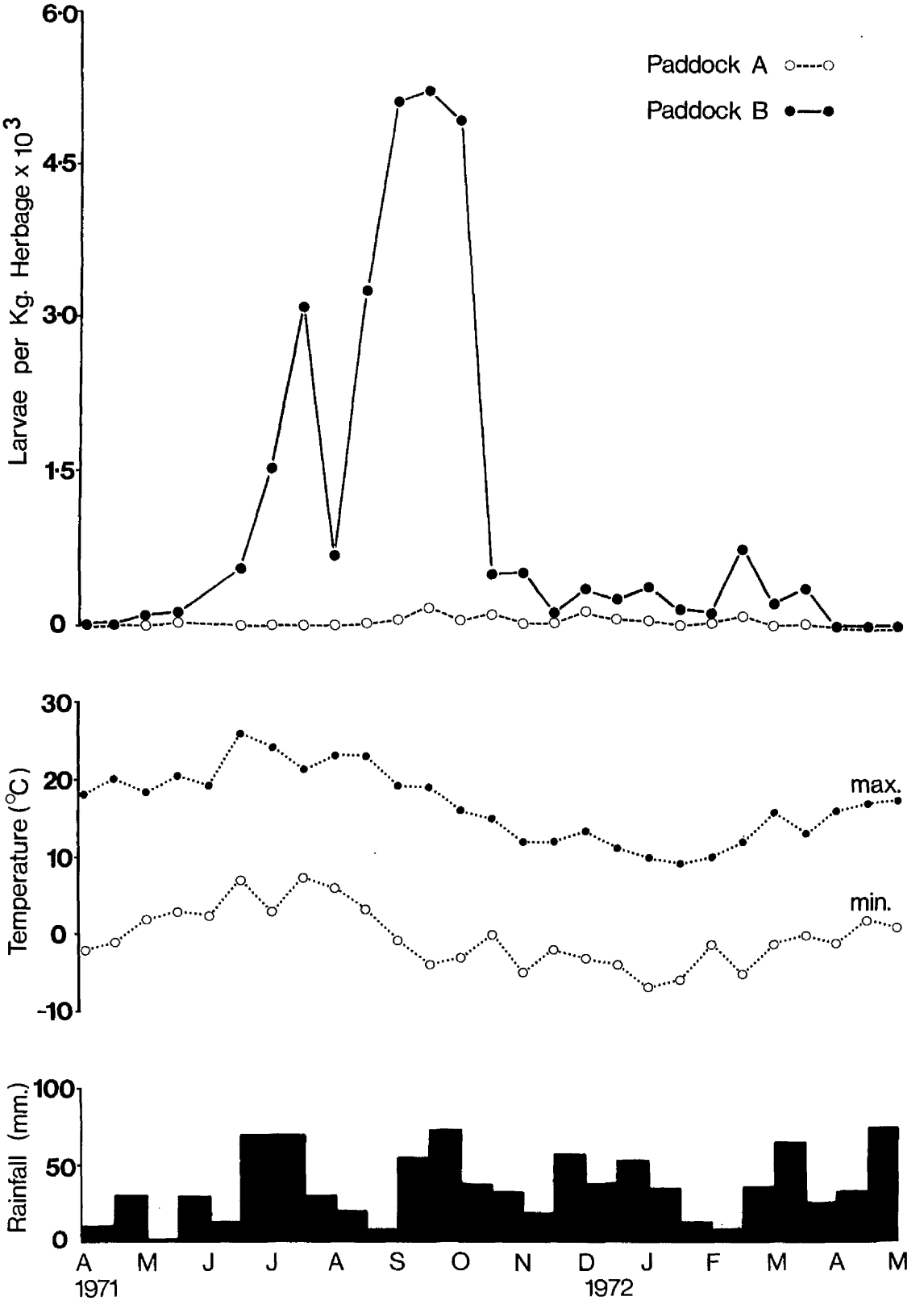


FIG. 40 Pasture larval counts from Paddocks A and B 1971-72 together with meteorological data during this period.

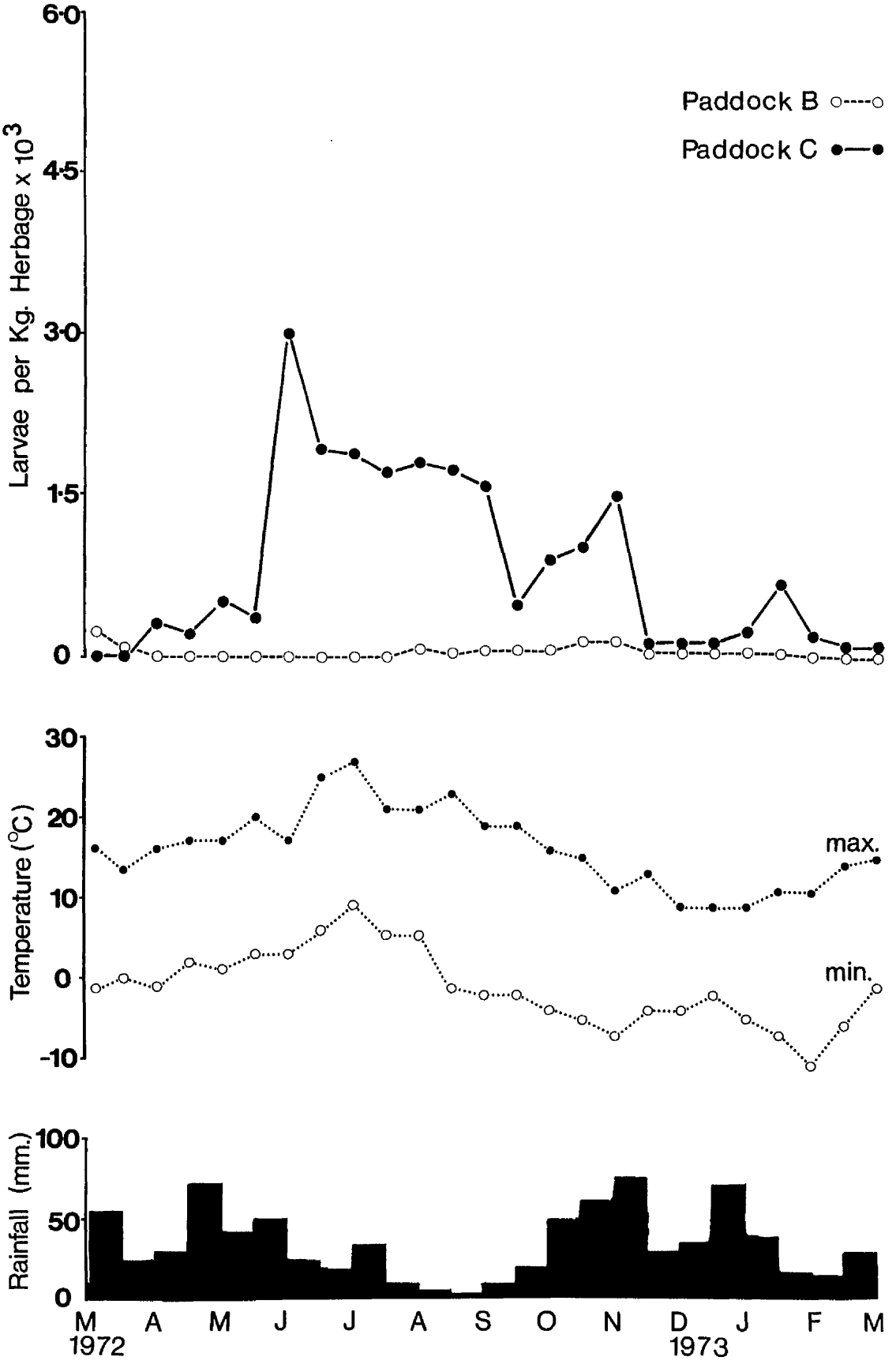


FIG. 41 Pasture larval counts from Paddocks B and C 1972-73 together with meteorological data during this period.

The situation on Paddock B in 1971-72 and Paddock C in 1972-73 were almost identical at the onset of each grazing season in that both were "clean" pastures grazed by the same number of infected pony mares and their foals. However in 1971 the mares went out to Paddock B in the middle of May whereas in 1972 the infected mares were put out to Paddock C in the middle of April. A level of 3,000 larvae/Kg. herbage was reached in Paddock B by the middle of August 1971 whereas a similar level occurred in Paddock C as early as the beginning of July 1972. Subsequently there was a marked increase in the pasture larval counts in Paddock B to approximately 5,000 larvae/Kg. in October 1971.

During the winter the levels of strongyle larvae recovered from both paddocks fell to a low level and by the early summer no larvae could be recovered from grass samples from either paddock.

An examination of the situation in the previously grazed or 'contaminated' paddocks, i.e. Paddock A in 1971 and Paddock B in 1972, shows that low levels of third stage strongyle larvae were recovered from both these pastures throughout the experimental period.

At the start of our observations in both 'contaminated' paddocks negative pasture larval counts were recorded although prior to this different degrees of pasture contamination had prevailed in each paddock.

Paddock A was grazed during the summer and autumn of 1970 by a varying number of infected ponies and pasture larval counts during this period confirmed the presence of a relatively small

number of infective larvae, e.g. 60 larvae/Kg. in December 1970.

As Paddock B had been used in our studies during 1971-72, details of previous grazing history and pasture larval levels were known at the start of the 1972 field studies. Very high levels of larvae had been recorded from Paddock B during the summer and autumn 1971 and infected foals continued to graze this paddock until February 1972. Thus strongyle eggs were deposited on this pasture throughout the winter 1971-72, unlike Paddock A which was vacated in November 1970.

In Paddock A in 1971 the mares received fortnightly anthelmintic treatment and pasture larval counts remained negative until mid-September 1971, i.e. 6 weeks after the establishment of patent infections in the foals from overwintered larvae (Fig. 38). Although the faecal egg counts of the foals rose gradually from September to February there was no corresponding increase in numbers of third stage larvae on the pasture. This was most likely due to weather conditions after October being unfavourable for the further development of strongyle eggs to the infective stage.

Since the mares grazing Paddock B in 1972 were treated with anthelmintic only on the evidence of a positive faecal egg count, some contamination of the pasture occurred from eggs in the faeces of the mares between treatments and accounts for the low pasture larval levels which occurred in July before patent infections were established in the foals by mid-August. As in Paddock A there was little further increase in pasture larval levels due to unfavourable climatic conditions from October 1972 onwards.

DIFFERENTIAL LARVAL COUNTS

Differential larval counts were carried out on both pasture samples and individual faecal cultures throughout the 2 years of the experiment. Because of the volume of these results they are presented in Appendix 3 but the most significant features in relation to S. vulgaris infection are shown in Table 7 and Figure 42.

From Table 7 it is apparent that during the 1971-72 studies there were distinct seasonal variations in the numbers of S. vulgaris larvae present in the mean differential larval counts of faecal cultures from the undosed mares and foals. Those of the mares contained a higher proportion of S. vulgaris larvae during the spring and summer whereas a relatively high percentage of S. vulgaris larvae occurred in the faecal cultures from all of the foals when they reached 9 - 11 months of age, i.e. during the winter months.

It is clear from Figure 42 that the highest proportion of S. vulgaris larvae were recorded in pasture samples taken between May and October from the paddocks where the mares remained untreated, i.e. Paddock B in 1971 and Paddock C in 1972.

PERIPARTURIENT EGG RISE

The results of individual faecal egg counts of 9 mares together with foaling dates are shown in Table 8. All of the

TABLE 7

MEAN DIFFERENTIAL LARVAL COUNTS - FAECAL CULTURES 1971 - 72

Date	PADDOCK A				PADDOCK B					
	Mares			Foals	Mares			Foals		
27/5			-ve		84	9	7	100	0	0
10/6			-ve		87	7	6		-ve	
24/6			-ve		92	2	6		-ve	
8/7			-ve		92	3	5		-ve	
22/7			few		94	3	3		-ve	
5/8			few		86	9	5		-ve	
19/8	N	100	0	0	92	5	3		few	
2/9		100	0	0	96	1	3	99	0	1
16/9	E	100	0	0	94	2	4	97	1	2
30/9	G	99	0	1	95	3	2	97	1	2
14/10		99	0	1	97	1	2	97	1	2
28/10	A	98	0	2	96	3	1	99	0	1
11/11	T	98	0	2	99	0	1	99	0	1
25/11		99	0	1	95	3	2	96	1	3
9/12	I	99	0	1	94	3	3	98	1	1
23/12	V	96	1	3	97	2	1	94	0	6
6/1		97	1	3	97	2	2	97	0	3
20/1	E	94	5	1	96	2	2	94	4	2
3/2		90	9	1	97	2	1	89	9	2
17/2		90	9	1	100	0	0	88	11	1
2/3		88	5	7	98	1	1	84	15	1
16/3		85	15	0	97	3	0	80	19	1

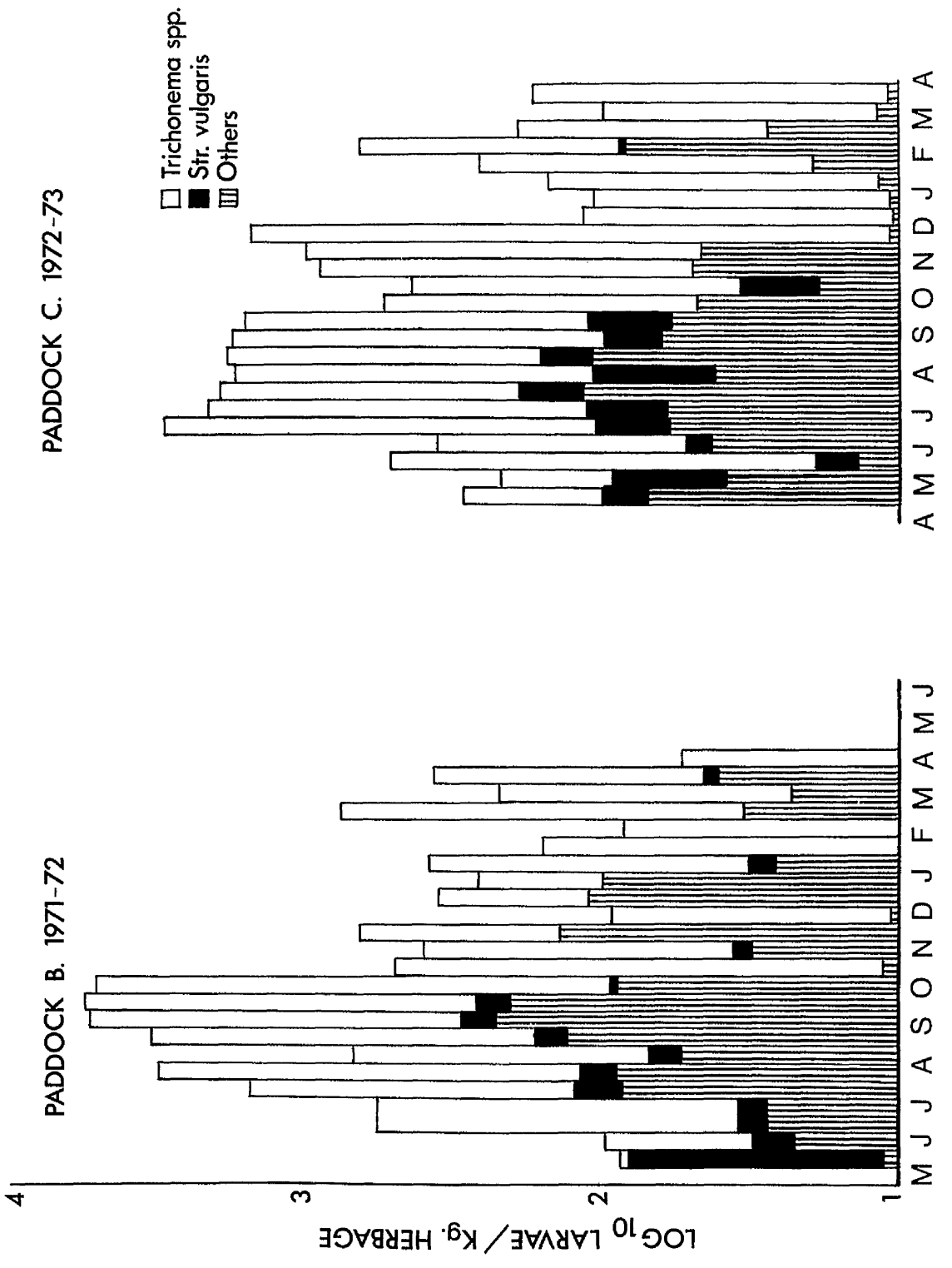


FIG. 42 Differential larval counts from paddocks where mares remained untreated during the grazing season.

mares foaled between January and March and parturition seemed to have little immediate effect on the numbers of strongyle eggs passed in their faeces. Three of the mares foaled in January and no subsequent increases in the strongyle egg counts were detected in faecal samples taken fortnightly until the middle of March. The remaining 6 mares foaled in February and March and although all of these mares showed increases in their faecal egg counts in May these increases appeared to be unrelated to individual dates of parturition.

DISCUSSION

From these epidemiological studies 2 important aspects clearly emerge. These will be discussed with particular reference to the 1971-72 season although the results were similar the following year. First, from a study of faecal egg counts it is clear that foals may be infected by either overwintered larvae on pasture or from eggs passed in the faeces of their dams but the latter is undoubtedly the most important source of infection. Thus patent infection occurred early in the season, i.e. July, in the Paddock A foals due to the ingestion of overwintered larvae in the spring but the level of infection in these foals remained low, i.e. between 100 - 300 e.p.g. throughout the period of the experiment. In contrast patent infections were established in the Paddock B foals 6 weeks later, i.e. September, but subsequently there was a rapid increase in the

TABLE 8

MIXED STRONGYLE FAECAL EGG COUNTS AND FOALING DATES OF NINE BROOD MARES

DATE	1	2	3	4	5	6	7	8	9
4/1/72	400	50	50						
	7/1/72	15/1/72	16/1/72						
18/1/72	650	100	300	150					
1/2/72	600	450	500	50	300				
					14/2/72				
15/2/72	300	200	250	-ve	50	-ve			
29/2/72	800	600	300		-ve	-ve	-ve	-ve	-ve
							7/3/72	7/3/72	
14/3/72	400	300	50	200	-ve	100	150	-ve	50
				29/3/72		15/3/72			15/3/72
11/4/72				450	-ve	50	100	-ve	-ve
25/4/72				700	-ve	50	-ve	-ve	N.S.
9/5/72				1300	450	3650	400	450	-ve
23/5/72				2000	N.S.	2400	1550	550	600

faecal egg counts of these foals and a mean of over 1,000 e.p.g. was recorded in November. The delay in establishment of patent infections in the foals from Paddock B is almost certainly due to the fact that the paddock was "clean" when the infected mares were put out in the spring and several weeks therefore elapsed before infective larvae were available on the pasture to infect the foals.

Secondly, from a comparison of the pasture larval counts in the 2 paddocks it is obvious that a high level of pasture contamination occurs when mares carrying a mixed strongyle infection remain untreated throughout the grazing season. In contrast routine fortnightly anthelmintic treatment of mares results in extremely low larval recovery, e.g. a level of over 5,000 third stage larvae per kilogram (L_3/Kg.) herbage occurred in Paddock B in October 1971 at the same time as the highest pasture larval count of 183 L_3/Kg. was recorded from Paddock A.

A similar pattern of pasture contamination and infection was observed during the 1972-73 studies but several points arose which merit further discussion.

For example, the mean faecal egg count of the foals which grazed Paddock C with their undosed dams was considerably higher than that of their counterparts in Paddock B in 1971, e.g. 2,500 e.p.g. in November 1972 compared to 1,000 e.p.g. in November 1971. This was a surprising finding considering the fact that not only were the mean faecal egg counts of the mares in Paddock B higher than those in Paddock C but the mares in Paddock B also remained

at grass for a total of 6 weeks longer than the Paddock C mares. As expected, this resulted in an extremely high level of pasture contamination in Paddock B although this was not subsequently reflected in the faecal egg counts of the Paddock B foals.

An explanation for this apparent anomaly can be found from an examination of the rate of development of significant levels of infective larvae on both paddocks in relation to the weather conditions which prevailed when the infected mares were put out to grass (Figs. 40 & 41). Since the mares went out to Paddock C in April 1972 faecal contamination occurred 1 month earlier than in 1971. Also the total rainfall during May and June 1972 was much higher than the corresponding period in 1971, i.e. 195.2 mm. and 70.5 mm. respectively. Thus in 1972 moist conditions favourable for pre-parasitic larval development occurred early in the grazing season. Similar conditions did not occur in Paddock B until July and August, the total rainfall for this period being 184 mm. compared to 81.5 mm. in 1972. Clearly these weather conditions were responsible for the establishment of a level of approximately 3,000 L_3/Kg. herbage on Paddock C by late June 1972 whereas a similar level did not occur in Paddock B till mid-August 1971.

The subsequent marked increase in pasture larval counts to approximately 5,000 L_3/Kg. in Paddock B by September/October 1971 was probably influenced by the following factors. First, as

mentioned above, the infected mares in Paddock B had higher faecal egg counts than those in Paddock C and remained at grass until the end of October 1971 whereas the mares were removed from Paddock C in the first week of September 1972. This effectively reduced the numbers of strongyle eggs deposited on Paddock C later in the grazing season. Secondly, the relatively high rainfall recorded during July, August and September 1971 provided favourable conditions for the development of large numbers of strongyle eggs to third stage larvae during this period. In contrast, fairly dry conditions existed during the same period in 1972.

Although it was evident from a comparison of the systems of management during the 2 years' observations that anthelmintic treatment of mares grazing previously contaminated pasture resulted in a relative reduction in the mean faecal egg counts and total worm burdens of their foals, this was less marked in the foals from Paddock B in 1972, e.g. 500 e.p.g. in December 1972 compared to 250 e.p.g. in December 1971 and approximately 7,000 worms in March 1973 compared to 3,500 in March 1972. This is largely explained by the fact that routine fortnightly anthelmintic treatment of mares in 1971 allowed infection of foals only from overwintered larvae whereas there were 2 sources of infection available for the foals in Paddock B in 1972, i.e. overwintered larvae and infective larvae which developed from eggs passed in the faeces of the mares between treatments.

One observation which indicated the unreliability of faecal egg counts as an estimation of adult worm populations was obtained from a comparison of the faecal egg counts of the foals in Paddocks B and C with their subsequent adult worm burdens at necropsy. In March 1973 these egg counts were of a similar level, i.e. approximately 1,000 e.p.g. In the case of the Paddock C foals this was a reduction from a previous high level of approximately 3,000 e.p.g. whereas in the foals from Paddock B this was the highest level reached. Although this tended to suggest that there would be a similarity in the total strongyle burdens of the 2 groups of foals this was not subsequently found at necropsy when the foals from Paddock B showed a mean adult strongyle burden of approximately 7,000 worms in contrast to the Paddock C foals where the mean was approximately 42,000.

The results of differential larval counts demonstrated the following seasonal pattern of infection with S. vulgaris in mares and foals at pasture. Mares produce high numbers of S. vulgaris eggs during the spring and summer which, given favourable climatic conditions, develop into third stage infective larvae on the pasture. Foals ingest these larvae during the summer and after approximately 6 months, the pre-patent period of S. vulgaris, a young adult population of S. vulgaris is established in the large intestine of these foals. These young adult worms are then responsible for the increase in numbers of the S. vulgaris larvae in the faecal cultures of the foals during January, February and March.

The situation regarding the small strongyle species only differs in that the rise in strongyle faecal egg counts of the mares in spring and summer is followed by an increased pasture larval level and subsequently an increase in the faecal egg counts of the foals in autumn and winter. However, due to the large number of different strongyle species present and variation in the length of their individual pre-patent periods, it is difficult to establish a pattern of infection for any one particular species.

It has already been shown by Poynter (1954) and Russell (1948) that most adult horses show a rise in their strongyle faecal egg counts during late spring and summer and during our studies we observed a similar rise in the faecal egg counts of nursing mares in late spring. This resulted in a substantial increase in the numbers of strongyle larvae on pasture during the summer months and subsequently the establishment of large numbers of adult strongyles in the large intestine of foals during the winter. Routine anthelmintic treatment of mares markedly reduced the total worm burdens of their foals. These results demonstrate that for any control measures to be effective it is of the utmost importance to minimise the level of infection in the mare during the spring and summer as it is during this period that climatic conditions on pasture favour the rapid development of large numbers of strongyle eggs to the infective stage. From October onwards there appears to be little further

pre-parasitic larval development and pasture larval levels gradually declined during the winter and early spring.

From our studies it is evident that overwintered larvae may persist on previously grazed paddocks until April the following year, but these larvae have died out by late May/early June. In the control of strongyle infection in the young animal it is therefore important to keep clean paddocks for nursing mares and foals or, where this is impractical, these animals should not go out to grass until June, by which time pasture larval levels will be negligible.

SUMMARY

In this section the significance of helminthiasis was studied in 2 separate systems of management over 2 years.

Basically 2 regimens were employed: one where untreated mares and foals went out in the spring to pastures never previously grazed by horses while in the other, mares, regularly treated with anthelmintics, and their foals went out to pasture which had been grazed the previous year by naturally infected ponies. These systems allowed 2 sources of infection for susceptible foals: where mares were treated, the main source of infection was from overwintered larvae, whereas in the situation where infected mares went out to clean pasture infection of the foals could only occur from larvae which developed from eggs passed in the faeces of the mare.

The results of these 2-year observations demonstrated the following points in the epidemiology of mixed strongyle infections.

First, foals going out to grass in the spring can be infected by both overwintered larvae and by eggs passed in the faeces of their dams but the latter is by far the more important source of infection.

Secondly, where horses remain untreated throughout the grazing season high levels of third stage strongyle larvae develop on pasture, whereas regular anthelmintic treatment at intervals of 2 - 4 weeks reduces pasture contamination to a minimum.

Thirdly, although high levels of infective larvae may occur on pasture grazed by infected animals during the summer and autumn, these levels fall during the winter and by May the following year virtually no third stage larvae can be recovered from these pastures.

Additional information was obtained on the pattern of infection with a single species, S. vulgaris, in mares and foals at pasture. Mares show a relative increase in the numbers of S. vulgaris eggs in their faeces in the spring and summer resulting subsequently in an increase in the numbers of S. vulgaris third stage larvae on pasture. Foals ingest these larvae during the summer, and after approximately 6 months, the pre-patent period of S. vulgaris, a young adult worm population is established in the large intestine of these foals.

A separate survey carried out on 9 mares indicated that there is a general increase in the strongyle faecal egg count of brood mares in late spring and early summer but this is not closely linked to the physiological events accompanying parturition in these mares.

In conclusion the results of these epidemiological studies provide a basis for the following proposals on the control of strongyle infection in the young susceptible animal during its first year at pasture.

1. Before going out to grass, pregnant or newly foaled mares should be dosed with a broad-spectrum anthelmintic.

2. Ideally mares and foals should go out to "clean" pasture, i.e. those not grazed the previous year by horses. Alternatively, since it has been shown that overwintered larvae have almost disappeared from pasture previously grazed by horses by the following May, it is advisable to delay the introduction of mares and foals to such pastures until that time.

3. Regular dosing of mares at monthly intervals during summer and autumn will markedly reduce the pasture levels of infective larvae to which grazing foals will be exposed and consequently the worm burdens of these foals at the end of the grazing season will be relatively insignificant.

4. It is evident from the high pasture larval counts observed during this 2-year study that any of the standard methods for reducing contamination of paddocks will also have a beneficial effect, e.g. removal of faeces from paddocks, avoiding overcrowded conditions, and mixed grazing.

CHAPTER 5

IMMUNITY TO *STRONGYLUS VULGARIS*

INTRODUCTION

Although little detailed information is available it has long been accepted that young horses, i.e. those under 2 - 3 years of age, are more susceptible to the effects of strongyle infection than older animals; for example Velichkin (1964) reported that the greatest losses due to strongyle infection occurred from 6 months to 3 years of age. Despite this a number of surveys based on strongyle faecal egg counts have shown a high prevalence of infection with adult strongyles in horses over 6 months of age. Thus, in a small number of adult ponies, Russell (1948) demonstrated a 100% incidence of infection with S. vulgaris adults. Subsequently Poynter (1970) examined faecal samples from over 3,000 horses of different ages and found an overall incidence of 70%, with the lowest incidence in foals (46.6%) and the highest in yearlings (89.6%). From differential larval counts he also observed that S. vulgaris accounted for a much higher percentage of the total worm egg count in animals under 3 years of age.

This high incidence of infection in horses of all ages is apparently paralleled by the ubiquity of arterial lesions due to migratory stages of S. vulgaris. At post mortem examination of 87 horses Ottoway and Bingham (1946) found gross arterial lesions due to S. vulgaris larvae in 95.4% of these animals. Poynter (1960) necropsied a further 43 animals and found 93% with arterial lesions attributable to S. vulgaris larvae. Both surveys confirmed that the anterior mesenteric artery is the most frequently affected site.

These qualifications on the incidence of strongyle infection and of arterial lesions due to S. vulgaris larvae do not of course negate the introductory statement that older horses are less susceptible to the effects of strongyle infection. The presence of strongyle eggs in the faeces in these circumstances is not a reliable index of the degree of susceptibility or pathogenicity and in the case of arterial lesions these may have been acquired early in life.

Enigk (1951) is the only worker to report an acquired immunity to experimental S. vulgaris infection in worm-free foals. He demonstrated that 2 foals developed a significant immunity during the course of repeated doses of S. vulgaris larvae. These foals survived the administration of 7,805 and 6,602 larvae in divided doses over a period of 42 and 105 days respectively, whereas a foal given a single dose of 800 larvae died due to acute intestinal infarction within 19 days.

A similar phenomenon was observed in the field during the epidemiological studies described in Chapter 4 in that foals continuously exposed to infection with S. vulgaris larvae on pasture during the summer months did not develop the acute syndrome seen in experimental infections. It is also the case that such a syndrome is rarely reported from the field by practising veterinary surgeons and one might therefore assume that some degree of immunity similar to that described by Enigk is relatively rapidly acquired.

Because of the paucity of information on the development of immunity to S. vulgaris infection, it was decided to carry out a number of experiments in an attempt to provide basic information on the immune reactions of ponies to experimental infection with S. vulgaris larvae. The design and results of these experiments are discussed under the following headings:

Age Immunity

Acquired Immunity

Vaccination.

Age Immunity

Experiment 1

The design and summarised results of a preliminary experiment on the role of age immunity is shown in Table 9.

In this experiment 2 ponies aged 2½ and 3 years which had been reared and maintained worm-free from birth were infected with a dose of 750 larvae and killed 1 month and 2 months after infection. The rectal temperatures of these ponies were taken daily and haematological estimations were carried out on blood samples collected twice weekly.

Neither animal used in this experiment showed any clinical signs throughout the experimental period.

TABLE 9

THE DESIGN AND RESULTS OF AN EXPERIMENT TO STUDY THE ROLE OF AGE IMMUNITY TO S. VULGARIS IN 2 WORM-FREE PONIES

TWO WORM-FREE PONIES	
$2\frac{1}{2}$ years	3 years
750 L_3	750 L_3
Killed Day 60	Killed Day 30
At P.M. examination 'scar' lesions in intestine due to larval penetration. Numerous fibrin tracts in aorta. Thrombosis of anterior mesenteric artery.	At P.M. examination penetration lesions in intestine. Fibrin tracts in arterial system. No thrombosis.
60 L_4 recovered	No L_4 recovered

The 3-year-old pony was killed 1 month post-infection and at necropsy nodules were present in the small intestine and caecum indicating the sites of larval penetration. On dissection of the arterial tree numerous fibrin tracts were visible in the aorta radiating from the origin of the anterior mesenteric artery but there was no thrombosis in the anterior mesenteric artery itself and no larvae could be recovered. The $2\frac{1}{2}$-year-old pony was killed 2 months after infection and once again 'scar' lesions were visible

in the intestine due to previous larval penetration. This animal, however, showed numerous fibrin tracts in the aorta and marked thrombosis with thickening of the anterior mesenteric artery and 60 fourth stage larvae 5 - 17 mm. in length were recovered.

The haematological results are presented in Appendix 4 and white blood cell changes similar to those described in previous experiments on the pathogenesis occurred in both ponies, i.e. increases in the total white blood cell counts with a rise in numbers of circulating polymorphonuclear leucocytes and eosinophils.

Experiment 2

As the results of the pilot experiment suggested that there was some degree of immunity in one of the ponies, i.e. the 3-year-old, another experiment was carried out, the plan and results of which are shown in Table 10.

Four 3-year-old ponies which again had been reared and maintained worm-free, and a 7-month-old worm-free control foal were used. The 4 older ponies were dosed with 1,000 S. vulgaris infective larvae whereas the 7-month-old foal received a dose of 750 larvae. Two ponies were killed 1 month after infection and the remaining 2 ponies and the control foal were killed 2 months after infection.

TABLE 10

THE DESIGN AND RESULTS OF AN EXPERIMENT ON THE ROLE OF AGE IMMUNITY TO S. VULGARIS IN 4 WORM-FREE 3-YEAR-OLD PONIES

4 worm-free 3-year-old ponies		Worm-free 7 month control
1000 L_3		750 L_3
2 ponies Killed Day 30	2 ponies Killed Day 60	Killed Day 60
At P.M. examination no gross lesions other than penetration 'scars' in intestine: on dissection of arteries variable fibrin tracts in aorta and intestinal arteries		At P.M. examination typical severe arterial lesion
No L_4 recovered		40 L_4 recovered

At post-mortem examination of the 4 ponies there were no visible gross lesions other than a few penetration 'scar' lesions scattered throughout the posterior small intestine, caecum and colon. Although on dissection of the arterial tree fibrin tracts due to the migration of early fourth stage larvae were visible on the endothelium of the aorta and intestinal arteries, no larvae could be recovered from the anterior mesenteric site. Figures 43 and 44 show the arterial preparations from these animals. The control foal, however, developed a typical severe arterial lesion (Fig. 45) in which fourth stage larvae were grossly visible. These

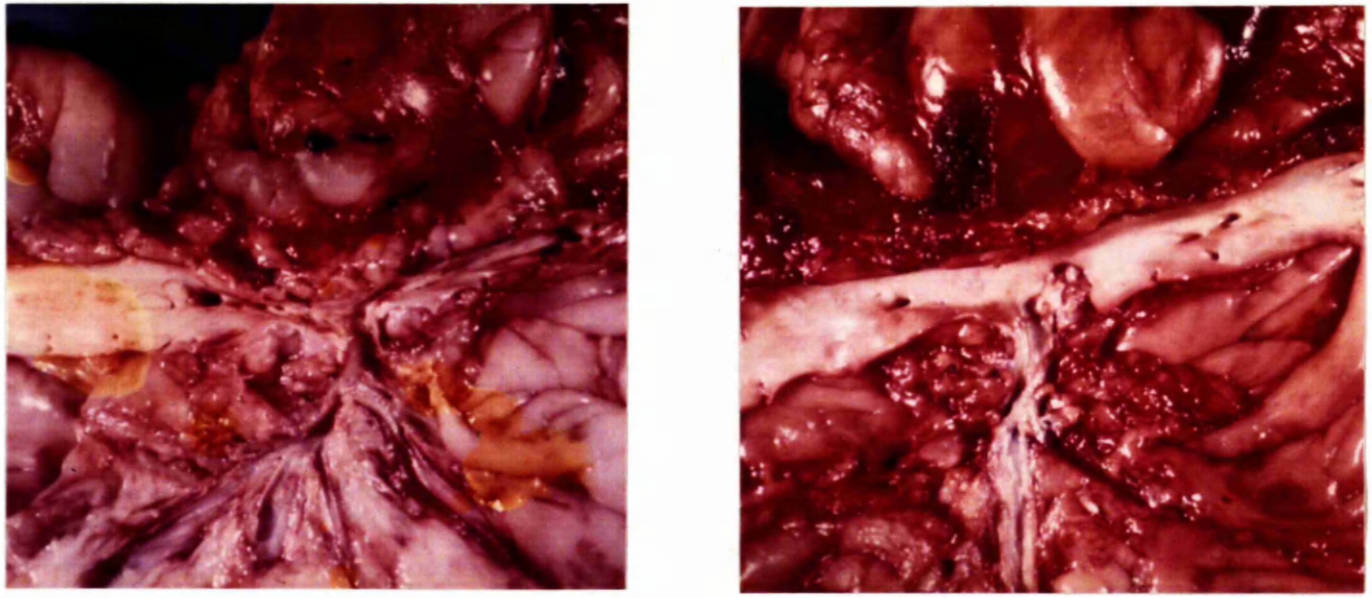


FIG. 43 Dissection of arterial preparations from two 3-year-olds at 1 month after infection

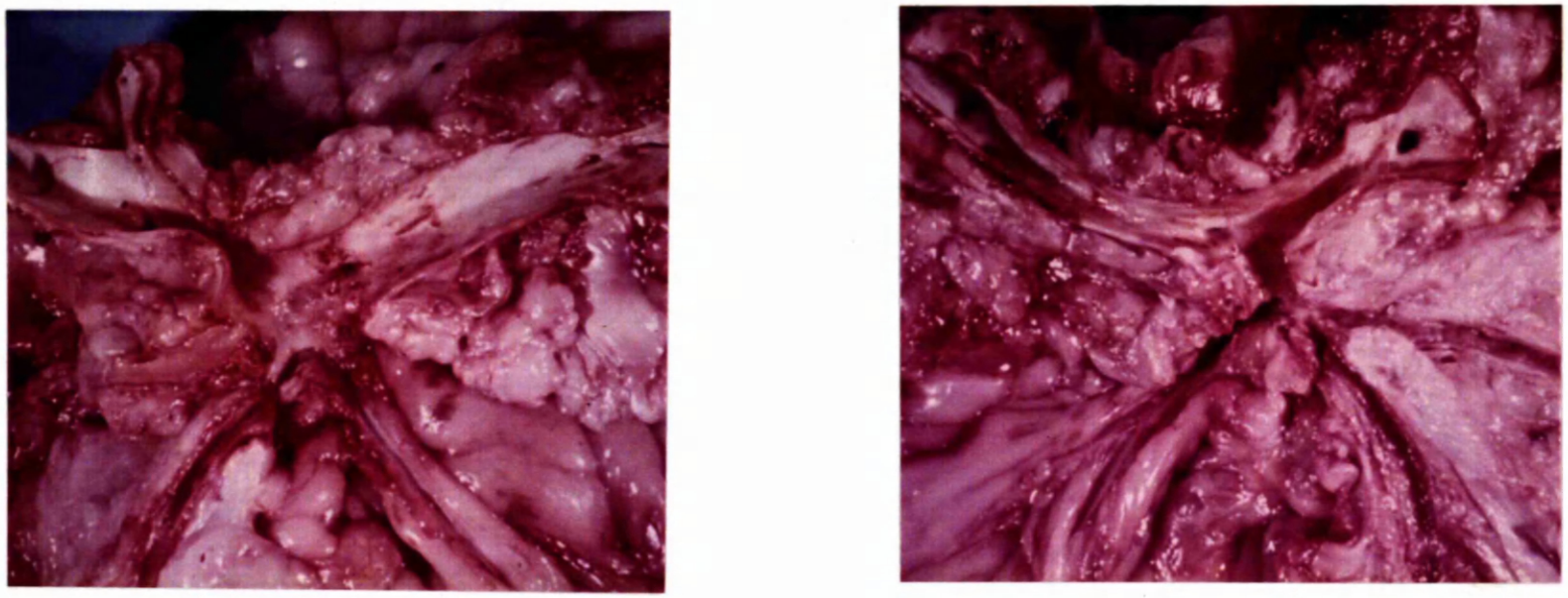


FIG. 44 Dissection of arterial preparations from two 3-year-olds at 2 months after infection

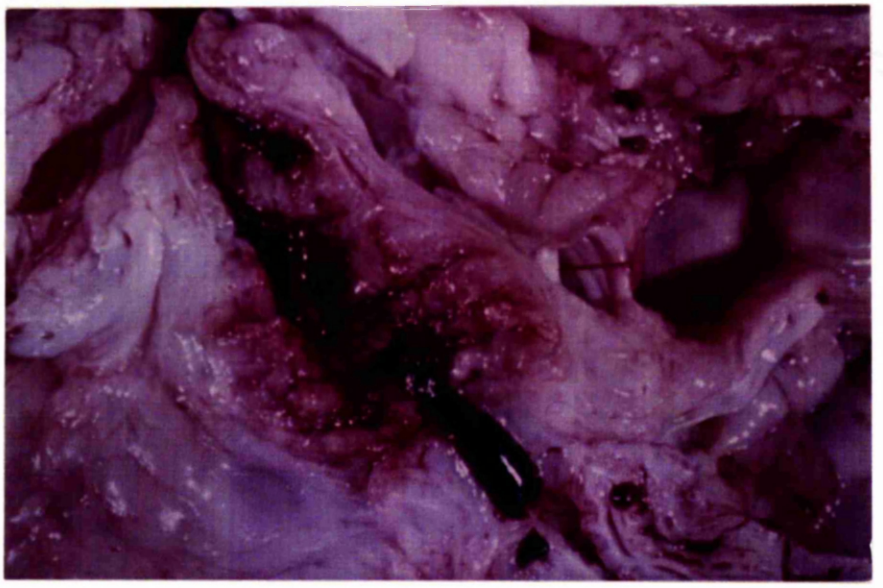


FIG. 45 Gross arterial lesion in control foal in age immunity experiment

results indicate that ponies reared worm-free until 3 years of age are apparently resistant to experimental infection with single large doses of S. vulgaris larvae.

Acquired Immunity

A number of experiments were carried out in an attempt to gain more information on the resistance of both naturally infected and experimentally infected animals to reinfection with S. vulgaris.

Naturally Infected Animals

The design and summary of results of experimental studies on the effect of single large doses of S. vulgaris larvae in 3 naturally infected aged pony mares and 3 naturally infected yearling ponies are summarised in Table 11.

The 3 aged mares were given doses of 1,500 infective larvae and 2 animals were killed 3 weeks after infection while the third mare was killed 2 months after infection.

The 3 yearlings were given 2,000 infective larvae and a control foal, aged 6 weeks, was dosed with 750 infective larvae at the same time: all 4 animals were killed 3 weeks after infection.

Clinical signs of infection were not observed in the 3 aged pony mares. At post-mortem examination there were signs of previous parasitic infection in all 3 animals, e.g. purulent intestinal nodules

TABLE 11

THE DESIGN AND RESULTS OF AN EXPERIMENT TO STUDY THE ROLE OF ACQUIRED IMMUNITY IN NATURALLY INFECTED PONIES

NATURALLY INFECTED PONIES		Worm-free 6-week-old control
3 Aged Mares	3 Yearlings	
1500 L_3	2000 L_3	750 L_3
2 animals killed day 21 / 1 animal killed day 60	Killed Day 21	Killed Day 21
No clinical signs	No clinical signs	Pyrexia Anorexia Abdominal Pain
At P.M. examination all animals showed evidence of previous parasitic infection including arterial lesions	At P.M. examination old arterial lesions containing mature L_4 fibrin tracts in aorta	At P.M. examination severe arterial lesion with thrombosis
No L_4 recovered	No L_4 recovered	160 L_4 recovered

containing fifth stage S. vulgaris larvae, calcified, gritty lesions and tracks in the mesentery and along the course of the caecal and colic arteries and various adult strongyles were present in the lumen of the large intestine. Our main interest in these animals, however, was concentrated on the arterial system. Although in each mare there were gross lesions in the anterior mesenteric artery (Fig. 46) containing mature S. vulgaris larvae, no immature fourth stage larvae from the single experimental infection could be recovered.

Clinical signs were also not apparent in the 3 yearling ponies but the control foal showed typical signs of infection, i.e. dullness, anorexia, pyrexia and abdominal pain. At necropsy all 3 yearlings had well-developed lesions in the anterior mesenteric artery (Fig. 47) which contained a number of mature fourth stage larvae; larval tracts were evident in the aorta but after scraping and digestion of the little thrombus material present, no immature S. vulgaris larvae were found. A severe lesion of the anterior mesenteric artery was present in the control foal (Fig. 48) and after scraping and digestion of the thrombus material present in this lesion 160 immature fourth stage larvae were recovered, i.e. 21% of the original dose.

The results of these studies, particularly those involving the yearling ponies, suggest that horses exposed to infection with S. vulgaris during life develop a significant degree of resistance to reinfection with single large doses of larvae.

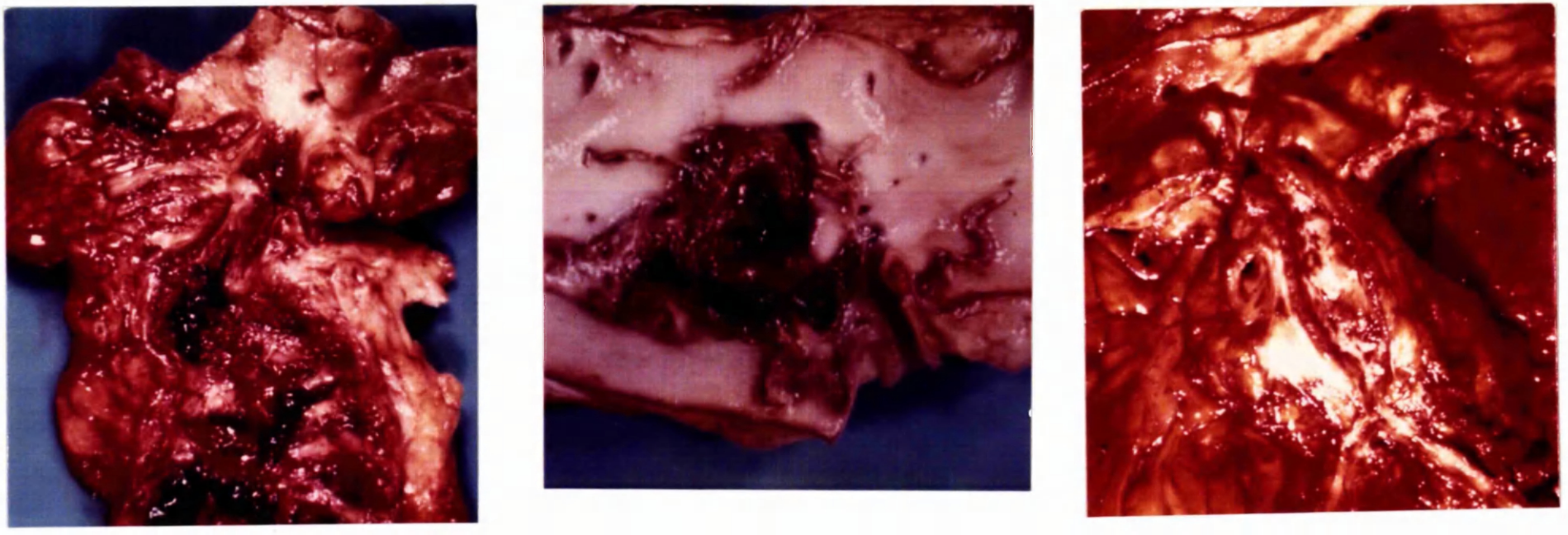


FIG. 46 Arterial preparations from 3 naturally infected pony mares

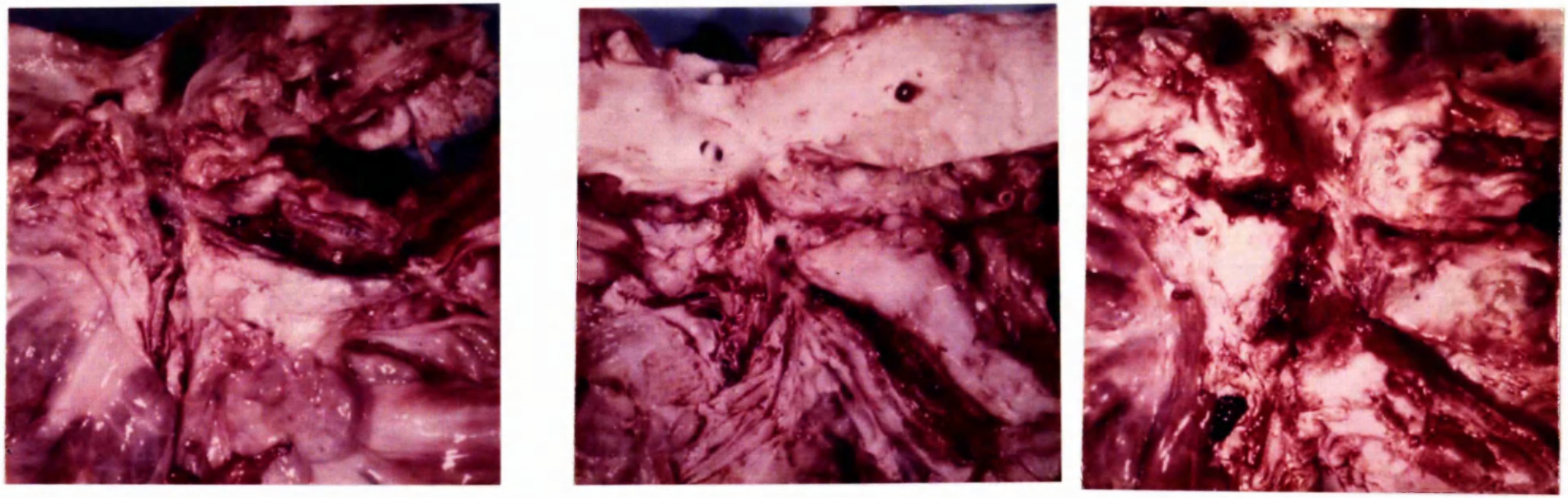


FIG. 47 Arterial preparations from 3 naturally infected yearling ponies

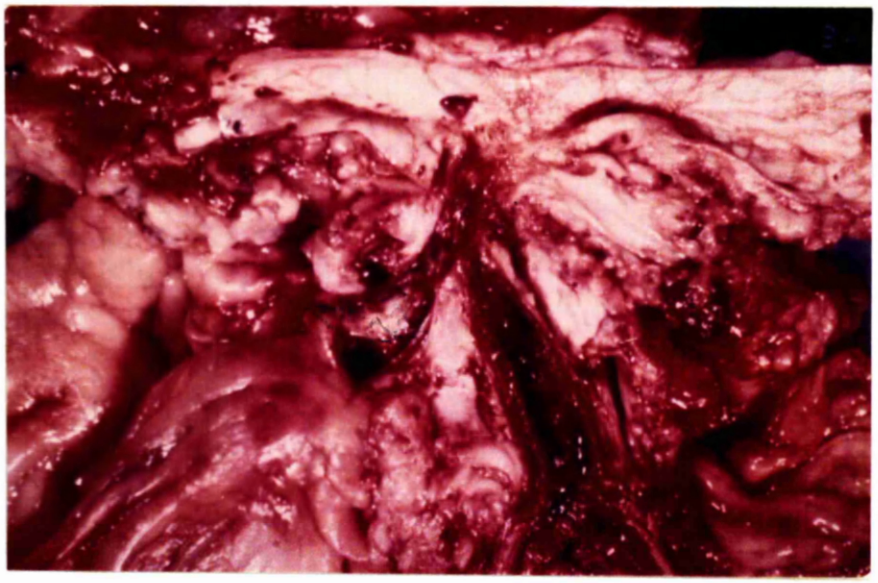


FIG. 48 Arterial preparation from control foal in naturally acquired immunity experiment

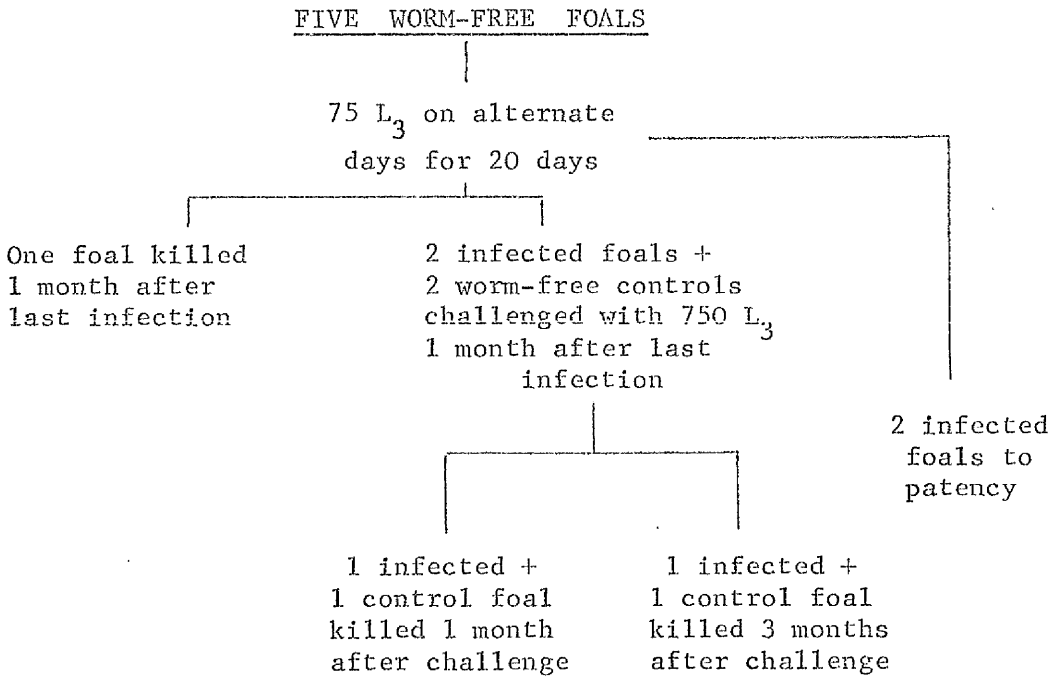
Experimentally Infected Animals

In our epidemiological studies it was shown that foals at pasture, although continually subjected to infection with substantial numbers of S. vulgaris larvae did not show any clinical signs. An experiment was therefore devised to investigate the effect of a challenge infection of 750 infective larvae on foals which had previously received small doses of larvae over a period of weeks.

The plan of this experiment is shown in Table 12.

TABLE 12

THE DESIGN OF AN EXPERIMENT TO STUDY THE EFFECT OF REPEATED LOW GRADE INFECTIONS ON SUBSEQUENT CHALLENGE WITH S. VULGARIS



Five worm-free foals were infected with 75 larvae on alternate days for 20 days, i.e. a total of 750 infective larvae were administered. One month after the last dose of larvae 1 of these foals was killed to check larval infectivity, and as this proved satisfactory 2 of the infected foals and 2 worm-free controls were challenged with a single dose of 750 larvae. One of the foals which had received the serial infection and 1 control were killed 1 month and 3 months after challenge, while 2 of the original foals were allowed to develop patent infections. The foals were checked daily, their rectal temperatures recorded and routine haematological examinations were carried out on blood samples taken weekly.

Results of the haematological estimations are given in Appendix 5.

Several interesting facts emerged from this experiment. First, the foals which received small doses of larvae showed no clinical signs during the serial infection or when subsequently challenged with a single dose of 750 larvae, while the challenge controls developed a typical syndrome of anorexia and dullness and one showed a marked temperature reaction. Secondly, although arterial lesions were present in the foals which received the serial doses of larvae before challenge, these were probably attributable to the effects of the initial infection since the larval burdens in their arteries were reduced when compared to the challenge controls. The major post-mortem findings in the 4 challenged foals are presented in Table 13.

TABLE 13

THE POST MORTEM FINDINGS IN 2 SERIALLY INFECTED AND 2 CONTROL FOALS AFTER CHALLENGE WITH 750 S. VULGARIS LARVAE

	2 foals killed 1 month after challenge		2 foals killed 3 months after challenge	
	Serial Infection Foal	Control Foal	Serial Infection Foal	Control Foal
S.I.	Few fibrous pale 'scar' lesions	Numerous pinkish/red lesions	One pale lesion visible. Ileal artery normal	Few lesions. Ileal artery thickened & nodular
CAECUM	Patchy haemorrhage 5 cms. at caecal tip	A few haemorrhagic lesions confined to tip of caecum	Gross nodular thickening along course of caecal arteries	Few nodular lesions in course of caecal arteries
DISSECTION OF ARTERIES	Gross lesions	Gross lesions	Gross lesions	Gross lesions
LARVAL RECOVERIES	64 L_4 > 1 cm. 10 L_4 < 1 cm.	80 L_4 < 1 cm.	38 L_4 early to late stages	60 L_4 Majority late stage

Figure 49 shows the larvae recovered from the foals killed 1 month after challenge. The fact that the serially infected foals received a total dose of 1,500 larvae whereas the controls received only 750 emphasises the reduction in arterial larval burdens, i.e. a mean of 56 from 1,500 larvae compared to a mean of 70 from 750 larvae.

Another experiment was carried out using two 22-month-old ponies, one of which had received a single infection of 750 infective larvae as a 4-month-old foal, while the other had been reared and maintained worm-free. Each of these ponies received 2,000 infective larvae and both were killed 6 weeks after infection. The pony which had experienced a single infection as a foal showed no clinical signs but the animal reared worm-free developed an acute syndrome with marked pyrexia, anorexia and colic (Fig. 50). On dissection of the arteries of the severely affected animal there was a marked arterial lesion in which numerous fourth stage larvae were visible (Fig. 51). In the animal which had shown no clinical signs of infection there was an old fibrous lesion of the anterior mesenteric artery and a few fibrin tracts in the endothelium of the aorta and intestinal arteries (Fig. 52) but no larvae were recovered.

Although these results are of necessity based on small numbers of animals, it nevertheless appears that after a primary infection with S. vulgaris, ponies may develop a significant degree of immunity to reinfection.

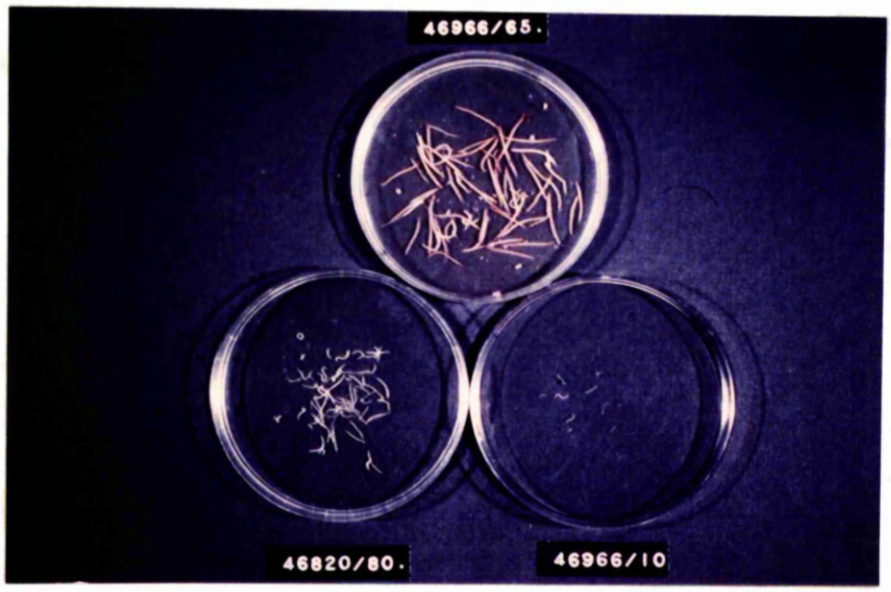


FIG. 49 Larvae from 2 foals killed 1 month after challenge in repeated infection and challenge experiment

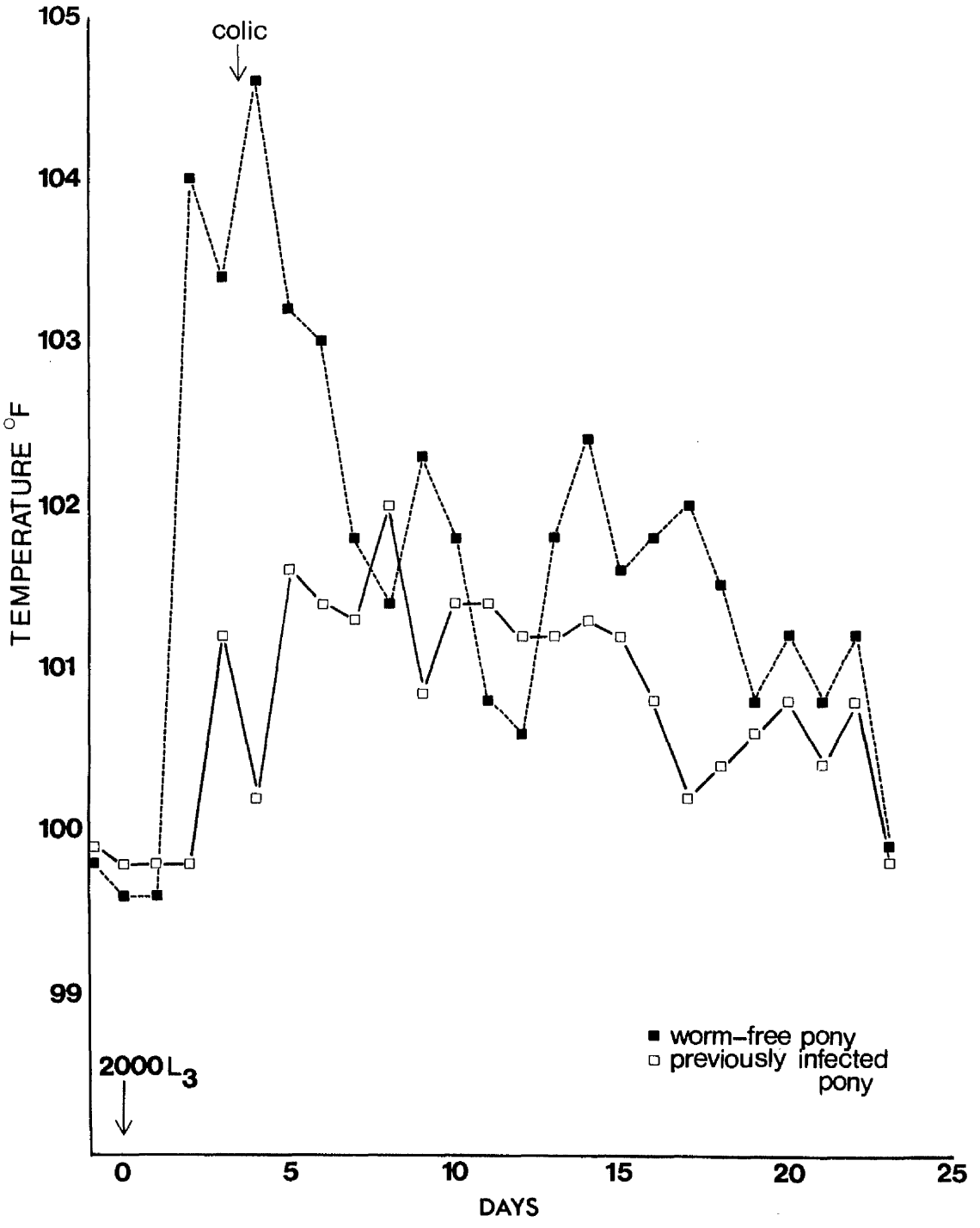


Fig. 50 Temperature reactions in two 22-month old ponies. Acquired immunity experiment.

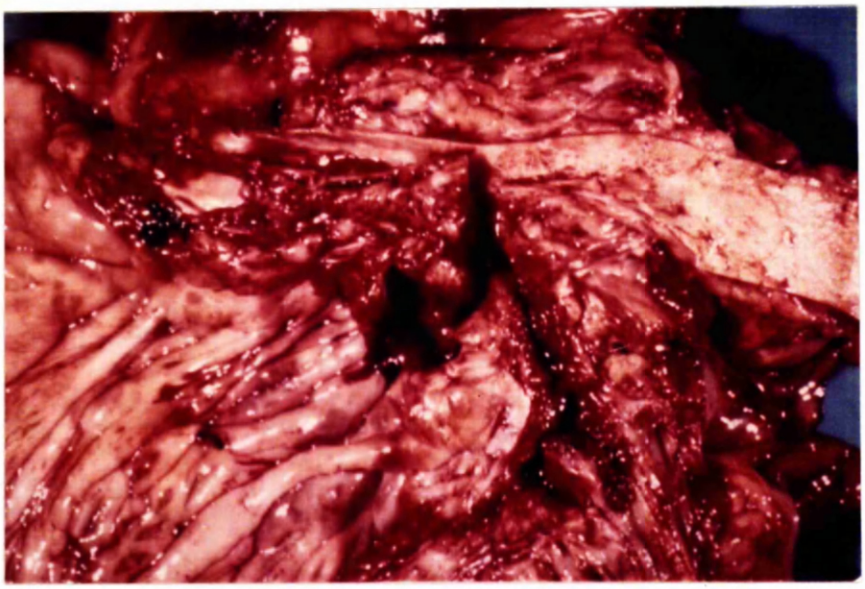


FIG. 51 Arterial preparation from previously worm-free 22-month-old pony

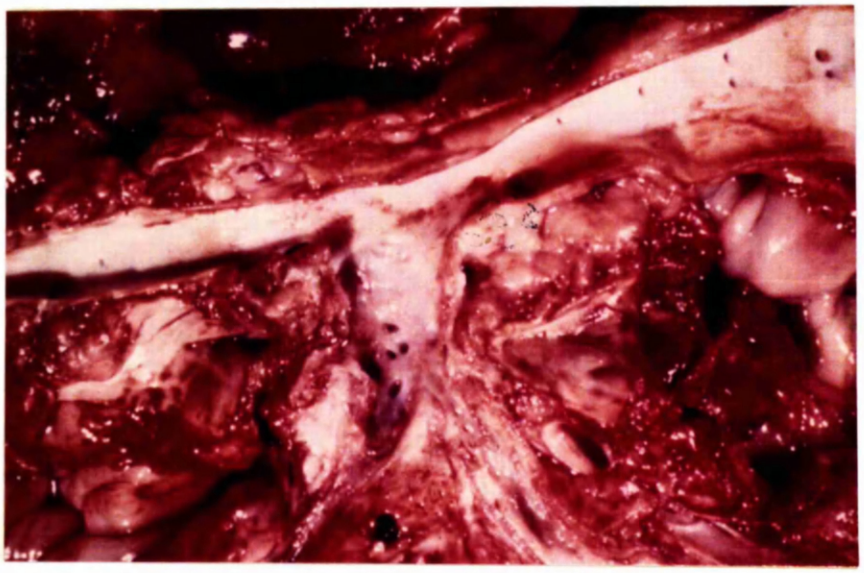


FIG. 52 Arterial preparation from previously infected 22-month-old pony

Vaccination

As our preliminary experiments had suggested that animals with previous experience of S. vulgaris infection acquired a significant degree of immunity to reinfection and since efficient irradiated larval vaccines had been developed against lungworm infection of cattle and sheep (Jarrett, Jennings, McIntyre, Mulligan & Urquhart, 1960; Jovanovich, Sokolic, Movsesijan & Cuperlovic, 1965) several experiments were devised to investigate the possibility of immunising worm-free foals with irradiated S. vulgaris larvae.

Due to the small numbers of experimental animals available only 3 foals were used in an initial irradiation dose titration experiment. The plan and results of this experiment are shown in Table 14.

Three worm-free foals aged between 6 and 8 months were each given a single dose of 750 larvae. One foal was used as a control and received normal larvae while the others received larvae subjected to 40 kr. and 80 kr. respectively. At post-mortem examination one month after infection, gross lesions were apparent in the caeca of the foals which received irradiated larvae but were not evident in the control foal (Fig. 53). Arterial lesions containing larvae were present in all 3 foals but these were least severe in the foal which received larvae irradiated at 80 kr. and only 1 immature fourth stage larva was recovered from this animal. Although the arterial lesion was most severe in the control foal, only 31 larvae could be recovered. This was a relatively low

TABLE 14

RESULTS OF AN IRRADIATION DOSE TITRATION EXPERIMENT USING 3 WORM-FREE FOALS

3 WORM-FREE FOALS 6-8 MONTHS OLD		
Foal 1	Foal 2	Foal 3
750 L_3	750 L_3	750 L_3
Normal	Irradiated at 40 kr.	Irradiated at 80 kr.
Killed Day 30	Killed Day 30	Killed Day 30
Penetration lesions scattered throughout intestine. Fibrin tracts in aorta. Marked arterial lesion.	Few penetration lesions but marked haemorrhagic lesion at caecal tip 2" diam. Few fibrin tracts. Moderate arterial lesion	Few penetration lesions. Gross haemorrhagic lesion 1½" diam. at caecal tip. Slight arterial lesion
31 early L_4	10 early L_4	1 early L_4

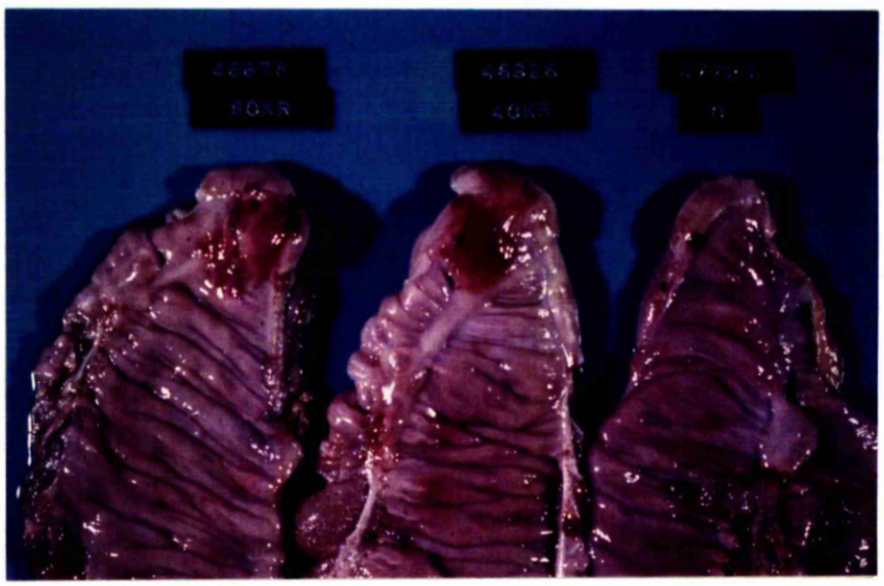


FIG. 53 The caeca of 3 foals used in irradiation dose titration experiment

"take" considering our previous experience where larval recoveries totalled 15 - 30% of the original infective dose but can possibly be explained on the basis of poor larval infectivity together with the fact that these foals were several months older than those used in our original experiments. Since the use of additional foals for another irradiation dose titration experiment would have resulted in further expense and delayed any vaccination experiments for another year, it was decided to carry out a preliminary vaccination experiment using 6 available worm-free foals.

Experimental Design

Four foals 5 - 8 weeks old were each given 2 doses of 250 and 500 irradiated larvae with an interval of 3 weeks between doses. Two of the foals received larvae which had been irradiated at 60 kr. while the other 2 foals were given larvae irradiated at 80 kr. These foals, together with 2 worm-free controls, were challenged with 1,000 normal larvae 4 weeks later.

The results of this vaccination experiment are presented in Table 15.

Although arterial lesions developed in both groups of vaccinated foals they were less severe in the animals which received larvae irradiated at 80 kr. The mean reduction in numbers of larvae recovered from the challenge infection was however very high in both vaccinated groups, i.e. over 80%. If the results are calculated on a basis of total numbers of larvae recovered from total numbers

TABLE 15

PATHOLOGY AND LARVAL RECOVERIES FROM 6 FOALS USED IN A PRELIMINARY VACCINATION EXPERIMENT

	80 kr. Vaccinates		60 kr. Vaccinates		Challenge Controls
ARTERIAL LESION	++ Mature lesion with little new thrombosis. Some fresh thrombosis in branches of intestinal arteries.		+++ Well developed lesion. Fresh thrombosis in arteries		+++++ Typical primary lesion. Severe arteritis. Gross thrombosis.
LARVAL RECOVERIES	* L_4 from Challenge	Total	L_4 from Challenge	Total	
FOAL 1	18	30	10	25	108 L_4
FOAL 2	21	23	27	45	98 L_4
MEAN	19.5	26.5	18.5	35	103
% REDUCTION IN TAKE IN VACCINATES	81%		82%		

* Conservative estimate based entirely on larval size.

administered, it is clear that in the vaccinated groups 1.5% and 2% of the total infection were recovered compared to over 10% in the challenge controls. This is again a reduction of approximately 80% in the "takes" of the vaccinated animals.

DISCUSSION

Although the experimental work presented in this chapter is based on a limited number of animals, it has nevertheless provided certain basic information on age, naturally acquired and artificially acquired immunity to S. vulgaris infection and it is proposed to discuss the results under these headings.

First, from our initial studies on the role of age immunity it appears that the 5 ponies which had been reared and maintained worm-free until they reached 3 years of age were subsequently resistant to the effect of experimental infection with doses of 750 - 1,000 S. vulgaris larvae. At post-mortem examination of these ponies 1 - 2 months after infection, intestinal lesions due to the penetration of third stage larvae, and fibrin tracts in the arterial system due to migration of early fourth stage larvae, were evident but no arteritis or thrombosis was apparent in the predilection site and no larvae were recovered. This is contrary to our experience of primary infections in worm-free animals up to 2 years of age where it has been shown that marked arterial lesions are present and fourth stage larvae are usually visible 1 - 2 months after infection. One

of the ponies used in this experiment, however, was 2½-years-old at the time of infection and when killed 2 months later a gross arterial lesion containing larvae was present. It is considered, therefore, that some immune mechanism in terms of resistance to experimental S. vulgaris infection comes into play in horses reared under worm-free conditions until 3 years of age.

Secondly, a study involving 6 naturally infected animals (3 yearlings and 3 aged pony mares) and 1 worm-free foal provided evidence that horses exposed to S. vulgaris infection during life rapidly acquire an immunity, in terms of resistance to the effect of reinfection with single doses of 1,500 - 2,000 S. vulgaris larvae.

This observation on the acquisition of immunity by previous infection was confirmed experimentally. In this experiment the sensitizing infections, administered to foals reared worm-free, were a series of small doses of larvae given over a period of several weeks. The fact that these foals showed no clinical signs either during the serial infection or on subsequent challenge, despite the fact that arterial lesions containing larvae were evident at necropsy, provides evidence of the development of an immunity in terms of resistance to the effects of reinfection. The larval recoveries from the foals used in this experiment yielded some interesting results. For example, in the 2 foals killed 1 month after challenge, 80 fourth stage larvae all less than 1 cm. in length were recovered from the control, whereas 64 larvae over 1 cm. long and 10 less than 1 cm. in length were

recovered from the foal which had received the serial infection before challenge. These 10 fourth stage larvae are therefore the maximum number which could possibly have developed from the challenge infection, i.e. a reduction of 87%, and it is even possible that these larvae were also the result of the initial serial infection. In the 2 foals killed 3 months after challenge it was more difficult to differentiate larvae based on size. The majority of the 60 larvae recovered from the control were however mature fourth stage, whereas the 38 larvae recovered from the serially infected foal varied from early to late fourth stage. In terms of total larval recoveries the serially infected foals had a mean of 56 from 1,500 larvae while the control foals had a mean of 70 from 750 larvae, i.e. a percentage development of 3.7% and 9.3% respectively. These experimental results suggest that at least a partial protective immune mechanism develops in young animals during continuous exposure to S. vulgaris larvae.

A further experiment was completed using two 22-month-old ponies, one of which had received a single experimental infection of 750 larvae as a 4-month-old foal, while the other had been reared and maintained worm-free throughout life. On challenge with 2,000 infective larvae the worm-free animal showed a typical clinical syndrome and gross arterial lesions containing larvae, whereas the pony which had experienced a single infection as a foal showed neither clinical signs nor arterial lesions. Although there were

only 2 animals involved in this experiment, it nevertheless appears that the animal which received a single experimental infection in foalhood was protected from the effects of reinfection at a later stage.

These experiments, which demonstrate that age and acquired immunity develop to S. vulgaris infection, pose several questions which as yet remain unanswered. Perhaps the most obvious of these is why, if there is a relatively significant age and naturally acquired immunity to S. vulgaris, is there such a high field incidence of infection with both adult and immature parasites.

There are several possible explanations for the apparent absence of immunity in horses born and reared under field conditions. It has been suggested (Taylor & Michel, 1953) that mature S. vulgaris larvae found in arterial lesions of field cases may in fact have been inhibited in their development and thus remain at the arterial site for considerable periods of time. However, a definitive experiment to confirm or refute this hypothesis has not been attempted. In our experience with experimental infections we have never encountered viable larvae in the arterial site beyond the point in time when they should have returned to the intestinal lumen.

Another possible explanation is that a degree of acquired immunological unresponsiveness persisting into adult life may develop in the young foal exposed at an early age to S. vulgaris infection but more experimental work would be required to confirm this.

The results of the vaccination experiment, although preliminary in nature, have shown that significant reductions in larval populations occur when foals given 2 doses of larvae irradiated at 60 kr. and 80 kr. are subsequently challenged but unfortunately the irradiated larvae themselves are responsible for arterial lesions. Further work is planned in an attempt to produce an immunity to reinfection without the development of significant arterial lesions.

SUMMARY

In a study of the possible existence of age immunity in five 3-year-old animals, reared and maintained worm-free, and dosed with 750 - 1,000 larvae, no gross arterial lesions developed and no fourth stage larvae were recovered. In contrast, in worm-free animals under 2½ years of age, severe lesions containing numerous larvae resulted from infections with 750 larvae.

The results of a series of subsequent experiments on acquired immunity demonstrated that both naturally and experimentally infected animals show a degree of resistance to the effects of reinfection with single large doses of S. vulgaris larvae. In one experiment 3 naturally infected yearling ponies and 3 aged pony mares dosed with 2,000 larvae showed no clinical abnormality and no larvae could be recovered from the experimental infections at necropsy. A 6-week-old control foal infected at the same time with 750 larvae showed typical signs of infection, i.e. pyrexia, anorexia and colic and at post-mortem 21% of the infective dose was recovered from the severe arterial lesion which was present in this animal. In a second experiment on acquired immunity, 2 foals which had received a total of 750 larvae divided into 10 doses over 20 days were challenged 1 month later, together with 2 controls, with single doses of 750 larvae. The foals which received the small doses of larvae showed no clinical signs either during serial infection or when subsequently challenged with a single dose of 750 larvae and the larval burdens in their arteries were reduced when

compared to the challenge controls, i.e. a mean of 56 from 1,500 larvae compared to a mean of 70 from 750 larvae.

A third experiment in which doses of 2,000 larvae were administered to two 22-month-old ponies, one of which had been infected with 750 larvae during foalhood while the other had been maintained worm-free, demonstrated that the animal which had experienced a single infection as a foal was resistant to the effects of the experimental reinfection.

A preliminary vaccination experiment was carried out on 4 foals given 2 doses of 250 and 500 larvae irradiated at 60 kr. and 80 kr. and challenged 1 month later with 1,000 larvae. The results of this experiment demonstrated that although arterial lesions developed in both vaccinated groups, these were less severe in the animals which received larvae irradiated at 80 kr. There was also a reduction of 80% in the numbers of larvae which developed as a result of the challenge infection in the vaccinated animals.

REFERENCES

ALEXANDER, F. (1970) Br. vet. J. 126. 604.

BAKER, D.W., SALISBURY, G.W. & BRITTON, J.W. (1939) Cornell Veterinarian 29. 297.

BOAG, B. & THOMAS, R.J. (1971) Res. vet. Sci. 12. 132.

CRONIN, M.T.I. & LEADER, G.H. (1952) Vet. Rec. 64. 8.

DACIE, J.V. & LEWIS, S.M. (1966) In Practical Haematology 3rd Edition. J. & A. Churchill Ltd., London.

DRUDGE, J.H., LYONS, E.T. & SZANTO, J. (1966) In Biology of Parasites. Ed. Soulsby, E.J.L. Academic Press, New York.

ENIGK, K. (1950) Z. Tropenmed. Parasit. 2. 287.

ENIGK, K. (1951) Z. Tropenmed. Parasit. 2. 523.

ENIGK, K. (1951a) Mh. Tierheilk. 3. 65.

ERSHOV, V.S. (1949) Veterinariya 26. 26.

ERSHOV, V.S. (1956) Parasitology and Parasitic Diseases of Livestock. Moscow State Publishing House.

FARELLY, B.T. (1954) Vet. Rec. 66. 53.

GERBER, H., CHUIT, P. & PAULI, B. (1971) Schweiz. Arch. Tierheilk. 113. 678.

GIBSON, T.E. & EVERETT, G. (1967) Parasitology 57. 533.

GIBSON, T.E. & EVERETT, G. (1971) Res. vet. Sci. 12. 602.

GORDON, H.McL. & WHITLOCK, H.V. (1939) J. Council Sci. Indust. Res. 12. 50.

GRAY, S.J. & STERLING, K. (1950) J. Clin. Invest. 29. 1604.

HERLICH, H. (1956) Proc. Helminth. Soc. Washington 23. 102.

JARRETT, W.F.H., JENNINGS, F.W., McINTYRE, W.I.M., MULLIGAN, W. & URQUHART, G.M. (1960) Immunology 3. 145.

JOVANOVIC, M., SOKOLIC, A., MOVSESIJAN, M. & CUPERLOVIC, K. (1965) Brit. Vet. J. 121. 119.

LOOS, A. (1901) Rec. Egypt. Govt. Sch. Med. p.25.

LOWE, J.E., HINTZ, H.F. & SCHRYVER, H.F. (1970) Am. J. vet. Res. 31 (6). 1109.

MAHAFFEY, L.W. & ADAM, N.M. (1963) Vet. Rec. 75. 561.

MAROTEL, G. (1931) Revue vét., Toulouse 83. 657.

MATHIESON, A.O. (1964) M.Sc. Thesis. University of Edinburgh.

MATTHEWS, C.M.E. (1957) Physics in Med. Biol. 2. 36.

McFARLANE, A.S. (1958) Nature 182. 53.

MILLER, N. (1953) Nature 171. 658.

NEILL, D.W. (1973) In Joyce-Loebl Review, Team Valley, Gateshead. Spring Number.

OGBOURNE, C.P. (1971) Parasitology 63. 289.

OGBOURNE, C.P. (1972) Parasitology 64. 467.

OLT, A. (1932) Dtsch. Tierarztl. Wschr. 40. 326.

OTTOWAY, C.W. & BINGHAM, M.L. (1941) Vet. Rec. 53. 275.

OTTOWAY, C.W. & BINGHAM, M.L. (1946) Vet. Rec. 58. 155.

PARFITT, J.W. (1955) Lab. Practice 4. 15.

PAULI, B., GERBER, H. & CHUIT, P. (1971) Schweiz. Arch. Tierheilk. 113. 685.

POYNTER, D. (1954) Vet. Rec. 66. 74.

POYNTER, D. (1960) Res. vet. Sci. 1. 205.

POYNTER, D. (1970) Proc. 2nd Int. Conf. Equine Infectious Diseases, Paris 1969. p.269. Karger, Basel/München/New York.

ROBERTS, F.H.S. & O'SULLIVAN, P.J. (1950) Aust. J. Agric. Res. 1. 99.

ROUND, M.C. (1969) J. Helminth. 43. 185.

ROUND, M.C. (1970) Proc. 2nd Int. Conf. Equine Infectious Diseases, Paris 1969. p.290. Karger, Basel/München/New York.

RUSSELL, A.F. (1948) J. Comp. Path. 58. 102.

SHUMAKOVITCH, E.E. (1940) Veterinariya 1. 109.

STERLING, K. (1951) J. Clin. Invest. 30. 1128.

TAYLOR, E.L. & MICHEL, J.F. (1953) J. Helminth. 27. 199.

VELICHKIN, P.A. (1964) Proc. Int. Cong. Parasit., Rome. Vol. II. p.868. Pergamon Press Ltd.

WEICHSELBAUM, T.E. (1946) Amer. J. Clin. Path. 10. 40.

WEISS, J. (1952) Nucleonics 10. 7.

WETZEL, R. (1942) Dtsch. Tierarztl. Wschr. 50. 443.

WETZEL, R. & ENIGK, K. (1938) Arch. Wiss. Prakt. Tierheilk. 73. 83.

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

APPENDICES 1 - 5

APPENDIX 1 - Table 1

Strongyle

Faecal Egg Counts 1971 - 72

Paddock A

	MARES			FOALS		
DATE	1	2	3	1	2	3
27/5/71	-ve	-ve	-ve	-ve	-ve	-ve
10/6/71	-ve	-ve	-ve	-ve	-ve	-ve
24/6/71	-ve	-ve	-ve	-ve	-ve	-ve
8/7/71	-ve	-ve	-ve	-ve	-ve	-ve
22/7/71	-ve	-ve	-ve	-ve	-ve	-ve
5/8/71	-ve	-ve	-ve	N.S.	100	100
19/8/71	-ve	-ve	-ve	50	-ve	50
2/9/71	-ve	-ve	-ve	250	100	-ve
16/9/71	-ve	-ve	-ve	N.S.	50	-ve
30/9/71	-ve	-ve	-ve	250	100	-ve
14/10/71	-ve	-ve	-ve	100	150	-ve
28/10/71	-ve	-ve	-ve	200	100	50
11/11/71	MARES REMOVED			100	350	50
25/11/71				100	100	150
9/12/71	FROM PADDOCK			600	100	100
23/12/71				150	100	150
6/1/72				250	200	-ve
20/1/72				100	400	100
3/2/72				300	300	400
17/2/72				400	-	400
2/3/72				200	-	100
16/3/72				450	-	300

APPENDIX 1 - Table 2

Strongyle

Faecal Egg Counts 1971 - 72

Paddock B

	MARES			FOALS		
DATE	1	2	3	1	2	3
27/5/71	50	650	1500	-ve	150	N.S.
10/6/71	-ve	600	750	-ve	-ve	-ve
24/6/71	-ve	1500	N.S.	-ve	-ve	-ve
8/7/71	150	2450	3000	-ve	-ve	-ve
22/7/71	250	3200	1150	-ve	-ve	N.S.
5/8/71	300	3200	950	-ve	-ve	-ve
19/8/71	350	1500	N.S.	-ve	-ve	-ve
2/9/71	300	N.S.	200	-ve	50	-ve
16/9/71	600	450	150	150	50	50
30/9/71	250	1400	50	250	200	150
14/10/71	400	550	150	600	400	150
28/10/71	450	1250	200	550	700	450
11/11/71	350	N.S.	100	N.S.	550	600
25/11/71	Mares removed from paddock			1800	550	850
9/12/71	150	250	50	400	600	1000
23/12/71	200	650	100	650	500	550
6/1/72	150	100	400	300	300	500
20/1/72	150	150	150	350	650	400
3/2/72	50	150	50	600	600	300
17/2/72	50	200	50	300	700	-
2/3/72	2150	1450	600	1250	250	-
16/3/72	100	300	350	350	1100	-

APPENDIX 2 - Table 1

Strongyle

Faecal Egg Counts 1972 - 73

Paddock B

	MARES			FOALS		
DATE	1	2	3	1	2	3
30/5/72	-ve	-ve	-ve	-ve	-ve	
14/6/72	-ve	50	-ve	N.S.	-ve	
28/6/72	-ve	-ve	-ve	-ve	-ve	-ve
11/7/72	-ve	100	-ve	-ve	-ve	-ve
25/7/72	-ve	-ve	-ve	-ve	-ve	-ve
8/8/72	-ve	-ve	-ve	-ve	50	-ve
22/8/72	-ve	150	-ve	-ve	N.S.	N.S.
13/9/72				50	50	-ve
5/10/72	MARES REMOVED			150	1000	-ve
13/10/72				200	600	-ve
27/10/72	FROM PADDOCK			200	350	-ve
10/11/72				300	600	800
24/11/72				500	550	450
8/12/72				400	650	400
21/12/72				500	550	450
4/1/73				550	600	600
18/1/73				200	500	700
2/2/73				750	650	150
16/2/73				2150	700	150
1/3/73				1700	800	500

APPENDIX 2 - Table 2

Strongyle
Faecal Egg Counts 1972 - 73

Paddock C

	MARES			FOALS		
DATE	1	2	3	1	2	3
25/5/72	150	1000	650	-ve	-ve	50
7/6/72	700	400	550	N.S.	50	-ve
22/6/72	200	50	600	50	-ve	-ve
5/7/72	300	400	800	-ve	-ve	-ve
19/7/72	450	450	650	50	-ve	-ve
2/8/72	200	250	1600	150	50	50
15/8/72	MARES REMOVED			-ve	50	50
30/8/72				-ve	50	-ve
14/9/72	FROM PADDOCK			-ve	-ve	-ve
5/10/72				850	750	100
13/10/72				500	3000	300
27/10/72				550	1800	500
10/11/72				2450	4400	600
24/11/72				700	4750	850
8/12/72				1500	2000	2100
21/12/72				2000	3000	1850
4/1/73				1800	3700	3200
18/1/73				2450	200	2200
2/2/73				850	550	150
16/2/73				2100	1150	200
1/3/73				1950	900	700

APPENDIX 3 - Table 1

Pasture Larval Counts 1971 - 72

L_3/Kg. Herbage

	PADDOCK A			PADDOCK B		
DATE	Tr	Sv	O	Tr	Sv	O
13/5/71	-ve	-ve	-ve	-ve	-ve	-ve
24/5/71	5	0	0	4	69	11
17/6/71	10	0	0	65	8	22
15/7/71	2	0	0	527	6	27
29/7/71	1	0	0	1401	35	85
12/8/71	-ve	-ve	-ve	2975	25	87
26/8/71	-ve	-ve	-ve	608	14	53
10/9/71	-ve	-ve	-ve	3099	35	126
23/9/71	65	0	3	4824	64	224
7/10/71	175	0	8	4959	54	200
21/10/71	26	1	8	4819	4	87
4/11/71	98	0	8	476	0	10
19/11/71	11	0	9	355	4	31
2/12/71	6	0	2	517	0	136
16/12/71	30	0	10	80	0	8
30/12/71	118	0	18	238	0	109
13/1/72	27	0	31	162	0	97
27/1/72	30	0	6	346	5	26
10/2/72	-ve	-ve	-ve	147	2	6
24/2/72	38	0	0	84	0	0
9/3/72	54	0	38	717	0	33
23/3/72	-ve	-ve	-ve	196	0	23
7/4/72	12	3	3	320	5	40
20/4/72	5	0	5	54	0	0
4/5/72	-ve	-ve	-ve	3	0	0
19/5/72	-ve	-ve	-ve	2	0	0
1/6/72	-ve	-ve	-ve	-ve	-ve	-ve
15/6/72	-ve	-ve	-ve	-ve	-ve	-ve

Tr = Trichonema spp.
Sv = S. vulgaris
O = Others

APPENDIX 3 - Table 2

Pasture Larval Counts 1972 - 73

L_3/Kg. Herbage

	PADDOCK B			PADDOCK C		
DATE	Tr	Sv	O	Tr	Sv	O
19/4/72	54	0	0	6	0	0
4/5/72	3	0	0	197	27	71
19/5/72	2	0	0	125	55	39
1/6/72	-ve	-ve	-ve	500	9	9
15/6/72	-ve	-ve	-ve	307	9	43
28/6/72	-ve	-ve	-ve	2895	45	60
12/7/72	6	0	2	2004	52	61
27/7/72	7	0	2	1731	69	119
9/8/72	5	0	3	1596	67	42
23/8/72	67	0	10	1655	49	109
6/9/72	28	0	3	1634	35	64
20/9/72	44	0	16	1483	54	59
5/10/72	53	0	15	500	0	48
19/10/72	59	0	8	418	0	19
3/11/72	46	0	5	864	16	19
17/11/72	120	0	20	980	0	50
1/12/72	124	0	10	1469	0	47
14/12/72	32	0	4	104	0	12
27/12/72	23	0	5	100	0	10
16/1/73	32	0	4	142	0	12
24/1/73	36	0	8	238	0	20
8/2/73	37	0	13	578	6	83
22/2/73	12	0	4	177	0	18
8/3/73	-ve	-ve	-ve	89	0	12
23/3/73	3	0	0	169	0	10
18/4/73	-ve	-ve	-ve	-ve	-ve	-ve
27/4/73	-ve	-ve	-ve	-ve	-ve	-ve

Tr = Trichonema spp.
Sv = S. vulgaris
O = Others

APPENDIX 3 - Table 3

Differential Larval Counts 1971 - 72

PADDOCK A

	MARES			FOAL 1			FOAL 2			FOAL 3		
DATE	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O
27/5/71					-ve			-ve			-ve	
10/6/71					-ve			-ve			-ve	
24/6/71					-ve			-ve			-ve	
8/7/71					-ve			-ve			-ve	
22/7/71	Faecal cultures from			Few	0	0	Few	0	0	Few	0	0
5/8/71	mares negative				N.S.			-ve		Few	0	0
19/8/71	throughout experimental				-ve		100	0	0	Few	0	0
2/9/71				100	0	0	100	0	0		-ve	
16/9/71	period				N.S.		100	0	0	Few	0	0
30/9/71				98	0	2	100	0	0	100	0	0
14/10/71				100	0	0	99	0	1	Few	0	0
28/10/71				95	0	5	100	0	0	Few	0	0
11/11/71				95	0	5	100	0	0	99	0	1
25/11/71				98	0	2	100	0	0	99	0	1
9/12/71				100	0	0	100	0	0	97	0	3
23/12/71				99	0	1	93	0	7	95	4	1
6/1/72				95	2	3	99	0	1	97	1	2
20/1/72				91	7	2	90	9	1	Few	0	0
3/2/72				93	6	1	91	7	2	84	15	1
17/2/72				90	8	2		-		81	19	0
2/3/72				84	15	1		-		79	0	21
16/3/72				95	5	0		-		61	39	0
30/3/72					-			-			N.S.	
14/4/72					-			-		97	2	1
28/4/72					-			-		83	15	2

Tr = Trichonema spp. Sv = S. vulgaris O = Others

APPENDIX 3 - Table 4

Differential Larval Counts 1971 - 72

PADDOCK B

	MARE 1			MARE 2			MARE 3			FOAL 1			FOAL 2			FOAL 3		
DATE	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0
27/5/71	Few	0	0	83	10	7	87	7	6		-ve		100	0	0		N.S.	
10/6/71	82	8	10	92	4	4	87	9	4		-ve			-ve			-ve	
24/6/71	Few	0	0	92	2	6		N.S.			-ve			-ve			-ve	
8/7/71	91	3	6	93	3	4	93	3	4		-ve			-ve			-ve	
22/7/71	96	2	2	95	2	3	90	6	4		-ve			-ve			N.S.	
5/8/71	92	3	5	94	2	4	72	21	7		-ve			-ve			-ve	
19/8/71	95	5	0	91	4	5		N.S.			-ve			-ve			-ve	
2/9/71	98	0	2		N.S.		95	2	3	Few	0	0		-ve		Few	0	0
16/9/71	93	4	3	95	2	3	94	1	5	99	0	1	100	0	0	98	0	2
30/9/71	93	5	2	98	2	0	96	1	3	94	2	4	99	0	1	98	0	2
14/10/71	98	0	2	96	2	2	95	2	3	95	2	3	100	0	0	96	0	4
28/10/71	93	4	3	96	4	0	97	2	1	98	1	1	100	0	0	99	0	1
11/11/71		Mares		Removed		From	Paddock				N.S.		100	0	0	99	0	1
25/11/71	96	3	1	92	5	3	98	1	1	96	2	2	96	0	4	97	1	2
9/12/71	94	1	5	93	4	3	97	3	0	98	0	2	99	0	1	97	2	1
23/12/71	96	3	1	98	1	1		N.S.		92	0	8	95	0	5	96	1	3
6/1/72	98	0	2	96	2	2	94	4	2	95	0	5	99	0	1	97	0	3
20/1/72	Few	0	0	98	1	1	95	2	3	97	0	3	98	1	1	88	10	2
3/2/72	100	0	0	98	1	1	93	5	2	88	11	1		N.S.		92	6	2
17/2/72	100	0	0	99	1	0	100	0	0	89	10	1	88	12	0		-	
2/3/72	98	0	2	97	1	1	98	2	0	84	16	0	85	14	1		-	
16/3/72	100	0	0	95	4	1	96	4	0	72	27	1	88	11	1		-	
30/3/72	Few	0	0	94	5	1	88	10	2		N.S.			-			-	
14/4/72		N.S.		94	4	2	75	24	1	92	6	2		-			-	
28/4/72		-			-			-		70	22	8		-			-	

Tr = Trichonema spp. Sv = S. vulgaris 0 = Others

APPENDIX 3 - Table 5

Differential Larval Counts 1972 - 73

PADDOCK B

	MARES			FOAL 1			FOAL 2			FOAL 3		
DATE	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0	Tr	Sv	0
30/5/72	Mares dosed on evidence of positive egg count. Faecal cultures from positive samples mainly *Trichonema* spp.				-ve			-ve				
14/6/72					-ve			-ve				
28/6/72					N.S.			-ve			-ve	
11/7/72					-ve			-ve			-ve	
25/7/72					-ve			-ve			-ve	
8/8/72					-ve		Few	0	0		-ve	
22/8/72					-ve			N.S.			N.S.	
13/9/72				100	0	0	99	0	1		-ve	
5/10/72				100	0	0	98	0	2	Few	0	0
13/10/72				100	0	0	99	0	1	Few	0	0
27/10/72				100	0	0	100	0	0		-ve	
10/11/72				100	0	0	98	0	2	100	0	0
24/11/72				100	0	0	100	0	0	100	0	0
8/12/72				100	0	0	99	0	1	99	0	1
21/12/72				100	0	0	98	0	2	100	0	0
4/1/73				100	0	0	96	3	1	99	1	0
18/1/73				100	0	0	97	3	0	100	0	0
2/2/73				100	0	0	92	8	0	100	0	0
16/2/73				97	2	1	Few	0	0	100	0	0
1/3/73				97	3	0	94	6	0	Few	0	0

Tr = *Trichonema* spp. Sv = *S. vulgaris* 0 = Others

APPENDIX 3 - Table 6

Differential Larval Counts 1972 - 73

PADDOCK C

	MARE 1			MARE 2			MARE 3			FOAL 1			FOAL 2			FOAL 3		
DATE	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O	Tr	Sv	O
25/5/72	100	0	0	98	1	1	96	3	1		-ve			-ve		Few	0	0
7/6/72	98	2	0	99	0	1	100	0	0		N.S.			-ve		Few	0	0
22/6/72	98	2	0	98	2	0	96	4	0		-ve			-ve			-ve	
5/7/72	96	3	1	99	1	0	97	3	0		-ve			-ve			-ve	
19/7/72	97	3	0		N.S.		95	4	1		-ve			-ve		Few	0	0
2/8/72	98	2	0	98	2	0	95	4	1		-ve			-ve		Few	0	0
15/8/72										Few	0	0	100	0	0	Few	0	0
30/8/72										Few	0	0	100	0	0	Few	0	0
14/9/72				MARES REMOVED						99	0	1	98	1	1	100	0	0
5/10/72										98	0	2	98	2	0	100	0	0
13/10/72				FROM PADDOCK						100	0	0	97	3	0	100	0	0
27/10/72										100	0	0	99	1	0	100	0	0
10/11/72										100	0	0	98	2	0	99	0	1
24/11/72										99	1	0	96	3	0	98	0	2
8/12/72										97	3	0	98	2	0	99	0	1
21/12/72										99	1	0	93	7	0	100	0	0
4/1/73										100	0	0		N.S.		98	1	1
18/1/73										99	1	0	97	3	0	98	2	0
2/2/73										100	0	0	99	1	0	100	0	0
16/2/73										100	0	0		-ve		Few	0	0
1/3/73										100	0	0	100	0	0	Few	0	0

Tr = Trichonema spp. Sv = S. vulgaris O = Others

APPENDIX 4

RED CELL INDICES - INITIAL AGE IMMUNITY EXPERIMENT

	2½ YEAR OLD							3 YEAR OLD						
Day No.	PCV %	RBC 10^6/cu.mm.	Hb g.%	WBC 10^3/cu.mm.	L %	E %	N %	PCV %	RBC 10^6/cu.mm.	Hb g.%	WBC 10^3/cu.mm.	L %	E %	N %
-5	35	7.54	14.4	11.5	80	0	20	31	7.20	10.4	11.7	69	2	29
0	34	6.38	13.8	10.0	67	1	32	28	6.76	11.0	11.1	79	0	21
6	38	8.20	15.0	13.0	68	2	30	41	9.50	17.2	15.1	69	1	30
9	33	9.12	13.2	19.2	72	1	27	43	5.40	16.8	13.7	53	0	47
13	40	7.84	14.4	15.3	50	3	47	43	7.62	15.6	15.2	47	10	43
16	34	9.60	13.7	11.8	51	4	45	44	10.02	15.0	13.7	71	5	24
20	36	8.10	14.7	12.2	41	10	49	40	9.14	15.3	12.5	61	8	31
26	33	6.09	12.2	8.7	59	10	31	39	7.70	15.0	12.8	68	4	28
30	29	6.74	11.3	10.0	66	3	31	41	8.76	15.9	10.8	57	9	34
34	34	8.18	13.5	10.7	62	12	26							
37	27	5.86	12.2	12.4	63	11	26							
41	34.5	6.70	13.0	19.9	64	8	28							
44	33	7.70	12.8	14.6	62	5	33							
47	29	5.54	11.9	10.9	67	7	26							
51	32.5	7.10	12.8	13.0	46	4	50							
55	30	6.38	11.6	12.1	55	2	43							
58	35	6.09	14.3	15.3	57	8	35							
62	33	5.78	12.8	12.5	54	6	38							

APPENDIX 5 - Table 1

MEAN RED CELL INDICES OF FOALS IN SERIAL INFECTION AND CHALLENGE EXPERIMENT

	SERIAL INFECTION			SERIAL INFECTION + CHALLENGE			CHALLENGE		
Day	PCV %	Hb gm.%	RBC 10^6/cu.mm.	PCV %	Hb gm.%	RBC 10^6/cu.mm.	PCV %	Hb gm.%	RBC 10^6/cu.mm.
0	35	13.9	6.19	33	13.0	6.00			
8	33	13.1	6.50	33	13.0	6.57			
14	32	12.4	6.51	29	12.1	6.26			
21	33	13.4	6.70	35	13.7	6.45			
28	33	12.4	7.41	33	13.3	7.10			
35	32	12.2	6.54	31	11.9	6.27			
42	33	12.3	6.49	31	11.1	6.76			
49	33	12.4	6.99	30	11.3	6.13			
56	34	13.7	6.93	30	12.5	6.34			
63	33	13.0	6.65	31	12.1	6.12	35	13.0	7.37
70	35	12.1	6.63	30	11.8	6.38	32	11.8	6.56
77	34	13.2	7.15	29	10.8	6.06	30	11.4	6.20
84	38	14.1	7.07	33	12.2	6.51	30	13.0	6.76
91	34	13.5	6.74	36	13.3	5.82	29	10.4	6.29
98	33	11.4	7.23	30	10.7	6.00	24	8.7	6.04
105	34	13.2	6.98						
112	35	13.6	6.81						
119	36	13.8	7.83						
126	36	13.5	7.72						
133	33	12.4	7.18						
140	35	13.1	6.76						
147	37	13.8	7.61						
154	31	10.4	6.76						
160	32	12.1	7.28						
167	34	12.3	7.10						

APPENDIX 5 - Table 2

MEAN WHITE CELL INDICES OF FOALS IN SERIAL INFECTION

AND CHALLENGE EXPERIMENT

	SERIAL INFECTION				SERIAL INFECTION + CHALLENGE				CHALLENGE			
Day	WBC 10^3/cu.mm.	L %	E %	N %	WBC 10^3/cu.mm.	L %	E %	N %	WBC 10^3/cu.mm.	L %	E %	N %
0	11.06	76	1.0	23	10.3	78	0.5	22				
8	10.9	N.S.	N.S.	N.S.	10.4	N.S.	N.S.	N.S.				
14	12.03	73	0	27	12.2	62	1.5	37				
21	13.1	67	1.6	31	12.6	65	0.5	35				
28	12.0	68	3.7	29	11.4	70	4.5	26				
35	10.0	61	6.0	33	10.5	62	10.0	29				
42	10.6	70	4.0	29	12.4	63	8.5	29				
49	12.3	67	4.3	29	10.8	52	2.5	46				
56	11.0	66	3.0	32	13.2	62	5.0	33				
63	11.3	57	2.5	41	11.0	68	3.0	29	11.0	80	1.0	20
70	13.3	69	2.0	30	13.1	59	2.5	39	12.9	62	2.0	37
77	12.6	70	3.0	27	13.6	68	5.5	27	12.5	58	2.5	40
84	10.7	68	1.5	31	12.5	64	3.5	33	15.2	60	1.5	39
91	13.6	69	2.5	29	16.9	61	4.0	35	15.3	56	4.5	40
98	13.2	63	2.0	36	13.4	56	8.5	36	16.5	57	4.0	39
105	21.4	57	2.5	41								
112	17.3	63	2.0	36								
119	15.6	56	3.5	41								
126	19.1	52	6.0	43								
133	19.7	62	5.0	33								
140	19.4	60	2.5	40								
147	15.2	62	1.5	37								
154	13.5	54	2.5	44								
160	17.2	58	2.0	41								
167	16.7	54	3.0	43								

STRONGYLUS VULGARIS INFECTION IN THE HORSE

Summary of a Thesis Submitted for the Degree
of Doctor of Philosophy of the University of Glasgow

by

James L. Duncan, B.V.M.S., M.R.C.V.S.

The work described in this thesis is concerned with studies on various aspects of infection with one of the most common and pathogenic of the equine helminths, Strongylus vulgaris.

In order to facilitate these experimental studies, an efficient technique was developed for the production of large numbers of S. vulgaris infective larvae in pure culture; in this, third stage S. vulgaris larvae were obtained by surgically transferring adult worms, collected from horses immediately after slaughter, directly into the caeca of worm-free yearling ponies.

Subsequently, various investigations on the life-cycle, pathogenesis, epidemiology and immunity of S. vulgaris infection were carried out.

Considerable controversy existed concerning the migratory route taken by developing parasitic larval stages of S. vulgaris in the tissues of the host. In order to elucidate this, worm-free pony foals were infected with a pure culture of infective larvae and killed at intervals over a period of 9 months. The results showed that infective larvae exsheathed and penetrated the intestine within a few days of infection. These larvae then moulted in the sub-mucosa, penetrated small arteries and had migrated within the lumina of the intestinal arteries to the anterior mesenteric site by 14 days after infection;

in this predilection site larvae developed from early to late fourth stage. After a period of 3 - 4 months the fourth moult was completed and the young adults returned to the intestine again via the lumina of the arteries. Nodules were formed with the subsequent release of young adults into the intestinal lumen. The pre-patent period was 6 - 7 months.

Studies on the pathogenesis of S. vulgaris infection showed that a distinct clinical syndrome occurred within 3 - 4 weeks of infection in foals given doses of 750 infective larvae. This syndrome was one of pyrexia, anorexia and colic associated with the presence of fourth stage larvae in the intestine and mesenteric arteries. Examination of tissues taken from foals killed over a period of 9 months demonstrated the sequence of pathological changes.

During the course of these studies infected foals showed a marked polymorphonuclear leucocytosis and an eosinophilia together with an increase in the serum globulin levels; the latter appeared to be due mainly to increases in the β-globulin levels. Subsequently using ^{125}I labelled albumin and ^{51}Cr labelled red blood cells it was shown that the rate of albumin catabolism was increased and the red cell survival time reduced in ponies with patent infections.

The epidemiology of equine helminthiasis was studied in 2 separate systems of management over 2 years.

Basically 2 regimens were employed: one where untreated mares and foals went out in the spring to pastures never previously grazed by horses while in the other, mares, regularly treated with anthelmintics, and their foals grazed contaminated pasture. These systems allowed 2

sources of infection for susceptible foals: where mares were treated, the main source of infection was from overwintered larvae; where infected mares went out to clean pasture infection of the foals could only occur from larvae which developed from eggs passed in the faeces of the mare.

The results demonstrated the following points.

First, foals going out to grass in the spring can be infected by both overwintered larvae and by eggs passed in the faeces of their dams but the latter is by far the more important source of infection.

Secondly, where horses remain untreated throughout the grazing season high levels of third stage strongyle larvae develop on pasture, whereas regular anthelmintic treatment at intervals of 2 - 4 weeks reduces pasture contamination to a minimum.

Thirdly, although high levels of infective larvae may occur on pasture grazed by infected animals during the summer and autumn, these levels fall during the winter and by May the following year virtually no third stage larvae can be recovered from these pastures.

The results of a series of experiments on the immunity to S. vulgaris infection provided the following information.

First, an age immunity was demonstrated when 3-year-old worm-free ponies were infected with S. vulgaris larvae. No clinical signs or gross arterial lesions developed in these animals and no fourth stage larvae were recovered at necropsy.

Secondly, a degree of acquired immunity in terms of resistance to reinfection was demonstrated in groups of naturally and experimentally infected animals.

Finally, the results of a preliminary vaccination experiment have shown that the arterial lesions are less severe and that there is an 80% reduction in larval numbers in vaccinated foals compared to challenge controls.