THE USE OF COMPARATIVE MORPHOLOGY OF THE INFECTIVE LARVAE, IN IDENTIFICATION AND DETERMINING THE INCIDENCE OF SOME COMMON NEMATODE PARASITES IN A HERD OF BEEF CATTLE

by

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INTRODUCTION

The present study deals with the morphological differentiation of infective larvae of some common nematode parasites of beef cattle. In addition, the morphological studies are applied to the following: (1) the diagnosis of parasitosis (2) the estimation of incidence of parasitosis, and (3) the influence of seasons on parasite burdens in range cattle.

The transmission of parasitic roundworm infections in cattle, with the exception of the genera Ascaris and Trichuris, takes place through the ingestion of third stage larvae. Roundworms in the genera Bunostomum and Strongyloides can also invade their host per cutaneously. It seemed desirable to conduct an investigation primarily to learn the microscopic characters of the larvae by which they could be specifically and easily distinguished in the laboratory and in the field.

It is exceedingly important to know the proper methods of diagnosis of every parasitic disease. Most of the parasites that look much alike may differ in their pathogenicity and habits, therefore, require different methods of control. The definitive diagnosis of parasitic diseases always has been based either on the identification of the parasite at post mortem examination, or the identification of the parasite's eggs in the feces of the diseased host. The latter method of diagnosis of helminthic infections is not reliable because of similarity in the size and the shape of roundworm eggs. Only the eggs of Ascaris vitulorum, Strongyloides papillosus, Nematodirus spp.,

Trichuris spp., and possibly Bunostomum spp. can be recognized with certainty in the fecal examination. The eggs of other nematodes of cattle can be placed in groups on the basis of their morphology i.e. the Haemonchus-Oesophagostomum-Ostertagia group, and the Cooperia-Trichostrongylus group.

Knowledge about the morphological characters of the third stage roundworm larvae would likewise be useful in estimating the incidence and fluctuations of worm burdens in beef cattle.

There are several factors that influence the increase or decrease in the worm populations and investigation on the frequent occurrence of out-breaks in different geographical vicinities with different climatic conditions. There is need of an intelligent approach to the problem. Dikmans (1948) emphasized that more specific and accurate information should be obtained at the state level on the geographic and seasonal distribution of cattle parasites, the frequency of this occurrence and economic importance. Ecological conditions within the state, such as rainfall and soil conditions greatly influence the development, behavior, distribution of free living stages and their survival on the pasture. Taylor (1935) suggested during his investigations on the parasitic gastritis in sheep that there may be marked differences in the larval concentration in different parts of the same field and as such there could be enormous differences between the suitabilities of various field conditions for the development of different larvae and probabilities for the ultimate appearance of the disease in the animals grazing there.

REVIEW OF LITERATURE

Not much information is available with regard to the species of nematode parasites present in range cattle in Kansas. The common nematode parasites of cattle, in general, have been reported by many workers from all parts of the world. Mayhew (1948) indicated 26 different species of nematodes having been recorded in the United States. Keith (1953) mentioned 26 species of roundworms recorded from the alimentary tract of cattle in Australia. The life histories of only a few of these nematode parasites have been worked out during the past 45 years. There has been considerable work done on the morphology of the infective larvae of sheep nematode parasites, whereas, very little work of this nature has been done with infective larvae of cattle nematode parasites.

Ransom (1906) worked out the life cycle of Haemonchus contortus and found that the free-living infective stage was ensheathed. He also found that infective larvae developed into adults in 2 to 3 weeks, after being ingested by sheep. Ransom (1911) described the presence of different species of nematode parasites in the alimentary tract of ruminants. Veglia (1915) worked out the life cycle of H. contortus.

Quite extensive work has been published on the life history of hookworms in man, dog, and ruminants. Cornadi and Barnette (1908) described the hookworm disease in cattle. Loss (1911) gave importance to the structure of the tail and the oesophagus for diagnosis of Ancylostoma duodenale. Further work was done

on the morphology of hookworm larvae by Cort (1925). Korke (1925) worked on A. duodenale and Necator americanus. Heydon (1927) described characteristic differences between the infective larvae of human hookworms. Svensson (1925), Hesse (1923), Cameron (1923) described the free-living stages of the Monodontus trigonocephalus, a hookworm of sheep. Cameron (1927) further described the infective larva of the sheep hookworm. Schwartz (1924) described the preparasitic stages of the cattle hookworm, Bunostomum phlebotomum. Ortlepp (1937) worked on the hookworm of sheep and goats. Mayhew (1939) and Sprent (1946) studied the life cycle of B. phlebotomum in detail and fully described the external and internal morphology of its infective larvae.

Goodey (1922), using the criterion of tail length, easily recognized the ensheathed larva of Trichostrongylus retortaeformis, a parasite of the rabbit. Monnig (1926) described the distinguishing characters of the infective larvae of T. instabilis and T. rugatus. Monnig (1930) studied the bionomics of the free-living stages of Trichostrongylus spp. Gordan (1933) differentiated the infective larvae of Ostertagia spp. and Trichostrongylus spp. of sheep.

Veglia (1924) worked out the life history of Oesophagostomum columbianum, the nodular worm of sheep and goats, and he described the structure of its infective larva. He also compared the morphology of the tails of the infective larvae of O. columbianum, Haemonchus contortus, Trichostrongylus extenuatus and Strongyloides papillosus. Goodey (1924) described the infective larva of Oesophagostomum dentatum, a parasite of the pig.

Andrews and Maldonado (1941) published work on the life history of O. radiatum, the common nodular worm of cattle. They recorded measurements and depicted by means of excellent diagrams differentiating characters of the infective larva.

Morgan (1928) described Ostertagia circumcincta and its larval stages. Threlkeld (1934) worked out the life cycle of O. circumcincta and described in detail the morphology of the infective larvae. The same author in (1946) published his work on the life cycle of another species O. ostertagia and described the morphology of its infective larva.

The life history of Nematodirus filicollis, a sheep nematode, was worked out by Boulenger (1915). He described the infective larva as the largest larva of all the nematode species found in sheep. This larva could easily be differentiated by its long filamentous tail. He included drawings and colored pictures of the larva.

From the foregoing review of the literature, it is evident that the life cycles of almost all the common nematode parasites of ruminants have been worked out. However, these species were mainly from sheep and goats with little information available in regard to the specific morphology of the infective larvae of cattle nematodes. Morgan (1930) published his observations on the third stage larvae of some common nematodes of sheep and goats. He placed much stress on the length and shape of the tail of the different larvae. He only described a few species. Monnig (1931 published a description of the sheep nematode

infective larvae. He described most of the common species with quite detailed information about the total lengths and measurements of the anterior and the posterior ends of the larvae. Dikmans and Andrews (1933) published a rather complete work on the morphology of the infective larvae of the most common nematode parasites of sheep.

While this thesis was being prepared, a publication by Keith (1953) appeared in the literature on the differentiation of the infective larvae of some common nematode parasites of cattle in Australia. His descriptions of morphology of larvae of cattle parasites conformed closely to the descriptions recorded for larvae of sheep parasites. Slight variations in the morphology of the larvae from sheep and cattle probably can be attributed to strain differences of the larvae.

An objective of the present work was to use the comparative morphology of the infective larvae obtained from fecal cultures in the differential diagnosis and estimation of the incidence of worm burdens in beef cattle. Not much information was available regarding the use of morphology of infective larvae in diagnostic work and in general survey work on nematode parasites of sheep and cattle. Taylor (1935) worked only on trichostrongylid fluctuations in sheep. Cushnie and White (1948) estimated the seasonal variations of worm burdens in sheep by egg counts. Rogers (1940) studied the effects of environmental conditions on the accessibility of third stage trichostrongyle larvae to grazing animals. Monnig (1930) recorded his observations on the bionomics of the free-living stages of Trichostrongylus spp.

and other parasitic nematodes. Crofton (1948) studied the ecology of immature phases of trichostrongyle nematodes. He further studied the effect of climate factors on the availability of the infective larvae of T. retartaeformis to the host. In 1949 he recorded his further observations on the effects of ecology on immature phases of trichostrongyle nematodes. Tetley (1949) studied in general the rhythms in nematode parasitism of sheep. Sprent (1946) recorded his observations on the bionomics of Bunostomum phlebotomum, a hookworm of cattle. Kates (1950) studied the survival on pasture, of free-living stages of some common gasto-intestinal nematodes of sheep on the basis of numbers of eggs recovered from the feces of animals which were fed on the pasture infested with infective larvae. He recorded the degree and percentage of infection recovered from these experimental animals. Morgan and Parnell (1950) and Morgan et al (1951) did similar work on Scottish sheep and recorded the seasonal variations in worm egg out-put. Spedding (1953) studied the day to day variation in the nematode egg counts of sheep.

All of the before-mentioned workers based their findings on incidence, fluctuations, and seasonal variations of the worm populations on the total and differential egg counts after feeding experimental animals infective larvae from pure cultures. Keith (1953) mentioned that the quantitative and qualitative data collected by Roberts and O'Sullivan (1951 unpublished) was based upon the identification and differential counts of larvae obtained from fecal cultures.

MATERIALS AND METHODS

The mature female worms which served as the source of eggs from which larvae were obtained, were collected from beef cattle slaughtered at a packing plant, near Manhattan, Kansas. Some worms were obtained from cattle examined in the post-mortem room of the Kansas State Veterinary College. Almost every genera of roundworms was collected except Trichostrongylus and Strongyloides. The female nematodes were washed several times in 0.7 percent salt solution to remove the debris and mucous adhering to them. The worms were allowed to remain in the salt solution in a Petri dish at room temperature or in an incubator for six to eight hours. During this time the female worms deposited their eggs in the Petri dish. This incubation of female worms in saline was necessary because it was learned that if the female worms were macerated immediately after they were collected, the eggs removed were either not fertilized or for some unknown reason did not develop into larvae. By leaving the worms in the salt solution, the eggs recovered were mostly fertile and in late segmentation stages. Subsequent to incubation the female worms were cut into several pieces with the aid of a fine scissors and the eggs remaining in the uterus of the female worms were recovered. The suspension of the eggs and the macerated worms was mixed with a culture medium.

The culture medium was prepared as follows: Ten grams of fresh cow dung were mixed thoroughly with 10 ml of distilled water in a beaker and boiled for 10 minutes. The contents of

the beaker were constantly mixed during the boiling operation. Boiling killed all of the pre-existing worm eggs and rendered the medium completely sterile for pure cultures. After the medium had cooled sufficiently, the egg suspension was thoroughly mixed with the medium with the aid of a spatula. The material was uniformly smeared on 3-inch square pieces of ordinary surgical gauze which were put into clean wide-mouth screw cap bottles (Plate I, Fig. 1). The culture bottles were stored for 10 days in a cabinet whose atmosphere was saturated with water. The temperature of the cabinet was maintained at 25°-28°C. For the first 48-hours, the bottles were not covered with lids. This insured a sufficient supply of oxygen for the developing larvae. The cultures were examined frequently during this period of incubation and a few drops of water were added if the cultures were found to get too dry. After 48-hours, the bottles were covered with lids and allowed to incubate for eight days. Each time a control culture bottle was also made to test the sterility of the medium in which no eggs were added.

After the 10-day incubation period, the cultures were removed from the bottles and put into Baermann funnels filled with warm water (Temp. 40-45°C). Plate I, Fig. 2 shows the general arrangement of these funnels. The warm water in the funnels stimulated the larvae so that they migrated quickly from the solid medium. The larvae settled to the bottom of the tubes in 4 to 6 hours, but the cultures were generally Baermannized overnight to ensure complete recovery of the larvae. The water from the funnels was poured off and the supernatent water in the

centrifuge tubes was removed by means of a pipette. The remaining sediment was examined for larvae.

For the study of the morphology of the larvae, fresh specimens were generally used. A drop of water containing larvae was placed on a clean slide along with a drop of saline solution. Then the slide was heated gently in order to straighten and kill the larvae. Care was taken not to apply too much heat to the slide since over heating of the slide would result in the destruction and the contraction of the larvae within their sheath. A cover glass was placed on the material and then the slide was examined. The morphological details could be seen very clearly, but sometimes a drop of weak solution of iodine was added in order to stain the internal structures. For the purpose of easy differential diagnosis in the laboratory and in the field the following measurements were utilized: (1) total length of the larva (the distance from the mouth to the tip of the tail sheath) (2) the length and shape of the tail sheath (3) length and shape of the oesophagus and (4) the shape and number of the intestinal cells. The recorded measurements were based upon a study of about 100 larvae in each genus or species obtained from pure as well as from mixed cultures.

The morphology of the infective larvae of Trichostrongylus spp., Strongyloides papillosus and Nematodirus spp. was studied from the mixed cultures, as it was not possible to collect the adult female worms from the slaughtered cattle.

When it was desired to preserve the larvae, they were heated gently on a slide until they were killed in an extended condition.

They were then transferred to hot 70 per cent alcohol and stored.

The camera lucida drawings of the larvae were made from both fresh and stained specimens. No significant shrinkage of the larvae occurred in the process of staining. The drawings of the whole larvae were made with low power except the drawings of the anterior and posterior ends of the larvae which were made with the high power objective and a 10 X ocular. All the drawings were made at approximately the same enlargement.

Photographs were taken of whole specimens of infective larvae magnified 100 times. The anterior and posterior ends of these larvae, both from fresh and stained specimens, were photographed at a magnification of 450 times.

The larvae were stained with aceto-carmine. Threlkeld (1934) used picro-carmine and Delafield hematoxylin and found it most satisfactory. Aceto-carmine was found to be very useful for this purpose. The advantage in using aceto-carmine was that the larva could be transferred directly from 70 per cent alcohol to aceto-carmine stain diluted 1:10 and then stained progressively. The larvae were left in the stain for about one hour. No destaining was required except the larvae were washed once or twice in 70 per cent alcohol. After the larvae were dehydrated in 85 per cent and 95 per cent alcohol for 20 minutes in each, they were cleared in pure glycerine for 2 to 3 days at which time all of the alcohol had evaporated. A drop of glycerine with stained larvae was placed on a clean ringed slide and sealed.

After the study of the comparative morphology of the infective larvae in the pure cultures was completed, it was then possible to estimate the incidence of the nematode infection on species level in a herd of beef cattle. The calves selected for this study, grazed on pastures 30 miles from Manhattan. These calves were born in the spring of 1952. Another group of calves born in the spring of 1953 was also studied. The object of studying this group was to see the effect of age factors along with the ecological factors in the incidence and the fluctuations in the worm burdens during different seasons. Twenty fresh fecal samples were collected weekly. The fecal samples were stored in the refrigerator until larvae counts could be made.

The following technique was used for culturing fecal samples collected from the calves. Three grams of feces were weighed and smeared on a 3-inch square piece of wet surgical gauze. Gauze saturated with water provided an adequate supply of moisture required for the hatching of the larvae. The pieces of smeared gauze were then put into wide-mouth bottles of 8 ounce capacity and kept in the humidity control cabinet for 10 days in a saturated atmosphere at 25-28°C. The bottles were not covered with lids for the first 48-hours in order to provide enough oxygen for developing larva. During this period care was taken to add a few drops of water twice a day to prevent any possible drying of the fecal cultures. The lids were put on the bottles after 48-hours and allowed to stay for another 8 days. At the end of this period the cultures were removed from the

bottles and put in a modified Baermann apparatus (Plate 1, Fig. 2). The funnels were filled with water (40-45°C). Immediately the larvae began to migrate from the cultures, and within 4 to 5 hours the larvae started settling to the bottom of the centrifuge tubes attached to the Baermann apparatus. The cultures were left in the funnels overnight which was sufficient time to recover almost all of the larvae. All but 0.3 ml of water was removed from the tubes with the aid of a pipette. The water remaining in the tubes contained the larvae. Next 0.1 ml of the thoroughly mixed sediment was placed on a slide. The slide was gently heated over an alcohol lamp in order to straighten out and kill the larvae. A large cover glass was then placed over the material. Total and differential larval counts were made. The total number of larvae in 0.1 ml corresponded to the total number of larvae in one gram of feces. The differential counts were recorded with the aid of a haematological denominator. At times, free-living soil nematode larvae as well as free-living protozoan were observed along with the larvae of parasitic nematodes. The presence of these free-living forms did not interfere with the routine counts. The soil nematodes could be easily recognized and distinguished by their size and morphology.

RESULTS

Description of Infective Larvae

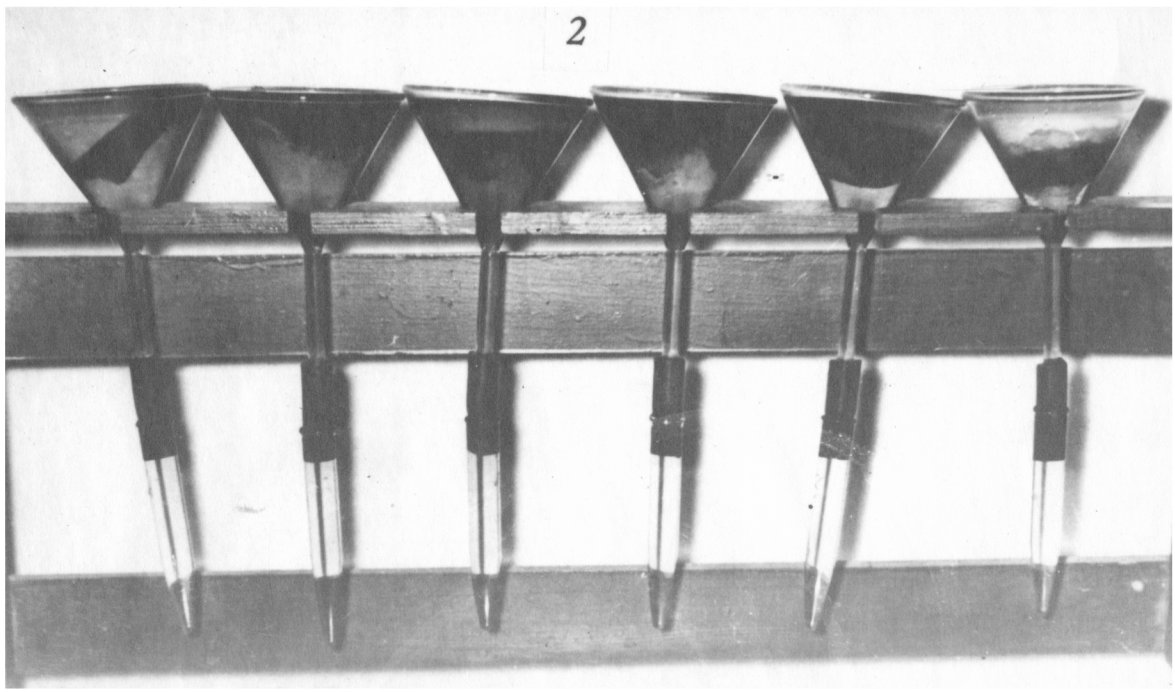
Bunostomum phlebotomum (Railliet, 1900). The infective

EXPLANATION OF PLATE I

Principle equipment for culturing and isolating the common infective nematode larvae of beef cattle.

1. Culture bottles
2. Baermann funnels

PLATE I



larvae of this species is much smaller than all other species of larvae reported in this study. It is easily recognized by its small size, filamentous whip-like tail sheath, and characteristic club-shaped oesophagus (Plate II, Fig. 1; Plate IV, Fig. 1).

The total length of the infective larvae varies from 0.46 to 0.62 mm long. The total length of a larva denotes the distance from the tip of the anterior end to the tip of the tail sheath. The intestine is composed of 16 cells which are more or less triangular in shape. The buccal capsule is small and funnel shaped as reported by Dikmans and Andrews (1933). The oesophagus is 0.13 to 0.18 mm long. The distance from the anterior end of the larva to the genital primordium measures 0.21 to 0.30 mm (Tables 1, 9; Plates VI, VII, VIII, IX, Fig. 1).

The following definition is used for the tail and of the tail sheath of larvae of B. phlebotomum and all other nematode larvae in this study. The tail of a nematode larva is that portion of its body lying between its anus and the posterior tip of its body, sheath excluded. The tail sheath is that portion of the sheath lying between the posterior tip of the body of the larva and the posterior tip of the sheath. The tail of B. phlebotomum larva measures from 0.04 to 0.06 mm long. The tail sheath is 0.06 to 0.10 mm long.

Trichostrongylus axei (Cobbold, 1879). The infective larva of this species is slightly larger than the Bunostomum phlebotomum larva. This species is easily identified by its medium size and the characteristic and conically shaped tail sheath which

Table 1. Comparative measurements of infective larvae of Bunostomum phlebotomum and B. trigonocephalum.

Host	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
*Sheep	-	-	-	0.09-0.1	Morgan (1930)
*Sheep	0.56-0.637	0.153-0.165	0.063	0.082	Monnig (1931)
*Sheep	0.514-0.678	0.14-0.175	0.055-0.068	0.098-0.115	Dikmans and Andrews (1933)
**Cattle	0.5-0.54	0.125-0.145	-	-	Schwartz (1924)
**Cattle	0.45-0.58	-	-	0.06-0.09	Sprent (1946)
**Cattle	0.453-0.633	-	-	-	Krug and Mayhew (1946)
**Cattle	0.5-0.583	-	-	0.059-0.083	Keith (1953)
**Cattle	0.46-0.62	0.13-0.18	0.04-0.06	0.06-0.096	Shivnani

* B. trigonocephalum
** B. phlebotomum

Table 2. Comparative measurements of infective larvae of *Trichostrongylus spp.*

Host	Species	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
Sheep	*T. axei* *T. instabilis* *T. rugatus*	0.656-0.772	0.151-0.181	0.063-0.071	0.034-0.039	Monnig (1931)
Sheep	*T. instabilis*	0.674-0.749	0.15-0.18	0.058-0.07	0.025-0.038	Dikmans and Andrews (1933)
Sheep	*T. vitrinus*	0.622-0.796	0.145-0.18	0.05-0.08	0.021-0.04	" "
Sheep	*T. colubriformis*	0.56-0.784	-	-	-	Gordan (1933)
Cattle	*T. axei*	0.619-0.762	-	-	0.025-0.039	Keith (1953)
Cattle	*T. axei*	0.6-0.74	0.15-0.18	0.04-0.07	0.025-0.04	Shivnani

ends in a more or less sharp point (Plate II, Fig. 2; Plate IV, Fig. 2).

The total length of the larva is 0.6 to 0.74 mm. The intestine consists of 16 cells. The oesophagus is 0.15 to 0.18 mm long. The distance from the anterior end of the larva to the genital primordium is 0.3 to 0.42 mm (Table 2, 9).

The tail of the larva is 0.04 to 0.07 mm long and has two tubercle-like projections at its posterior end as described by Dikman and Andrews (1933). The tail sheath is typically short measuring 0.025 to 0.040 mm in length (Plates VI, VII, VIII, IX, Fig. 2).

Haemonchus contortus (Rudolphi, 1803). The infective larva of this species is also of medium size measuring in total length from 0.68 to 0.78 mm (Plate II, Fig. 3; Plate IV, Fig. 3). The intestinal tract consists of 16 cells and the cells are rectangular in shape. The length of the oesophagus is from 0.13 to 0.16 mm. There is a typical globular space present in the buccal cavity as reported by Dikman and Andrews (1933). The distance between the anterior end of the larva and genital primordium is 0.32 to 0.40 mm (Tables 3, 9).

The tail of the larva is 0.04 to 0.06 mm and is pointed. The tail sheath is fairly long and filamentous and is sharply pointed. It shows a characteristic kink just behind the tail of the larva. The kink was observed and described by Monnig (1931), Dikman and Andrews (1933), and Keith (1953). This characteristic is of great diagnostic value for this species. The tail sheath measures 0.05 to 0.08 mm long (Plates VI, VII, VIII, IX,

Fig. 3).

Cooperia punctata (Linstow, 1907). The larva of this species is fairly large in size (Plate II, Fig. 4; Plate IV, Fig. 4). Its globular buccal cavity resembles the buccal cavity of the Haemonchus contortus larva. Unlike the latter larva, C. punctata larva possesses two oval structures at the anterior end of its oesophagus (Plates VI, VII, VIII, IX, Fig. 4). These oval structures are distinctly characteristic of the genus Cooperia. It is rather difficult to differentiate the species of Cooperia from one another. C. onchophora can be distinguished from other species in the genus by the size of its tail. This species has a tail sheath of medium size and is sharply pointed.

The total length of larva of C. punctata varies from 0.76 to 0.86 mm. The oesophagus is from 0.14 to 0.17 mm long. The intestine has 16 cells, which may be either triangular or rectangular in shape. The genital primordium is located 0.37 to 0.44 mm from the anterior end of the larva (Tables 4, 9).

Oesophagostomum radiatum (Rudolphi, 1803). The infective larva of this species is easily recognized by its medium size and a characteristic long, fine, filamentous tail (Plate III, Fig. 5; Plate V, Fig. 1). The body is comparatively much broader than any of the larvae studied. This larva usually has transverse ridges on the sheath but this character is not of much value.

The total length of the larva is from 0.75 to 0.86 mm, almost the same size as Cooperia punctata (Tables 5, 9).

Table 3. Comparative measurements of infective larvae of Haemonchus contortus.

Host	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
Sheep	-	-	0.06	0.07-0.084	Morgan (1930)
Sheep	0.694-0.772	0.127-0.145	0.063-0.071	0.082	Monnig (1931)
Sheep	0.65-0.751	0.122-0.15	0.054-0.068	0.065-0.078	Dikmans and Andrews (1933)
Cattle	0.749-0.85	-	-	0.087-0.119	Keith (1953)
Cattle	0.68-0.78	0.13-0.16	0.04-0.06	0.05-0.08	Shivnani

Table 4. Comparative measurements of infective larvae of *Cooperia* spp.

Host	Species	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
Sheep	*C. fuelleborni* *C. serrata* *C. antidorca*	0.772-0.83	0.101- 0.165-	0.071	0.055-0.071	Monnig (1930)
Sheep	*C. curticei*	0.711-0.85	0.13-0.162	0.058-0.071	0.039-0.052	Dikmans and Andrews (1933)
Cattle	*C. punctata*	0.666-0.866	-	-	0.047-0.071	Keith (1953)
Cattle	*C. punctata*	0.76-0.86	0.14-0.17	0.05-0.075	0.04-0.065	Shivnani

The intestine is made up of 16 cells, typically triangular in shape. The oesophagus measures from 0.13 to 0.16 mm long. Anterior to the oesophageal region, there is generally noticed a triangular area with the base directed anteriorly as described by Dikmans and Andrews (1933). The distance between the anterior end of the larva and its genital primordium is 0.3 to 0.4 mm.

The tail of the larva is 0.05 to 0.075 mm long. It usually tapers bluntly but sometimes is slightly round. The tail sheath projects 0.13 to 0.18 mm from the posterior end of the larva (Plates VI, VII, VIII, IX, Fig. 5).

Ostertagia ostertagia (Stiles, 1892). The infective larva of this species is one of the largest larvae studied. The tail sheath is of medium size and is bluntly pointed. Like the Haemonchus larva, this larva also has a kink in the tail sheath (Plate III, Fig. 6; Plate V, Fig. 2).

The total length of this larva is from 0.85 to 0.92 mm. The intestine is made up of 16 cells which are triangular in shape. The length of the oesophagus is from 0.14 to 0.18 mm. The genital primordium is situated from 0.37 to 0.43 mm from the anterior end of the larva (Tables 6, 9).

The tail of the larva measures from 0.055 to 0.075 mm. The sheath of the tail extends from the end of the larva proper, from 0.045 to 0.07 mm. Sometimes the sheath tapers to a sharp point and sometimes to a blunt point (Plates VI, VII, VIII, IX, Fig. 6).

Table 5. Comparative measurements of the infective larvae of *Oesophagostomum radiatum*.

Host	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
*Sheep	0.791-0.849	0.157-0.172	0.074		Monnig (1931)
*Sheep	0.771-0.923	0.15-0.17	0.06-0.08	0.125-0.16	Dikmans and Andrews (1933)
**Cattle	-	-	-	0.177	Andrews and Maldonado (1941)
**Cattle	0.726-0.857	-	-	0.134-0.182	Keith (1953)
**Cattle	0.75-0.86	0.13-0.16	0.05-0.075	0.13-0.18	Shivnani

* *O. columbianum*
** *O. radiatum*

Cooperia onchophora (Railliet, 1898). The infective larva of this species is second to the larva of Nematodirus spp. in total length. The larva of the latter species is the largest of all the species of larvae studied. The larva is characterized by having two oval pear-shaped bodies in the buccal cavity immediately anterior to the oesophagus as described by Dikmans et al (1933) (Plate III, Fig. 7; Plate V, Fig. 3).

The total length varies from 0.82 to 0.95 mm. The oesophagus measures from 0.15 to 0.18 mm in length. The genital primordium is situated 0.39 to 0.48 mm from the anterior end of the larva (Tables 7, 9).

The tail of the larva is broadly rounded at the end and measures from 0.06 to 0.08 mm long. The tail sheath projects behind the tail of the larva proper from 0.065 to 0.1 mm and is sharply pointed (Plates VI, VII, VIII, IX, Fig. 7).

Nematodirus spp. This species of nematode parasite is of rare occurrence in cattle in Kansas. The percentage of infective larvae in the mixed fecal cultures of calves was found to be very low. The infective larva of this species is the largest so far described and is easily identified by its large size and the long filamentous whip-like tail.

The total length of this larva varies from 0.98 to 1.150 mm, including the tail sheath. The sheath of the tail extends beyond the tail of the larva proper from 0.21 to 0.27 mm (Table 9).

Strongyloides papillosus (Wedl, 1856). This species of parasite is usually encountered in young calves. The infective

Table 6. Comparative measurements of infective larvae of Ostertagia spp.

Host	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
*Sheep	0.83	-	0.065	0.04-0.045	Morgan (1930)
*Sheep	0.888-0.907	0.172-0.188	0.078	0.039	Monnig (1931)
*Sheep	0.656-0.880	-	-	-	Gordan (1933)
*Sheep	0.797-0.866	0.15-0.190	0.062-0.080	0.03-0.040	Dikmans and Andrews (1933)
**Sheep	0.825-0.91	0.15-0.18	0.05-0.070	0.04-0.060	" "
***Cattle	0.85-0.90	-	-	-	Threlkeld (1946)
***Cattle	0.784-0.928	-	-	0.055-0.075	Keith (1953)
***Cattle	0.85-0.920	0.14-0.180	0.055-0.750	0.045-0.070	Shivnani

* O. circumcinta
** O. mentulata
*** O. ostertagia

Table 7. Comparative measurements of the infective larvae of Cooperia onchophora.

Host	Total length (mm)	Length of oesophagus (mm)	Length of larval tail (mm)	Extension of tail sheath (mm)	Authors
Sheep	0.804-0.924	0.152-0.18	0.06-0.07	0.062-0.082	Dikmans and Andrews (1933)
Cattle	0.809-0.976	-	-	0.079-0.111	Keith (1953)
Cattle	0.82-0.95	0.15-0.18	0.06-0.08	0.65-0.1	Shivnani

larva of this species was recovered only in the mixed fecal cultures of the young calves and not in the yearling calves (Plate III, Fig. 8; Plate V, Fig. 4).

The larva is easily recognized by its slender body, absence of sheath and the long oesophagus. The oesophagus covers more than one-third of the entire length of the larva. The tail of the larva is slightly notched at the top (Plates VI, VII, VIII, IX, Fig. 8).

The total length of the larva is from 0.52 to 0.67 mm. The length of oesophagus varies from 0.2 to 0.25 mm (Tables 8, 9).

Seasonal Incidence of Nematode Parasites in Young Beef Cattle

On the basis of the comparative morphology of infective nematode larvae, the following nematode parasites were identified in fecal cultures from yearling calves (born in spring 1952) and young calves (born in spring 1953): (1) Haemonchus contortus (2) Bunostomum phlebotomum (3) Nematodirus spp. (4) Trichostrongylus axei (5) Cooperia punctata (6) Ostertagia ostertagia (7) Oesophagostomum radiatum (8) Cooperia onchophora (9) Strongyloides papillosus.

From the data it was observed that the degree of infection in the herd of beef cattle was very low as compared to the degree of infection recorded by other workers in Australia, Texas, and other parts of the world where severe out-breaks of gastro-enteritis due to parasites are known to occur frequently.

Table 8. Comparative measurements of infective larvae of Strongyloides papillosus.

Host	Total length (mm)	Length of oesophagus (mm)	Authors
Sheep	0.574-0.71	-	Schwartz and Alicata (1930)
Sheep	0.598-0.675	0.227-0.259	Monnig (1931)
Cattle	0.524-0.678	-	Keith (1953)
Cattle	0.52-0.67	0.2-0.25	Shivnani

Table 9. Summary of measurements and other characters of infective larvae of some common nematodes of beef cattle in Kansas.

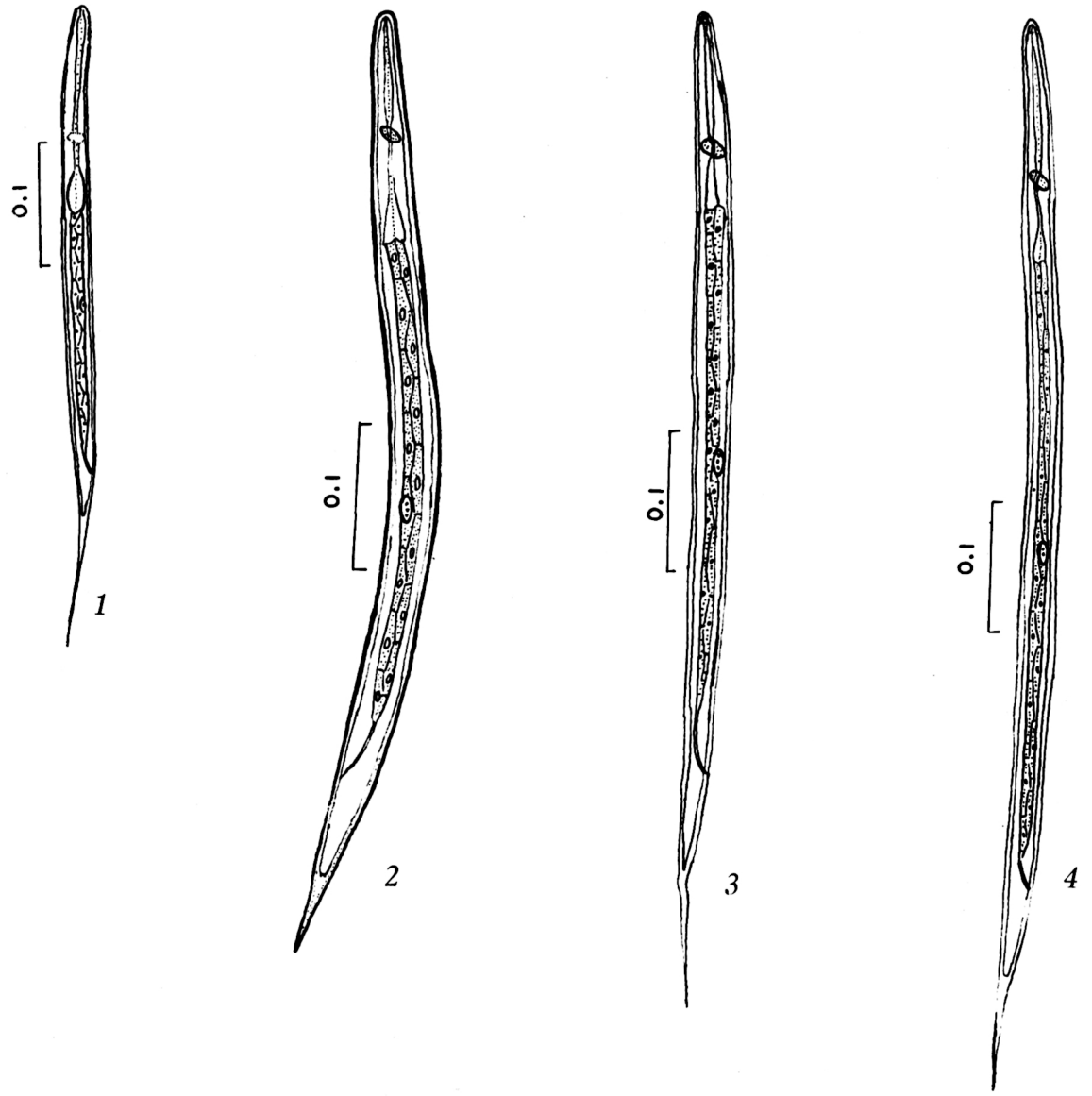
Species	Total length (mm)	Length of oesophagus (mm)	Length from anterior end to genital primordium	Length of larval tail (mm)	Extension of tail sheath (mm)	Salient characteristics
Bunostomum phlebotomum	0.46-0.62	0.13-0.18	0.21-0.3	0.04-0.06	0.06-0.096	Small size; filamentous tail, bulb shape oesophagus and buccal cavity funnel shape.
Trichostrongylus axei	0.6-0.74	0.15-0.18	0.3-0.42	0.04-0.07	0.025-0.04	Tail short, conical in shape.
Haemonchus contortus	0.68-0.78	0.13-0.16	0.32-0.4	0.04-0.06	0.05-0.08	Buccal cavity globular, definite kink in the tail sheath posterior to the tail of the larva proper.
Cooperia punctata	0.76-0.86	0.14-0.17	0.37-0.44	0.05-0.075	0.04-0.065	Posterior end of the larva proper round. Tail sheath medium size and sharply pointed, two oval shape bodies in the buccal cavity.
Oesophagostomum radiatum	0.75-0.86	0.13-0.16	0.3-0.4	0.05-0.075	0.13-0.18	Long filamentous tail, intestinal cells 16-24, triangular in shape.
Ostertagia ostertagia	0.85-0.92	0.14-0.18	0.37-0.43	0.055-0.075	0.45-0.07	Buccal cavity tubular, kink in the tail sheath, tail of sheath bluntly pointed.
Cooperia onchophora	0.82-0.95	0.15-0.18	0.39-0.48	0.06-0.08	0.065-0.1	Tail sharply pointed, two pear shaped bodies in the buccal cavity, larval tail bluntly pointed.
Nematodirus spp.	0.98-1.15	-	-	-	0.21-0.27	Long filamentous tail sheath, largest in total body length including the tail sheath. Tail of the larva proper fork shape.
Strongyloides papillosus	0.52-0.67	0.2-0.25	-	-	-	Oesophagus as long as one-third of the body length. Tail end slightly notched.

EXPLANATION OF PLATE II

Infective larvae of common nematode parasites of beef cattle. Camera lucida drawings with the scale in millimeters.

1. Bunostomom phlebotomum
2. Trichostrongylus axei
3. Haemonchus contortus
4. Cooperia punctata

PLATE II

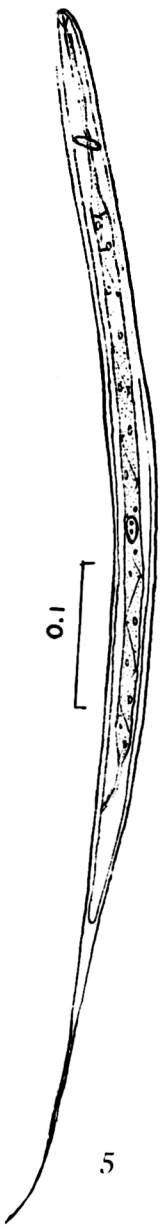


EXPLANATION OF PLATE III

Infective larvae of common nematode parasites of beef cattle. Camera lucida drawings with the scale in millimeters.

5. Oesophagostomum radiatum
6. Ostertagia ostertagia
7. Cooperia onchophora
8. Strongyloides papillosus

PLATE III



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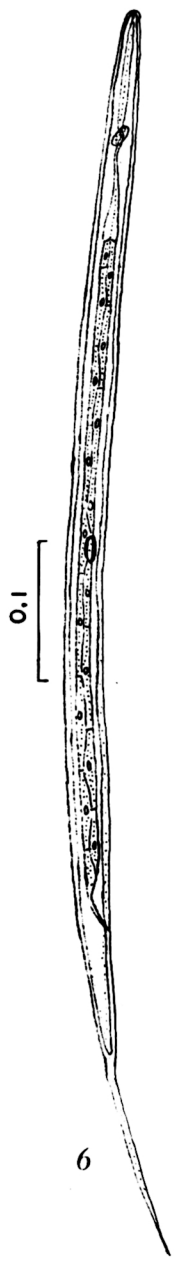

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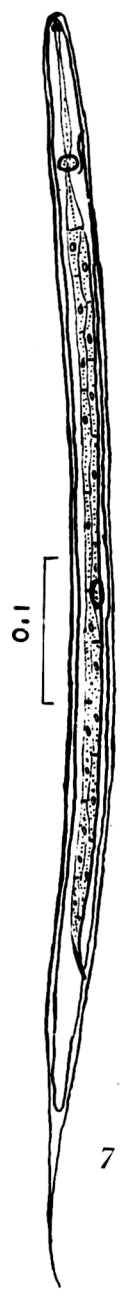

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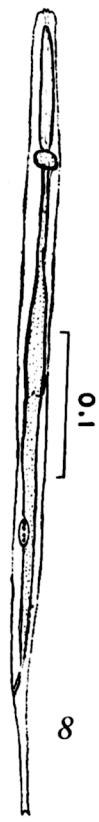


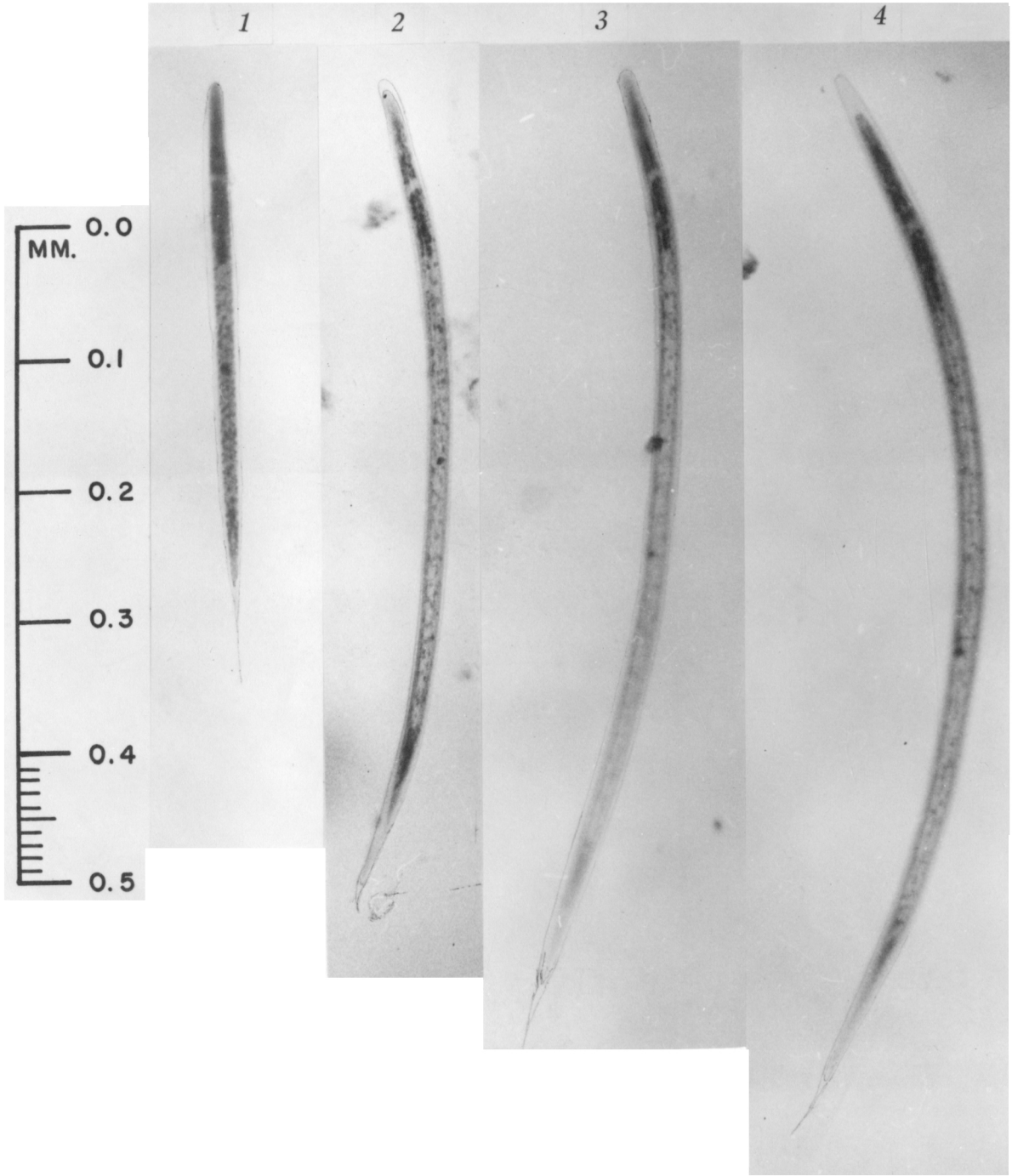
8

EXPLANATION OF PLATE IV

Photographs of infective larvae of common nematode parasites of beef cattle.

1. *Bunostomum phlebotomum*
2. *Trichostrongylus axei*
3. *Haemonchus contortus*
4. *Cooperia punctata*

PLATE IV



EXPLANATION OF PLATE V

Photographs of infective larvae of common nematode parasites of beef cattle.

1. Oesophagostomum radiatum
2. Ostertagia ostertagia
3. Cooperia onchophora
4. Strongyloides papillosus

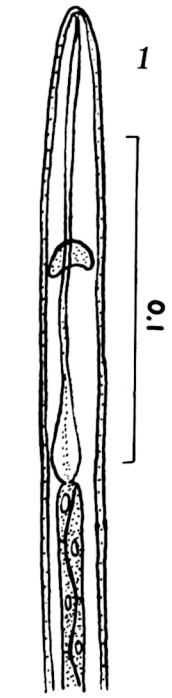
PLATE V

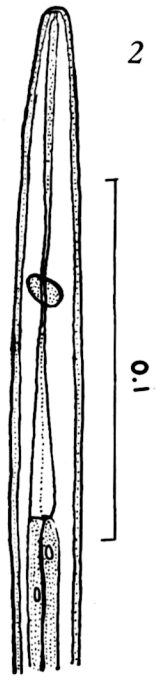
EXPLANATION OF PLATE VI

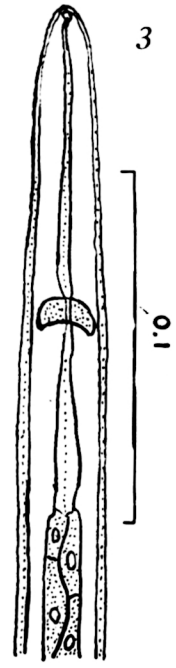
Anterior ends of common infective nematode larvae of beef cattle. Camera lucida drawings with the scale in millimeters.

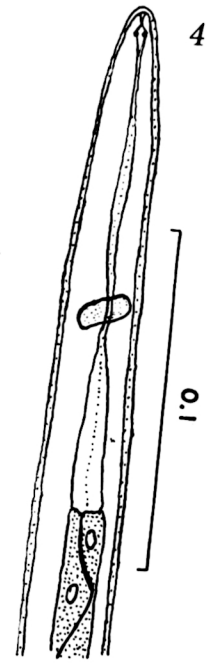
1. Bunostomum phlebotomum
2. Trichostrongylus axei
3. Haemonchus contortus
4. Cooperia punctata
5. Oesophagostomum radiatum
6. Ostertagia ostertagia
7. Cooperia onchophora
8. Strongyloides papillosus

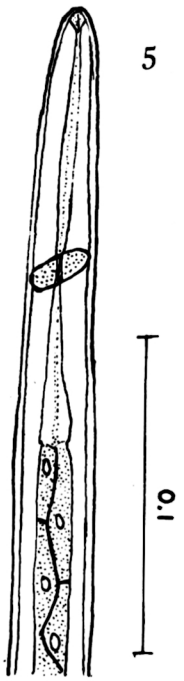
PLATE VI

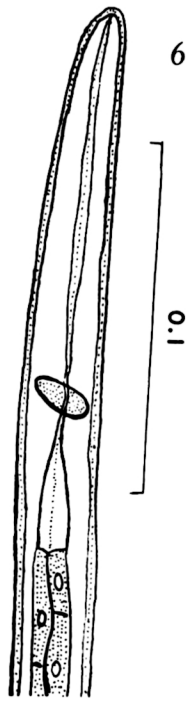


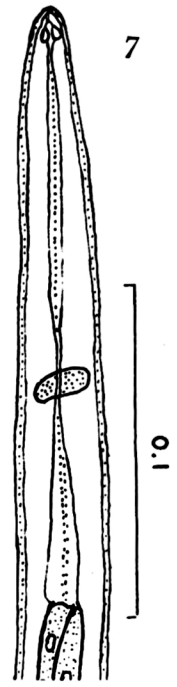


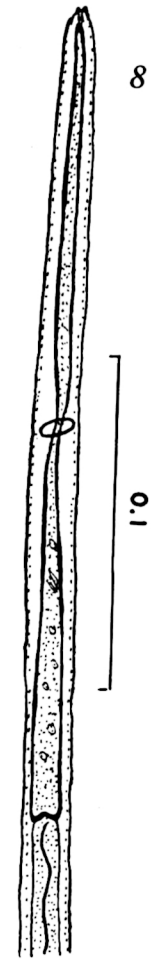












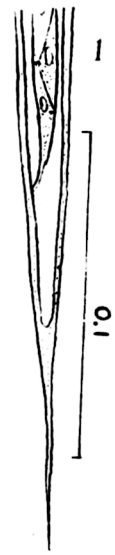
EXPLANATION OF PLATE VII

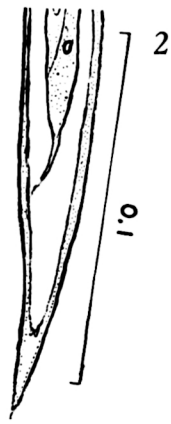
Posterior ends of common infective larvae of beef cattle.

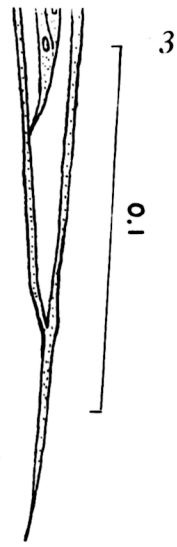
Camera lucida drawings with the scale in millimeters.

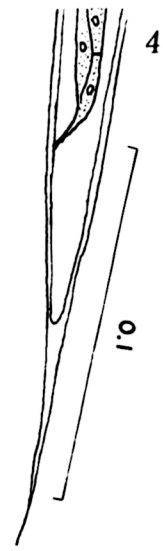
1. Bunostomum phlebotomum
2. Trichostrongylus axei
3. Haemonchus contortus
4. Cooperia punctata
5. Oesophagostomum radiatum
6. Ostertagia ostertagia
7. Cooperia onchophora
8. Strongyloides papillosus

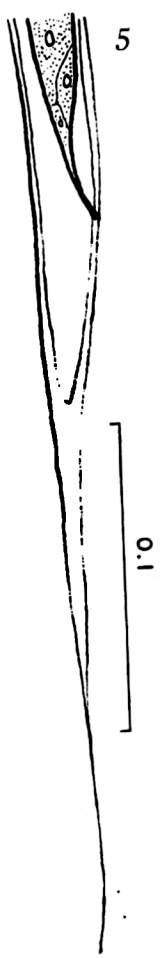
PLATE VII

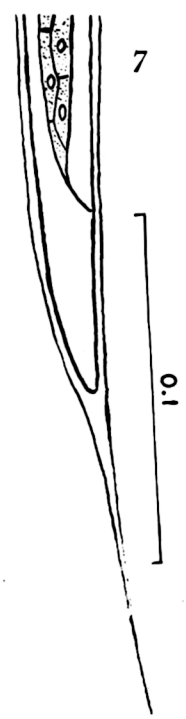


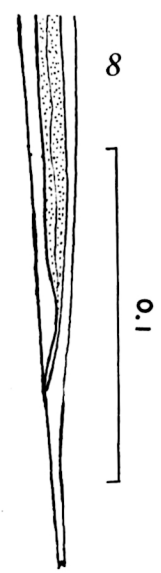










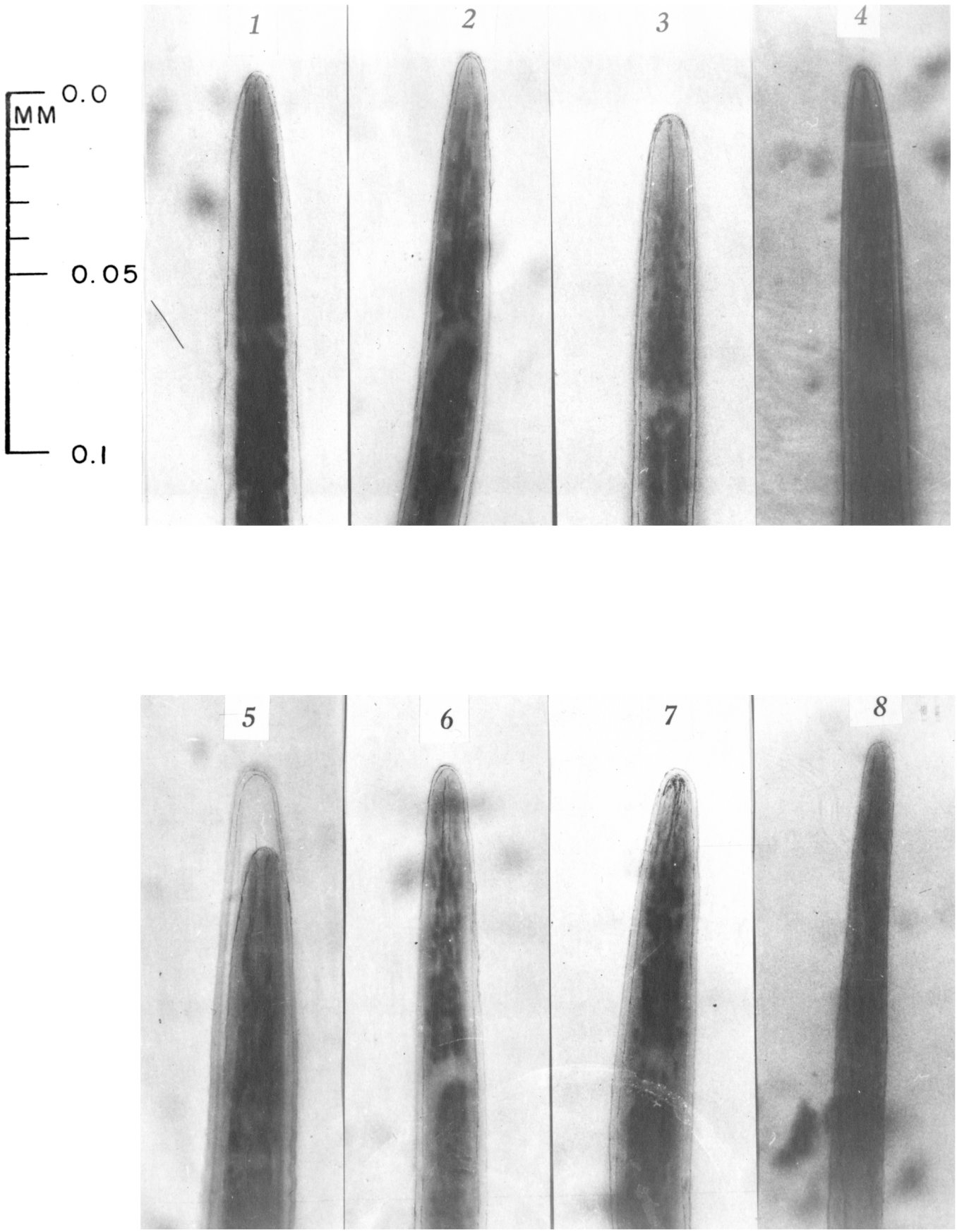


EXPLANATION OF PLATE VIII

Photographs of anterior ends of common infective nematode larvae of beef cattle.

1. *Bunostomum phlebotomum*
2. *Trichostrongylus axei*
3. *Haemonchus contortus*
4. *Cooperia punctata*
5. *Oesophagostomum radiatum*
6. *Ostertagia ostertagia*
7. *Cooperia onchophora*
8. *Strongyloides papillosus*

PLATE VIII

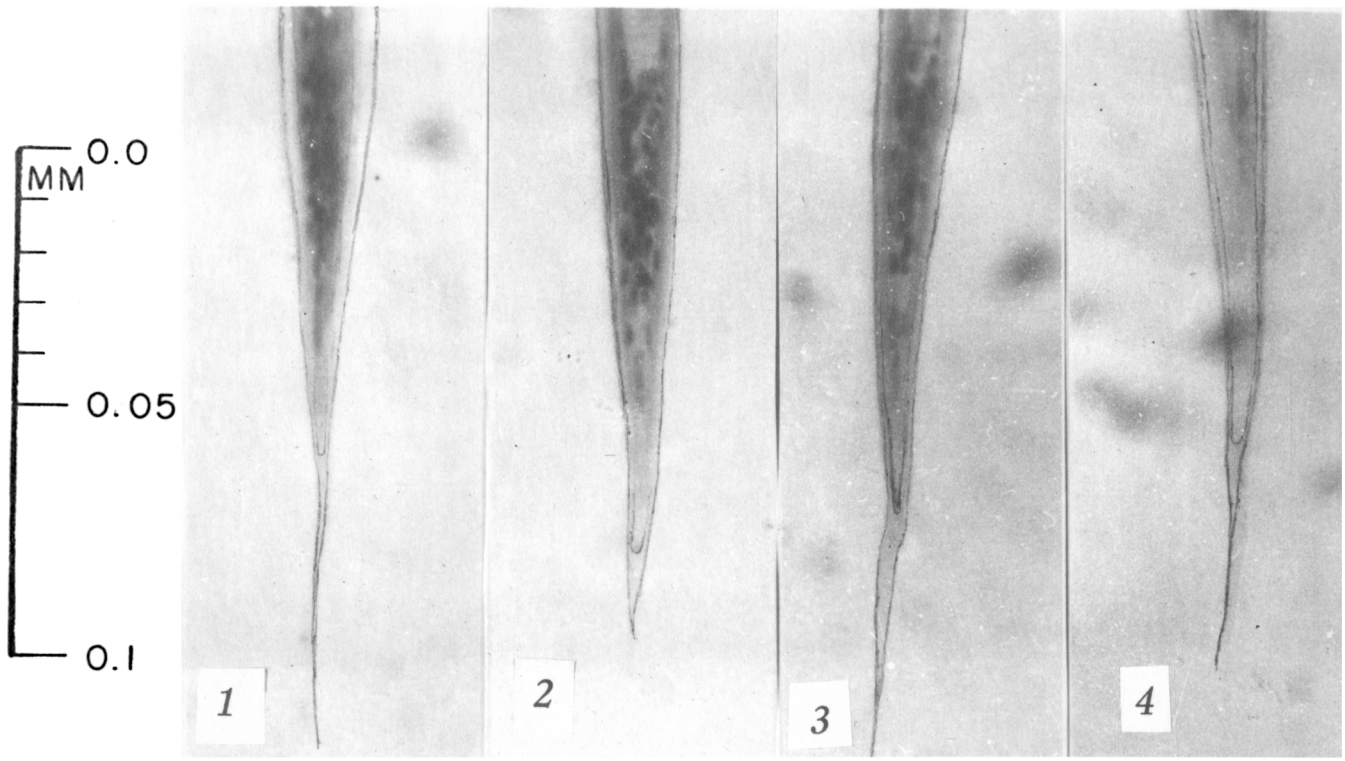


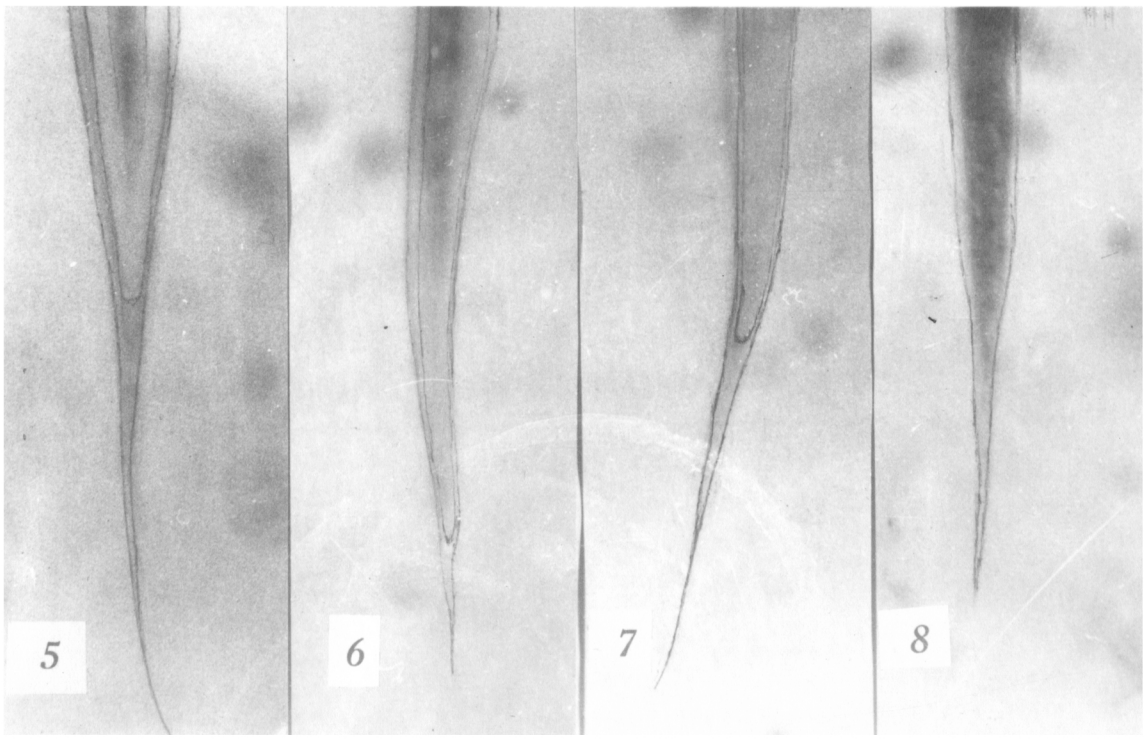
EXPLANATION OF PLATE IX

Photographs of the posterior ends of common infective nematode larvae of beef cattle.

1. *Bunostomum phlebotomum*
2. *Trichostrongylus axei*
3. *Haemonchus contortus*
4. *Cooperia punctata*
5. *Oesophagostomum radiatum*
6. *Ostertagia ostertagia*
7. *Cooperia onchophora*
8. *Strongyloides papillosus*

PLATE IX





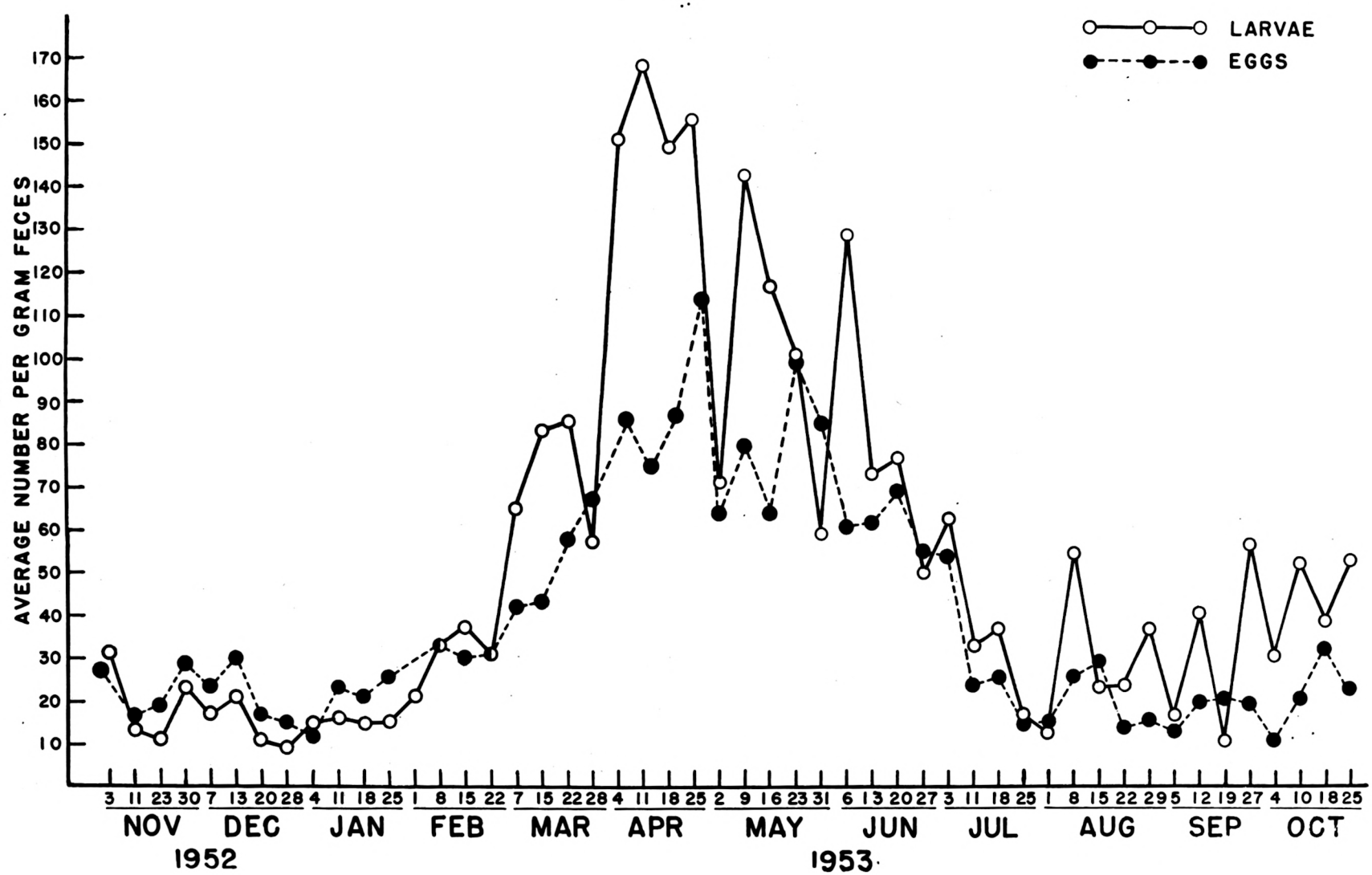
The total larvae counts beginning November, 1952 to the second week of February, 1953 were constantly low i.e. between 10-30 larvae per gram (Plate X). From the first week of March, 1953, the larvae counts began to show a general rise until a peak was reached in the second week of April, 1953. During the month of April, the total counts showed a maximum rise i.e. varying from 150 to 170 larvae per gram. There was a fairly high level of larvae counts during the month of May, 1953, except during the first week of May, when the counts suddenly fell. This abrupt drop in counts was attributed to the moving of the calves to new summer pasture. There was a noticeable gradual fall in the larvae counts beginning in early June and remaining very low during the rest of the summer.

The total larvae counts from the weekly fecal cultures of the yearling calves are compared with total egg counts of the same fecal samples for the corresponding period (Plate X). The data on egg counts were furnished by L. W. Dewhirst, Department of Zoology, Kansas State College. The same fluctuations were noticed in the egg out-put as were evident in the larvae counts. Beginning in February, 1953, the larvae counts were somewhat higher than the egg counts, which probably could be attributed to the varying concentration of the eggs in different portions of the feces. However, the general trend of fluctuations in the numbers of larvae and in the numbers of eggs was similar and gave a clear cut indication of the reliability of the larvae counts employed in the present study. The number of larvae recovered from the fecal cultures depends upon the number of

EXPLANATION OF PLATE X

Comparison of weekly average larvae and egg counts in yearling calves (born in spring, 1952).

PLATE X


LARVAE
EGGS
AVERAGE NUMBER PER GRAM FECES
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
3 11 23 30 7 13 20 28 4 11 18 25 1 8 15 22 7 15 22 28 4 11 18 25 2 9 16 23 31 6 13 20 27 3 11 18 25 1 8 15 22 29 5 12 19 27 4 10 18 25
NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT
1952
1953

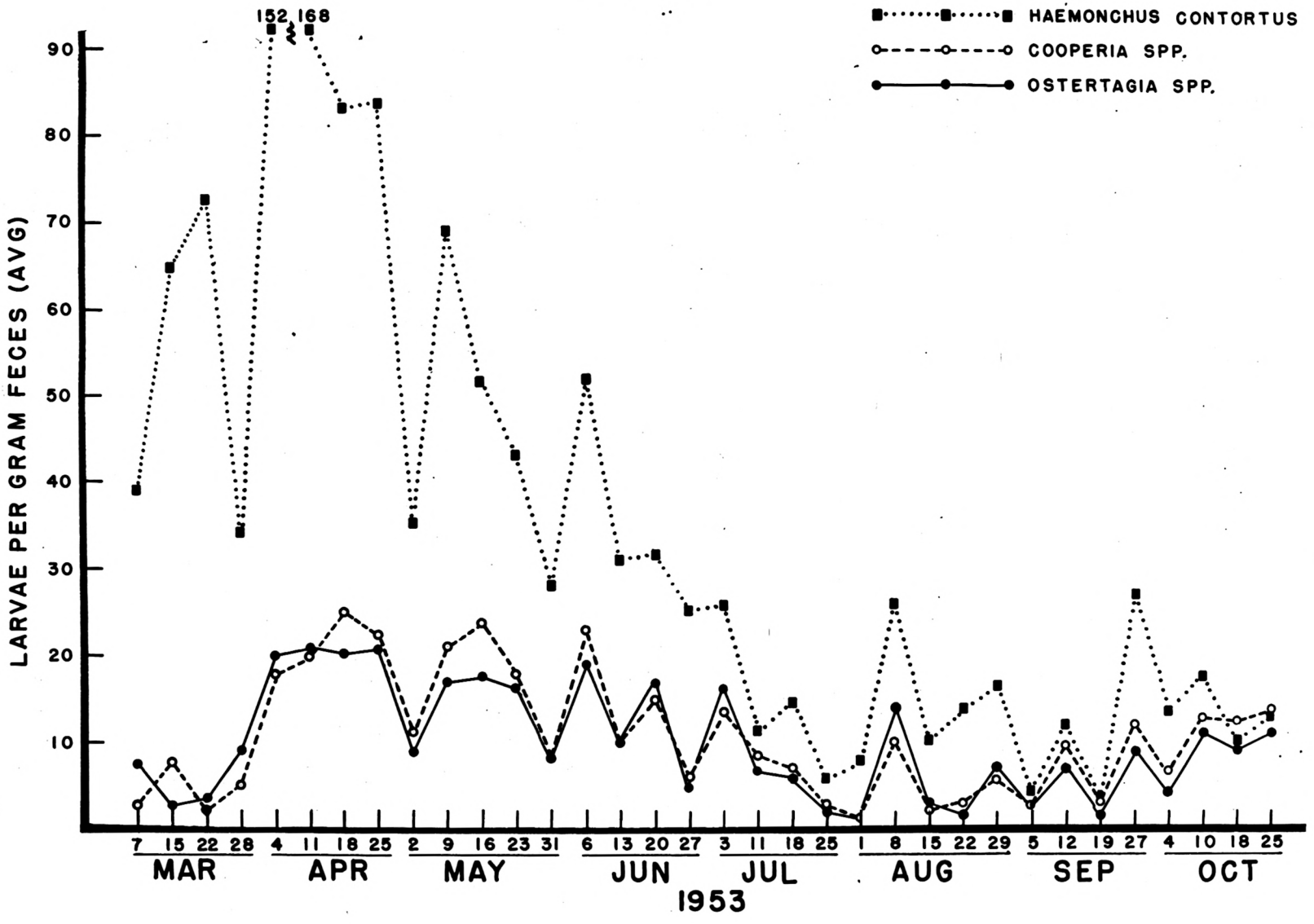
eggs present in the feces and their fertility as well as on the egg producing capacity of the female worms present in the digestive tracts of the cattle.

The pattern of the differential larvae counts of the yearling calves varied with the species of worms (Plate X). Haemonchus contortus predominated throughout the year and reached a high peak in April. Haemonchus larvae began to decrease considerably during the summer i.e. in early June and showed minimum counts in September, 1953. Kates (1950) showed by a series of experiments that Haemonchus larvae were not resistant to extreme weather conditions. Ostertagia ostertagia and Cooperia spp. were the next most abundant and were more capable of surviving in dry and warm weather than were Haemonchus. The present study showed that Cooperia and Ostertagia were highly resistant to varying climatic conditions. Kates (1950) showed that the Ostertagia was most resistant to the effects of weather. There was a low per centage of Oesophagostomum radiatum in early spring followed by a gradual rise in numbers until they reached their highest peak in June (Plate XII). The worms most abundant in April and May were H. contortus, Ostertagia ostertagia, and Cooperia spp. Kates (1950) observed that the larval stages of Oesophagostomum radiatum had low resistance, but the present study showed that this species was fairly consistent throughout the year with a high peak in summer. There was no significant rise or fall in the genera Bunostomum, Nematodirus, or Trichostrongylus although they were comparatively available in larger numbers during the spring. All three

EXPLANATION OF PLATE XI

Weekly average differential larvae counts in yearling calves (born in spring, 1952).

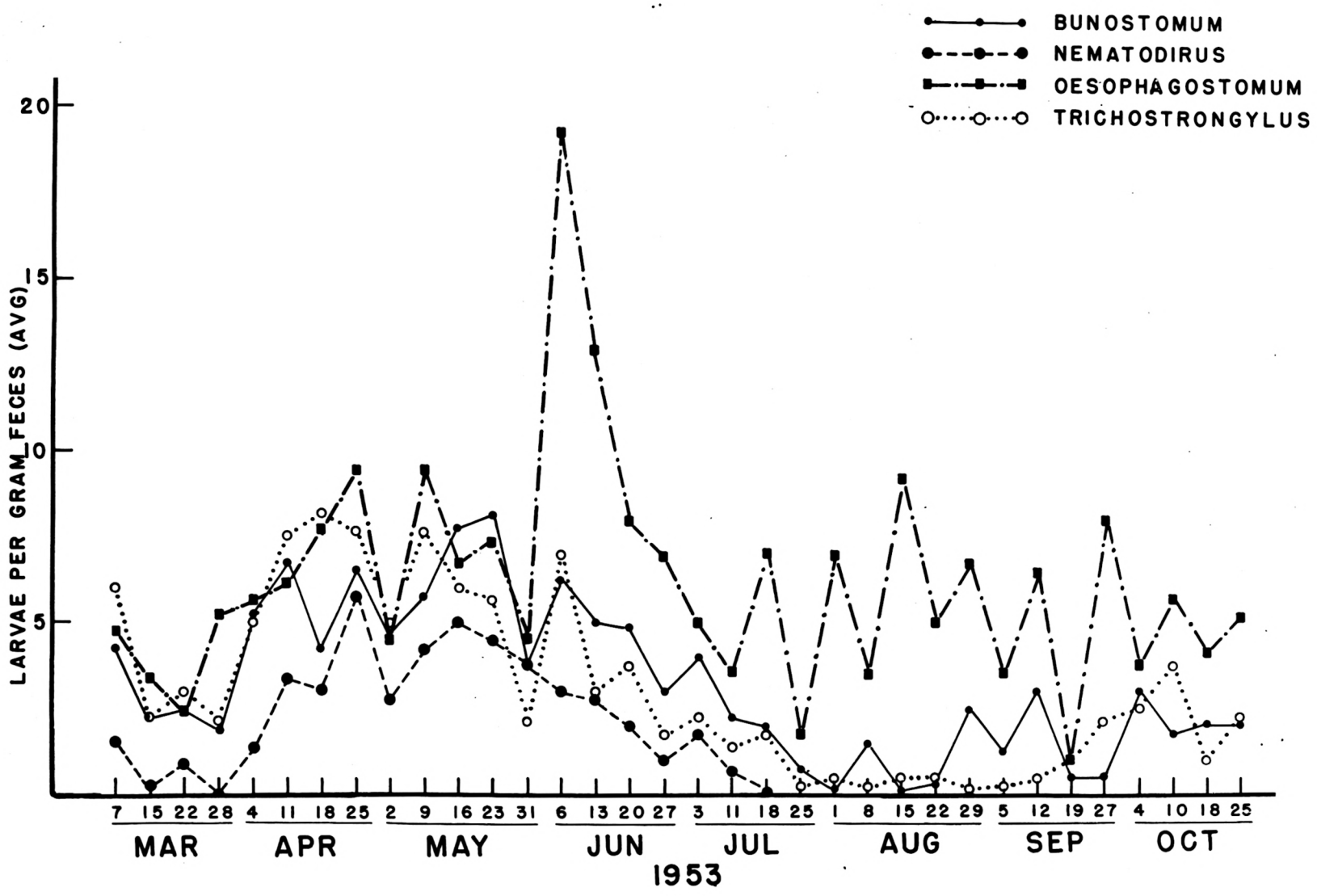
PLATE XI


152, 168
HAEMONCHUS CONTORTUS
COOPERIA SPP.
OSTERTAGIA SPP.
LARVAE PER GRAM FECES (AVG)
90
80
70
60
50
40
30
20
10
7 15 22 28
4 11 18 25
2 9 16 23 31
6 13 20 27
3 11 18 25
1 8 15 22 29
5 12 19 27
4 10 18 25
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
1953

EXPLANATION OF PLATE XII

Weekly average differential counts in yearling calves (born in spring, 1952).

PLATE XII


BUNOSTOMUM
NEMATODIRUS
OESOPHAGOSTOMUM
TRICHOSTRONGYLUS
LARVAE PER GRAM FECES (AVG)
20
15
10
5
7 15 22 28
4 11 18 25
2 9 16 23 31
6 13 20 27
3 11 18 25
1 8 15 22 29
5 12 19 27
4 10 18 25
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
1953

species showed a very low level in the summer months and seemed to be the least resistant. Nematodirus spp. were seldom recovered and were never recovered from the fecal cultures after the third week of July, 1953 (Plate XII). The low incidence of Nematodirus was possibly due to very small numbers of larvae available on the pasture. This species was found to have a very low rate of egg production and very low rate of egg hatchability as observed in studies using pure cultures.

From the data collected thus far, it was evident that infective larvae of the genera Haemonchus, Cooperia, Ostertagia, and Oesophagostomum were available on the pasture in greater numbers than the larvae of the other genera. Plates XIII and XIV illustrate the comparison of differential counts of larvae and the total egg counts on the basis of larger groups such as the Haemonchus-nodular group and the Cooperia-Ostertagia-Trichostrongylus group. The larvae counts of the Cooperia-Trichostrongylus group are similar to the egg counts of the same group reported by Dewhirst (1953). But the counts of the Haemonchus-nodular group were slightly different and varied from the egg counts of this group during the spring. The larvae counts were much higher than the egg counts of the Haemonchus-nodular group.

The total larvae counts in feces from new born calves showed a slightly different picture from the total counts in feces from the yearling calves (Plate XV). There was a gradual rise in May, 1953, which was mainly due to the presence of Strongyloides papillosus. In the third week of June, there was an

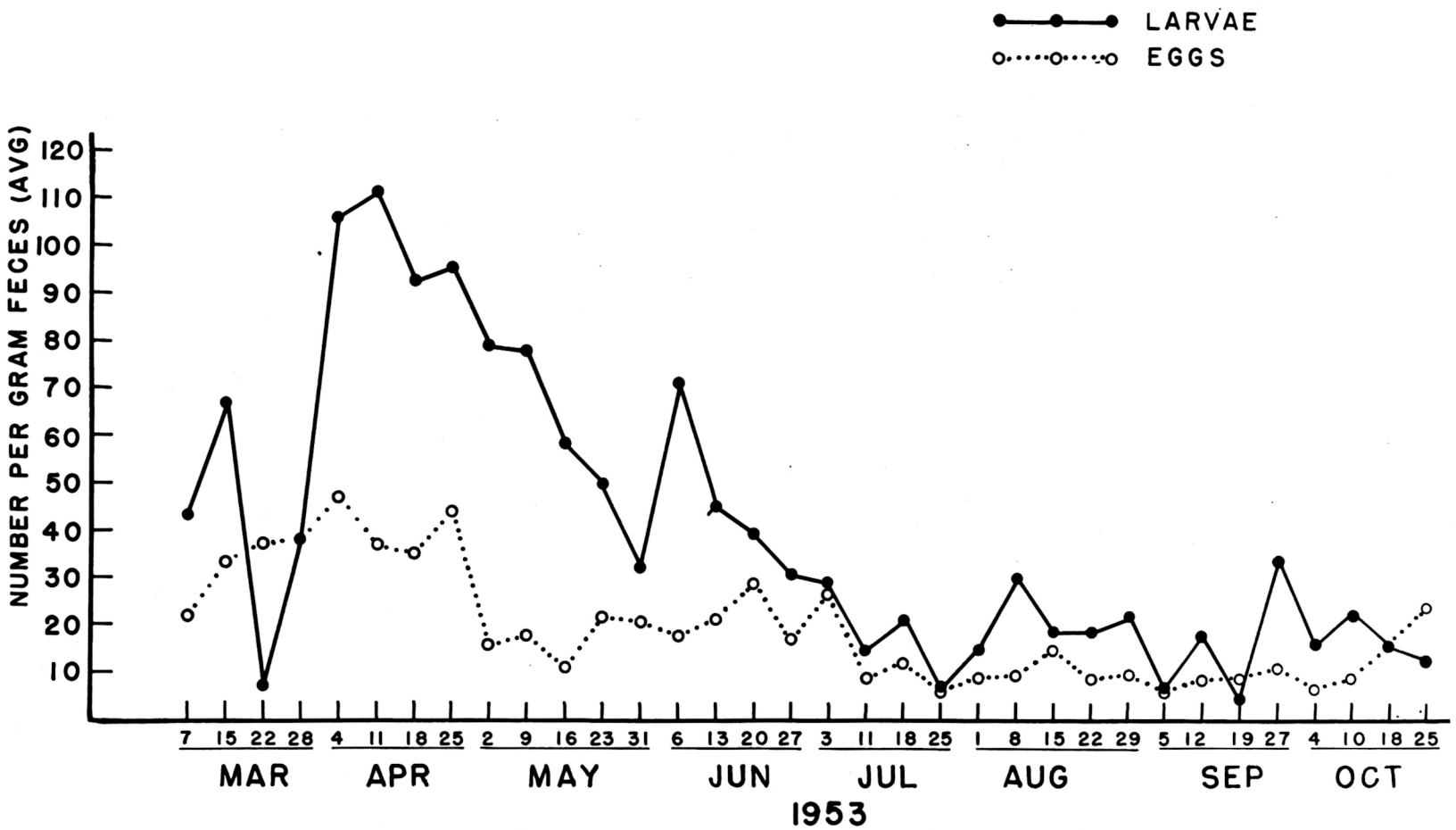
increase in the counts due to a greater in-take of larvae, but again there was a decline in the level of infection during July and August accompanying warm and dry weather. The larvae counts then increased from mid-September and showed their highest peak in October. October was the weaning time of the calves and probably afforded better opportunities for the calves to ingest greater numbers of infective larvae as a result of grazing on the pasture. This high rise was due to the genera of Haemonchus, Ostertagia, Cooperia, and partially to Oesophagostomum. Haemonchus showed a considerable decrease in summer in the yearling calves, whereas, in the young calves, Haemonchus maintained a fairly high level (Plate XVI). It seemed that the young calves being more susceptible offered better opportunities for these species to get established. As a result, the rate of growth and the egg laying capacity of the worms was enhanced. The data obtained from the differential larvae counts revealed that the worm burden for the first four to six weeks was practically nil except for Strongyloides which is a common parasite of very young calves. Infections with Strongyloides were usually first to appear, but the results showed that the infection gradually declined and terminated when the calves were about 18 weeks old (Plate XVII). Haemonchus contortus began to appear in the second week of June and reached its highest peak in October, 1953. Oesophagostomum radiatum maintained a low level until the second week of September and reached a peak in October, 1953. In general all other worm larvae began to appear in fecal cultures when calves were five to six weeks old; Haemonchus

EXPLANATION OF PLATE XIII

Comparison of average weekly larvae and egg counts of Haemonchus-nodular group in yearling calves (born in spring, 1952).

PLATE XIII

HAEMONCHUS—NODULAR GROUP

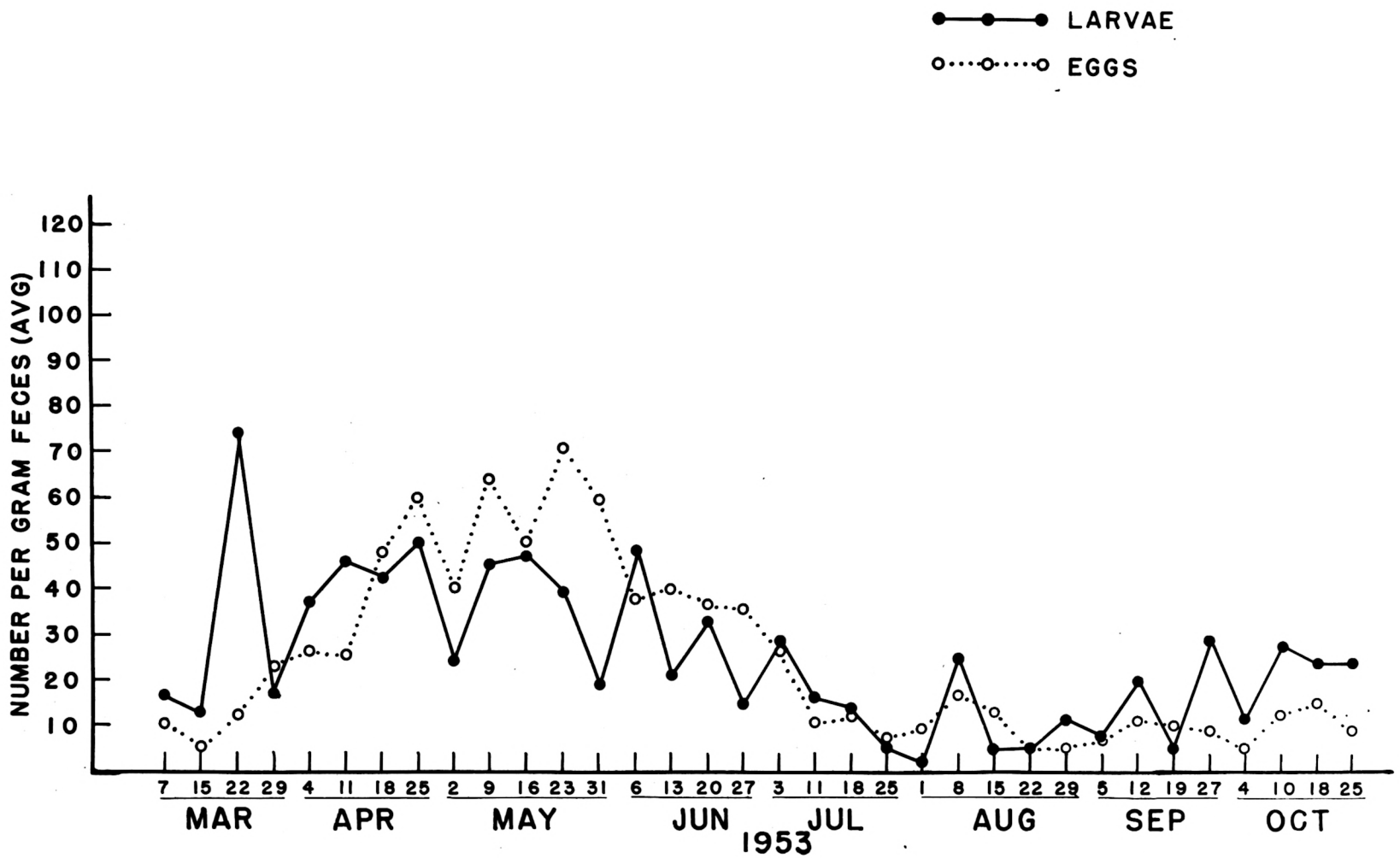


EXPLANATION OF PLATE XIV

Comparison of larvae and egg counts of Cooperia-Trichostrongylus group in yearling calves (born in spring, 1952).

PLATE XIV

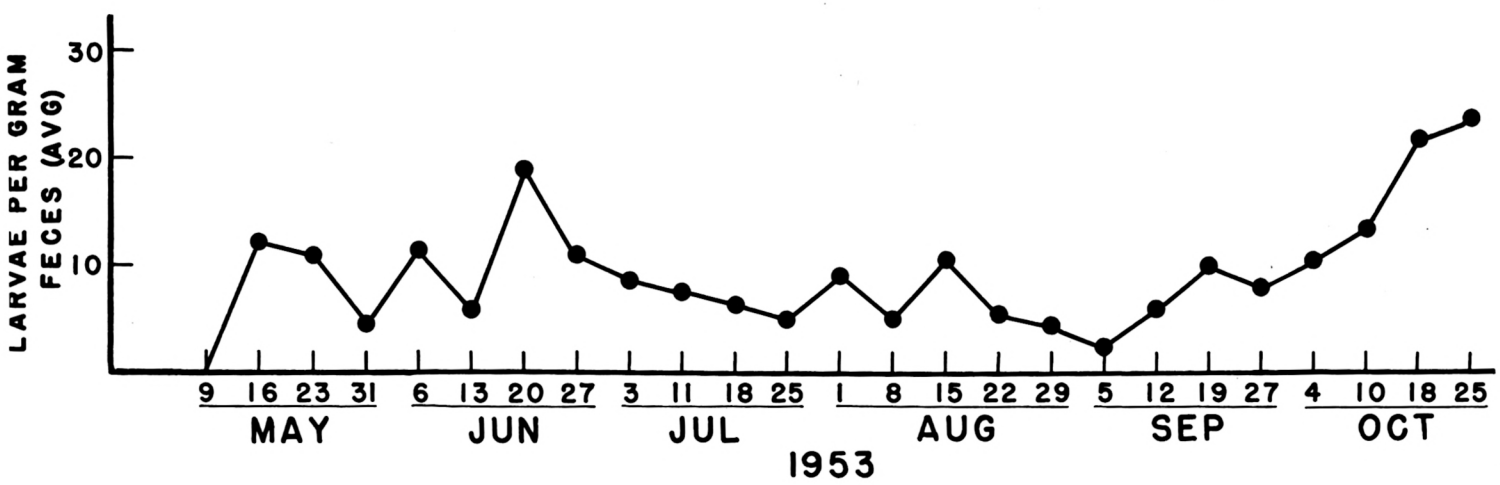
COOPERIA—TRICHOSTRONGYLUS GROUP



EXPLANATION OF PLATE XV

Weekly average larvae counts in young calves (born in spring, 1953).

PLATE XV


LARVAE PER GRAM FECES (AVG)
30
20
10
9 16 23 31
MAY
6 13 20 27
JUN
3 11 18 25
JUL
1 8 15 22 29
AUG
5 12 19 27
SEP
4 10 18 25
OCT
1953

being first to appear followed by Ostertagia and other species. This different picture of worm burdens in the young calves from that of the yearlings suggested that the age factors played an important part in parasitic infections in calves, along with the effects of climatic factors. The infection with Trichostrongylus and Bunostomum was consistently very low, Nematodirus being entirely absent in the young calves (Plate XVII). Bunostomum and Trichostrongylus reach a peak in June and then decline throughout the remainder of the year.

DISCUSSION

Morphology of Infective Larvae

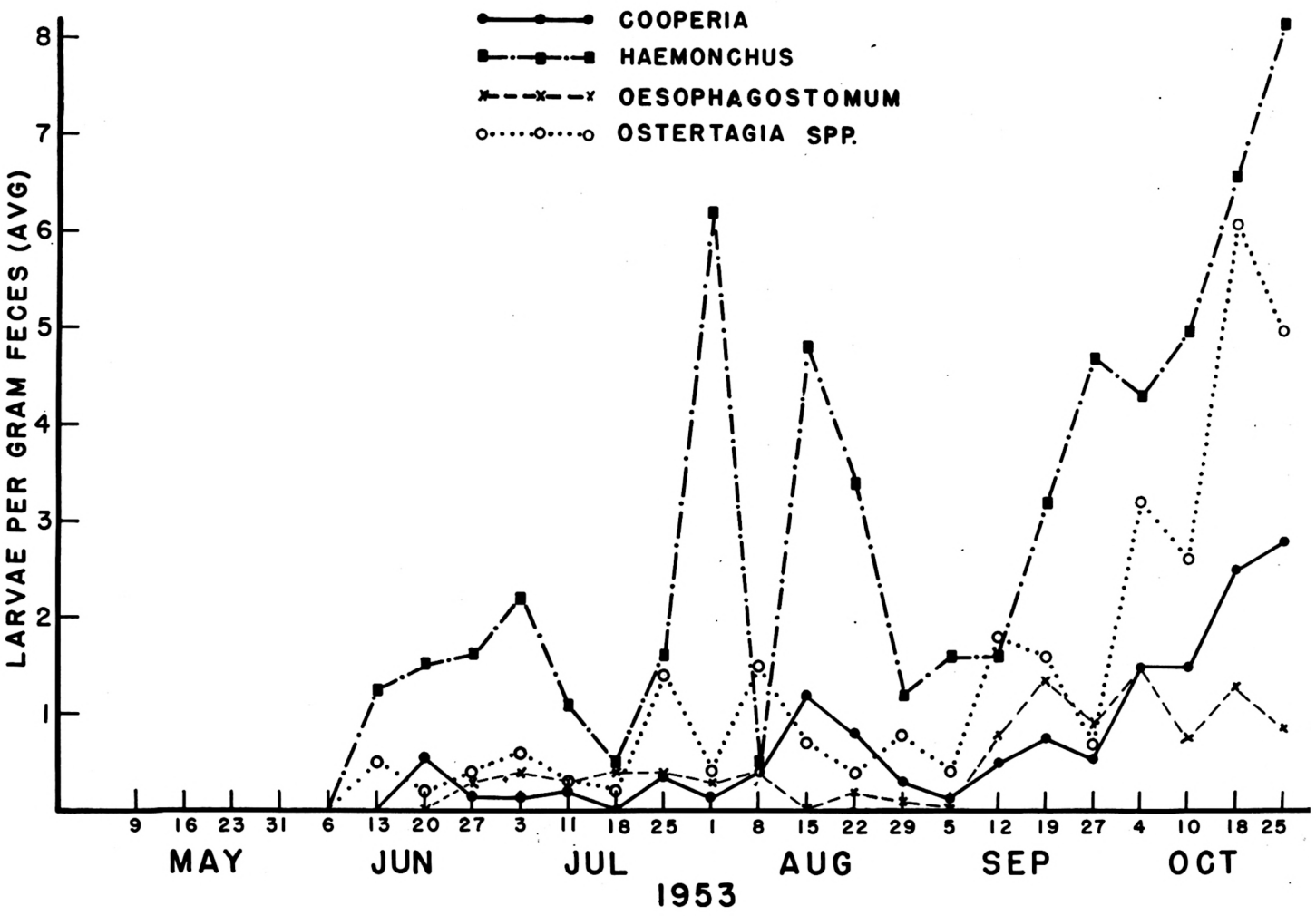
Except for slight variations of infective larvae of cattle nematodes, the measurements recorded in this study conformed with those recorded by Monnig (1931), Dikmans and Andrews (1933) and Keith (1953). Most of the species found in sheep are also found in cattle and other ruminants; thus these variations in the sizes of infective larva, suggest a possible strain difference.

Diagnosis of common nematode parasites of cattle can be successfully done from fecal cultures on the basis of differential morphological characters presented by each species of nematode larvae. For the purpose of easy and quick identification as to the genera and species, two morphological characters were important; (1) the total length of the larva including the sheath and (2) the length of the tail sheath and its appearance.

EXPLANATION OF PLATE XVI

Weekly average differential larvae counts in young calves (born in spring, 1953).

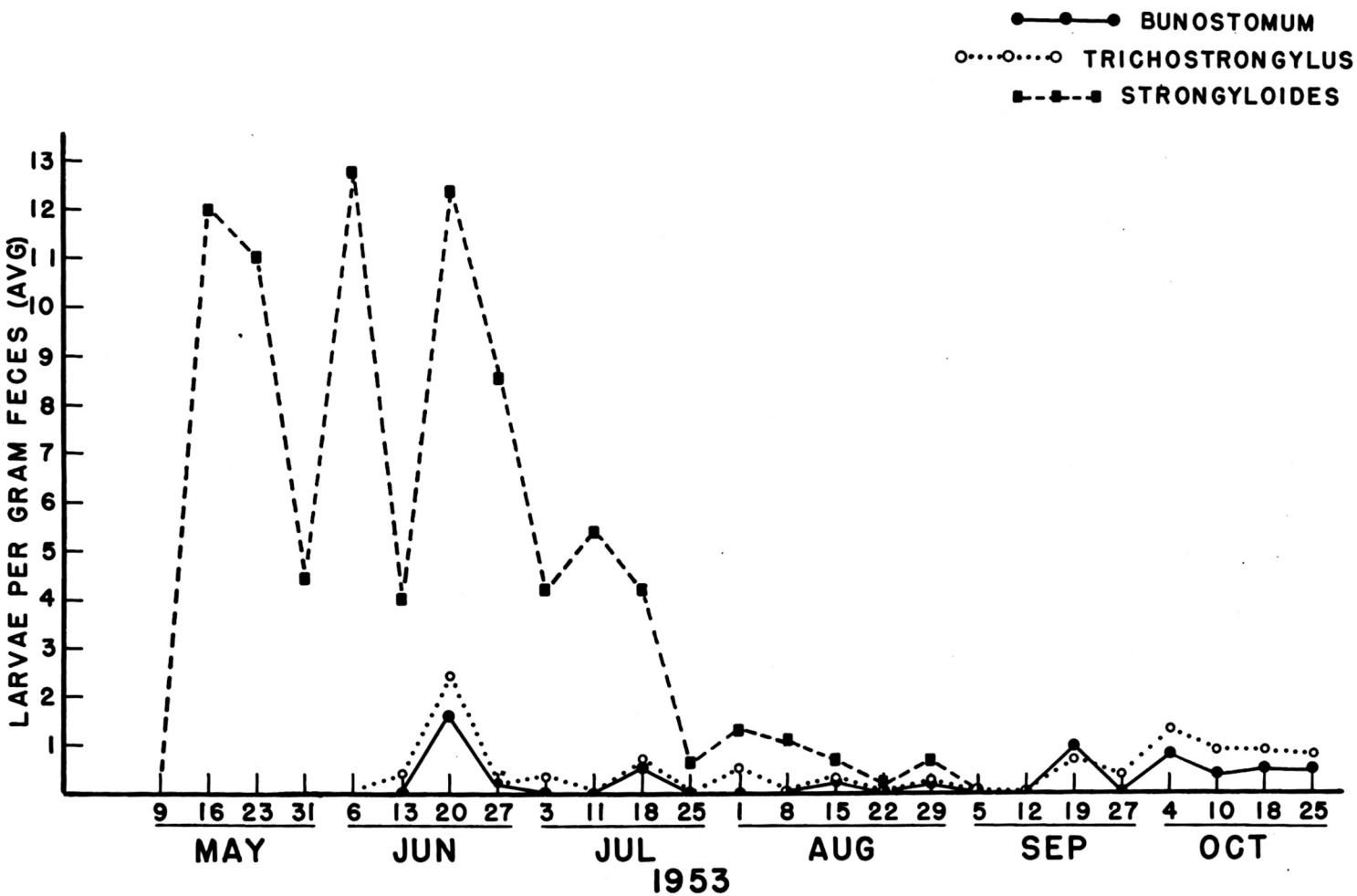
PLATE XVI


COOPERIA
HAEMONCHUS
OESOPHAGOSTOMUM
OSTERTAGIA SPP.
LARVAE PER GRAM FECES (AVG)
8
7
6
5
4
3
2
1
9 16 23 31 6 13 20 27 3 11 18 25 1 8 15 22 29 5 12 19 27 4 10 18 25
MAY JUN JUL AUG SEP OCT
1953

EXPLANATION OF PLATE XVII

Weekly average differential counts in young calves (born in spring, 1953).

PLATE XVII


BUNOSTOMUM
TRICHOSTRONGYLUS
STRONGYLOIDES
LARVAE PER GRAM FECES (AVG)
13
12
11
10
9
8
7
6
5
4
3
2
1
9 16 23 31
6 13 20 27
3 11 18 25
1 8 15 22 29
5 12 19 27
4 10 18 25
MAY
JUN
JUL
AUG
SEP
OCT
1953

After some experience, the above mentioned two characteristics are useful for species diagnosis, although there are variations in the total sizes of the same group and overlappings in sizes are common. In such cases, there are other minute differentiating characters which aid in making a definite diagnosis. During the course of the present study, the following differentiating characteristics described by Dikmans and Andrews (1933) were taken into consideration; (1) end of larval tail (2) structure of oesophagus (3) number and shape of intestinal cells and (4) shape of the buccal cavity.

The width of the larva was not of much value in identification, although the writer found that the infective larva of Oesophagostomum radiatum was much broader than any other species. However, both slender and broader forms were noticeable in the same group.

The infective larvae of Haemonchus, Cooperia and Ostertagia are generally confused due to their great similarity in size and general appearance. Many authors found it exceedingly difficult to differentiate some larvae from others, until Dikmans and Andrews (1933) and Andrews (1935) came out with an excellent description of the infective larvae of sheep nematode parasites. They described the most out-standing characteristics which were of great value in species diagnosis. The species of Nematodirus and Oesophagostomum could be recognized with little difficulty on the basis of their total length, although both have long filamentous tail sheaths. Hegner and Cort (1921) used the structure of the buccal cavity in differentiating the larvae

of Ancylostoma and Necator from that of Strongyloides stercoralis. Heyden (1927) found that the structure of the buccal cavity and the tail length were the most important features in identifying and differentiating hookworms of dogs. Veglia (1924) emphasized that the total length and tail ends and other characters of the larvae were of diagnostic value and gave the comparative diagrams of the tail ends of the larvae of O. columbianum, H. contortus, Trichostrongylus externatus and Strongyloides papillosus. Monnig (1926) was unable to differentiate the larva of two species of T. instabilis and T. rugatus due to overlapping of their sizes. Morgan (1930) could not differentiate the infective larva of Oesophagostomum venulosum and Chabertia ovina, because both of these worms have a long whip-like tail. Monnig (1931) compared the infective larvae of several species on the basis of total length, length of oesophagus, length of larval tail and tail sheath. He could not find good distinguishing characters between the different species in genus Trichostrongylus as they looked very similar. The species in this genus showed slight variation in the tail length, and were easily confused with the larvae of Ostertagia. Monnig (1931), Dikmans and Andrews (1933) described a characteristic kink in the tail sheath of Haemonchus contortus. Monnig (1931) reported the tail of the infective larvae of Ostertagia as being larger than the tail of Trichostrongylus but shorter than the tail of Cooperia. He further stated that the infective larvae of Ostertagia, Cooperia, and Haemonchus were the most easily confused and difficult to distinguish in mixed culture.

Dikman and Andrews (1933) discovered that there was considerable overlapping in the length of the larval tails and tail sheaths of the infective larvae of the genera, Haemonchus, Cooperia, Ostertagia and Trichostrongylus. They stressed the importance of taking other minute characteristics into consideration when making a definite diagnosis. They were able to differentiate the infective larva of Oesophagostomum and Chabertia by the number and shape of intestinal cells and length of tail sheath in each species. Oesophagostomum had 16-24 triangular shaped intestinal cells and a long tail sheath. The infective larva of Chabertia ovina was separated from others by having 24-32 intestinal cells rectangular in shape.

Several authors in the past, as mentioned above, experienced difficulty in making an accurate generic diagnosis of infective larvae in cultures. It is the opinion of the present writer that the following characteristics would aid in fairly reasonable and accurate diagnosis of the infective larvae at the generic level.

Bunostomum phlebotomum. The infective larva of this species could be easily identified by its small size, typical bulb shaped oesophagus and whip-like filamentous tail sheath.

Trichostrongylus axei. The infective larva of this species has a short, stubby tail sheath, which is more or less conical in shape. Another characteristic, as described by Dikman and Andrews (1933) is the presence of two tubercles on the posterior end of the larva proper.

Haemonchus contortus has a typical globular space in the

buccal cavity and a definite kink in the tail sheath immediately behind the posterior end of the larva proper. The tail sheath is sharply pointed.

Cooperia punctata. The infective larva of this species also has a globular space, but has two oval structures anterior to the oesophagus. The posterior end of the larva proper is rounded. The tail sheath is sharply pointed and is smaller than the tail sheath of the Haemonchus larva.

Cooperia onchophora. The infective larva of this species is one of the largest larvae. It has two oval and pear-shaped bodies anterior to the oesophagus. The tail sheath is sharply pointed and is much longer than C. punctata. The tail end of the larva proper is broadly rounded.

Ostertagia ostertagia. The infective larva of this species may be differentiated by the characters of the larval tail and the tail sheath. This larva is also of fairly large size. The tail of the larva proper is bluntly pointed and the tail sheath is comparatively shorter than Cooperia.

Oesophagostomum radiatum. The infective larva can easily be distinguished from others by its medium sized, long filamentous tail sheath. The intestinal cells number from 16-24 and are triangular in shape. It has a triangular area anterior to the oesophagus.

Nematodirus spp. The infective larva is recognized by its great length and the long fine whip-like tail.

Strongyloides papillosus. The infective larva is characterized by the absence of a sheath, the very slender body and

the long oesophagus which is nearly one-third of the entire body length. The tail end, under high power, is seen to be notched.

Seasonal Incidence of Worm Burdens

The data collected for the present study in regard to the incidence of nematode parasites in beef cattle show that the degree of infection was very low. The maximum number of larvae recorded during the spring months varied from 150-170 per gram of feces, which when compared with the figures recorded by other workers in other places seemed very low, because ecological conditions such as rainfall, temperature and soil conditions vary between the areas and greatly influence the development, behavior, and distribution of the infective larvae of the parasites. The weather conditions during the past year in the area of investigation were unusually dry and the rainfall was low. The low incidence could be attributed to unusually dry weather conditions. In order to arrive at a definite conclusion about the effects of varying climatic conditions on the infective larvae in a particular area, an intensive study for a period of at least two years is required. However, the writer was able to get a fairly reasonable general idea about the incidence, general distribution, frequency of occurrence and the fluctuations in different seasons from his studies of one year.

The seasonal variations in the larvae counts were observed during the course of the investigation. The minimum count occurred in the winter. There was a gradual rise in the month of

February, with the highest peak in the spring months and a gradual fall in the summer. All these variations give a clear cut indication of the worm burden pattern and the potential egg out-put.

Monnig (1930) with his extensive study on the bionomics of free living stages of Trichostrongylus spp. and other parasitic nematodes of sheep showed that a cold and dry winter retarded the development of Trichostrongylus eggs and larvae. Usually these eggs and larvae are desiccated before they reach the infective stage. Low temperatures caused slower development. High temperatures caused rapid development followed by deterioration and death. It was the actual degree of dryness of the larvae which determined the results. Morgan et al (1951) presented the data of their study on a flock of 159 Scottish sheep of varying ages for a period of two years, showing that the spring peak followed by the summer fall was evident in all groups of sheep. They were of the opinion that the increase in the worm burden in spring was due to greater numbers of larvae picked up at that time. They further indicated that the level of infestation was influenced more by the host's resistance than the actual intake of infective larvae. The resistance could be low in the spring after a winter's exposure to adverse weather conditions and a low level of nutrition. Cushnie and White (1948) also noticed that a peak of egg production by parasitic helminths was reached in the spring and was followed by a marked drop during the summer. They thought that the spring rise was due to a greater egg laying activity by worms already

present rather than from new infections.

The writer obtained similar results to those of Cushnie and White (1948) and Morgan et al (1951) who reported an increase in the worm burden in calves during the spring months. This increase in worm burden could be due to intake of greater numbers of infective larvae on the pasture during that time and also to an increased egg producing capacity of the worms already present in the digestive tract. The low larval level during the winter months could be due to the inhibitory effect of cold weather on the hatchability of the eggs passed in the feces on the pasture, consequently, resulting in a very low rate of intake of larvae.

Crofton (1948 and 1949) did remarkable work on the effects of ecological factors on the survival of infective larvae on the pastures. He thought that the availability of the infective larvae to the host was based on two factors; the presence of parasites in the third larval stage and the position of the infective larvae on the pasture. He showed that climatic factors at that time played an important part in the migration of the larvae on the different parts of the vegetation, which had originally influenced hatchings and survival of the larvae. He further emphasized that a high death rate of the larvae occurred in the warm weather and hatching of larvae was retarded by low temperatures.

Taylor (1935) explained the fluctuations as due to the effects of dietary changes and the relative resistance of the host, but not due to increased larval intake. He further

discussed that the possible explanation of fluctuations as due to the rate of egg production of the adult worms rather than to fluctuations in the rate of intake of infective larvae.

Rogers (1940) observed in his experiments with sheep in Australia that the egg out-put of female nematodes decreased as the worm population increased, irrespective of the climate. He thought overcrowding of worms inhibited the egg laying capacity of the female worms. Consequently the fluctuations in the pasture infectivity occurred not only by direct inhibitory action of the climate on the free living stages but, also, indirectly through concurrent fluctuations in the output of eggs by female hookworms.

Sprent (1946) in his studies on hookworms of cattle concluded that dryness of the pastures would prevent development of the larvae. Spedding (1953) observed that the egg concentrations in sheep feces were not constant and were highly variable throughout the day as well as from day to day. Kates (1950) showed that Ostertagia and Nematodirus were the most highly resistant of all nematode larvae to low temperatures. The larvae of Oesophagostomum, Haemonchus contortus, Chebertia, and Bunostomum showed a low resistance to drying and low temperatures. Haemonchus and Oesophagostomum were the least resistant to the effects of weather. Ostertagia was the most resistant to cold weather.

SUMMARY

1. Distinguishing morphological characters of the

infective larvae of some common nematode parasites of beef cattle were studied, from pure and mixed cultures.

2. The morphological studies were applied to the following: (a) the diagnosis of nematode parasites, (b) the estimation of incidence of parasitism in a herd of beef cattle, and (c) fluctuations in the worm burdens in different seasons.

3. Overlappings in the size and the shape of larvae of the genera Haemonchus, Cooperia, and Ostertagia were common, but they can be differentiated from one another by the following characteristics:

Haemonchus contortus infective larva was distinguished by its medium size, a definite kink in the tail sheath, and the sharply pointed tail sheath. The buccal cavity was globular.

The Ostertagia ostertagia infective larva was distinguished by its large size, medium size and bluntly pointed tail sheath. Sometimes the tail sheath presented a kink-like appearance.

Cooperia spp. The presence of two oval bodies in the buccal cavity was characteristic of this genus. C. punctata and C. onchophora could be separated from one another by their total size and the size of the tail sheath. The former had a medium sized, sharply pointed tail sheath. The latter was longer in total length and had a much longer, sharply pointed tail sheath. The end of the larval tail was rounded in both species.

The Bunostomum phlebotomum larva was the smallest larva of all the species and had a fine filamentous tail sheath. It had

a distinct bulb shaped oesophagus.

The infective larva of Oesophagostomum radiatum was recognized by its medium size and long whip-like tail. The intestinal cells were typically triangular in shape.

The infective larva of Nematodirus spp. was easily recognized by its largest size and a characteristically long filamentous tail sheath.

The Strongyloides papillosus infective larva was recognized by having a long oesophagus, a slender body and no sheath.

4. The following listed species of nematode parasites were identified from the mixed fecal cultures from calves during a period of one year: (1) Haemonchus contortus (2) Cooperia onchophora (3) Cooperia punctata (4) Ostertagia ostertagia (5) Oesophagostomum radiatum (6) Trichostrongylus axei (7) Bunostomum phlebotomum (8) Nematodirus spp. (9) Strongyloides papillosus.

5. The data showed that the degree of infection was very low for all species of nematodes present in the herd. Comparison of total larvae and egg counts of Haemonchus-nodular and Cooperia-Trichostrongylus groups correlated with one another.

6. This study recorded lowest counts in winter followed by a gradual rise in early spring until a peak was reached in the spring months. The larvae counts gradually fell in the summer.

7. Infective larvae of Haemonchus, Cooperia, Ostertagia, and Oesophagostomum reached a high peak in the spring months. Haemonchus contortus predominated throughout the year. Cooperia

and Ostertagia were next abundant. Bunostomum and Trichostrongylus maintained a constantly low level. Nematodirus was not very abundant.

8. In new born calves, the level of infection was fairly low during all the months except in October when it reached its highest peak. No infection was recorded for the first six weeks except Strongyloides papillosus. Haemonchus was the first to appear in these calves.

9. The seasonal pattern of larvae output has been very clearly defined in relation to the different seasons.

10. The method of using larvae counts in following seasonal variations in nematode infections shows promise.

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THE USE OF COMPARATIVE MORPHOLOGY OF THE INFECTIVE LARVAE, IN IDENTIFICATION AND DETERMINING THE INCIDENCE OF SOME COMMON NEMATODE PARASITES IN A HERD OF BEEF CATTLE

by

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AN ABSTRACT OF MASTER'S THESIS

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Department of Zoology

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It is exceedingly important to know the proper methods of diagnosis of every parasitic disease. The diagnosis of parasitic infections in animals has always been based on the identification of either parasites recovered from the host on post-mortem examination or the eggs from the feces of the sick animal. Because of much similarity in the size and shape of the round-worm eggs, the specific diagnosis is rather difficult. Therefore, the present study was initiated, primarily to learn the distinguishing morphological characters of the infective larvae by which they could be specifically differentiated from one another in the laboratory and in the field. This study was also applied to the following: (1) the diagnosis of common nematode parasites of range cattle. (2) the estimation of degree of infection. (3) the influence of seasons on the fluctuations in worm burdens.

The following materials and methods were employed in this study. The female nematode worms that served as a source of eggs for the culture of larvae were obtained from the beef cattle slaughtered at the packing plant and from cattle brought to the post-mortem room of the School of Veterinary Medicine, Kansas State College. The eggs were mixed with sterile feces, smeared on three inch square pieces of surgical gauze, and stored in the wide-mouth bottles. These cultures were incubated at 28°C. for ten days. The larvae were separated from the cultures by a modified Baermann apparatus. This apparatus was made by attaching glass funnels to 15 ml centrifuge tubes by means of thick rubber tubing. The funnels were filled with warm water (Temp. 40°C-45° C.).

A herd of about five hundred young beef cattle, in the area of Flint hills, was used for the study of incidence of nematode parasites and their seasonal fuctuations. Twenty fresh fecal samples were collected every week. The cultures were made by smearing three grams of feces on a piece of gauze which was subsequently incubated for ten days. The larvae were separated from the cultures in the same manner as indicated above. The water was removed from the centrifuge tubes by means of a pipette leaving only 0.3 ml of water containing the larvae at the bottom of the tube. One-tenth of a milliliter of well mixed sediment was placed on the microscopic slide and gently heated to kill and to straighten the larvae. The number of larvae counted in 0.1 ml of the mixture represented the total number of larvae per gram of feces.

The results of this study were as follows:

1. The infective larvae of nematode parasites of cattle presented two important characteristics which were mostly used in identification and differentiation from one another i.e. (a) the total length of the larva including the tail sheath and (b) the length and the shape of the larval tail and the sheath of the tail.

2. Difficulty in identification of the infective larvae of the genera Haemonchus, Cooperia, and Ostertagia, was experienced because of similarity in their size and shape. With the help of the following out-standing characteristics, they could be differentiated from one another without any trouble; (1) size and shape

of oesophagus, (2) number and shape of intestinal cells, and (3) the shape of the buccal cavity.

3. The most distinguishing characters of the infective larvae of some common nematode species, identified during the present study are described below.

Haemonchus contortus infective larva is characterized by its medium size, sharply pointed tail sheath, a typical kink in the tail sheath immediately behind the larval tail, and a globular buccal cavity.

Ostertagia ostertagia infective larva is characterized by its length and by its medium sized, bluntly pointed tail sheath. The buccal cavity is tubular.

Cooperia spp. The infective larvae of this genus are characterized by the presence of two oval bodies in the buccal cavity. The tail sheath is sharply pointed. C. punctata and C. onchophora are separated from one another by their total length and the size of the tail sheath. C. punctata is of medium size and has sharply pointed medium sized tail sheath. C. onchophora is of large size and the tail sheath is much smaller and more sharply pointed than the former species. The end of the larval tail in both species is rounded.

Bunostomum phlebotomum infective larva is the smallest of all the species and has a fine filamentous tail sheath. The oesophagus is club shape.

Oesophagostomum radiatum larva is easily recognized by its medium size and its long whip-like tail. The intestinal cells are 16-24 in number and typically triangular in shape.

Nematodirus spp. is characterized by its largest size and a characteristic long filamentous tail sheath.

Strongyloides papillosus is easily recognized by its slender body, and by the absence of a shaeath. The oesophagus extends more than one-third of total body length.

4. During the course of investigation on the incidence of nematode parasites in beef calves, the following species of nematodes were diagnosed by the morphological characters of their infective larvae. (1) Haemonchus contortus (2)Cooperia onchophora (3) Cooperia punctata (4) Ostertagia ostortagia (5) Oesophagostomum radiatum (6) Trichostrongylus axei (7) Bunostomum-phlebotomum (8) Nematodirus spp. (9) Strongyloides papillosus.

5. The degree of infection was found to be very low in the herd of beef cattle in Kansas as compared with the higher rate of infection of beef cattle reported by various workers from other parts of the world, where severe outbreaks of parasitic gastro-enteritis are known to occur. The maximum number recorded in yearling calves in this study was 150-170 per gram of feces and the minimum number of larvae was 10-30 per gram of feces.

6) Variations in the total as well as the differential larvae counts were observed in different seasons. It was found that seasonal fluctuations in the larvae out-put paralleled the total egg counts. Lowest counts were recorded during the winter months, followed by a gradual rise in early spring months. The larvae counts decreased considerably in the summer.

7. The data on the differential counts in the yearling calves varied with the species of worms. Haemonchus contortus predominated throughout the year. The peak Haemonchus larva count occurred during the spring months and the lowest peak occurred during the summer and winter months. These variations showed that Haemonchus was the least resistant to the effects of climate. The worms responsible for high peak counts were Haemonchus, Cooperia spp., Ostertagia spp., and Oesophagostomum. There was no significant rise or fall in the Bunostomum phlebotomum, Nematodirus spp., or Trichostrongylus larvae counts. Cooperia and Ostertagia larvae were found to be most resistant to the effects of the climatic conditions.

8. The total larvae counts in the young calves presented a different picture from that of the yearling calves. The larvae counts remained at a low level throughout the year, except in October when a high peak counts was reached. Haemonchus contortus showed considerable high counts in the summer. Ostertagia and Cooperia also showed a high level of counts in October. Strongyloides papillosus was present in large numbers for the first six to eight weeks and showed a considerable decrease as the calves grew older. Bunostomum and Trichostrongylus larvae counts were the lowest recorded. Nematodirus spp was entirely absent.

9. Larvae counts and the egg counts showed grat similarity when they were compared on the basis of larger groups such as Haemonchus-Nodular group and Cooperia-Trichostrongylus group.

10. All variations noticed during the period of study in their seasonal occurrence, gave a clear cut indication of the worm burden pattern, the potential egg out-put and the kind of larvae available on the pastures.