Report on some experiments in connection with the life history of Strongylus (or Haemonchus) contortus, the wire-worm of sheep and goats, and also some notes on the Zoological structure of the wire-worm and of the nodular worm, Oesophagostomum columbianum.

By R. C. LEWIS, M.A. (Cape).

These two worms are common in sheep and goats in South Africa, and are responsible for loss of condition and often death, when present in large numbers. Numerous vermicides have been employed for their destruction, but have met with only partial success, and the researches of the American authorities on these parasites show that the best means of securing comparative freedom are (1) rotation of pasture, (2) choosing pastures on high well-drained ground, (3) giving a plentiful supply of fresh water, (4) seeing that the drinking troughs are raised well above the ground so that the water may not be contaminated by the faeces, (5) isolating such animals and seeing that the pastures are not overstocked.

Eggs are laid in large numbers and pass out in the faeces, so that the presence of the parasites is readily detected by a microscopic examination of the faeces, when the egg capsules are seen. The usual method employed for detecting whether the egg capsules were numerous or not, and accordingly whether the infection was heavy, was by simply smearing a small portion of the faeces on a slide, diluting with water and teasing out, and then counting the number of egg capsules under an 18 m.m. coverslip, but very successful results were obtained by the following method:—Make a paste of the faeces with water and press through fine muslin to remove the coarser particles. Then shake up with water and decant several times, this is done in order to remove soluble matter. The egg capsules have a higher specific gravity than water and sink, so that they are all contained in the sediment. A solution of $Ca\ Cl_2$ is then made up of specific gravity 1.25, and added to the sediment and shaken up. This mixture is then centrifugalized, and the solid matter sinks to the bottom, but the egg capsules float in the superficial liquid. The upper portion of this liquid is then drawn off and water is added. Then this liquid is again centrifugalized and the lower layer of liquid, which contains the egg capsules, is drawn off by means of a pipette. In this way the egg capsules are freed from the faeces and finally lie in pure water. Permanent preparations of these egg capsules may then be obtained by pouring the water containing them into a mixture of 100 parts 70 per cent. alcohol and 5 parts glycerine, heated to nearly boiling point, constantly stirring so that the egg capsules are well mixed in the glycerine. This fluid is then placed in an incubator at 50° for 2

days so that all the alcohol and water is evaporated off, and the egg capsules lie in pure glycerine. Place a drop of this on a glass slide and melt more glycerine gelatine on a spatula over a flame. The glycerine gelatine consists of 20 parts gelatine, 100 glycerine, 120 parts distilled water, and 2 parts carbolic acid. Add a drop or two to the glycerine on the slide, stir, and then quickly put on the coverslip. When the gelatine has cooled and hardened, paint the edges of the coverglass with varnish. As many as 93 egg capsules with embryos inside, all well preserved, were counted under an 18 mm. coverslip in a preparation made in this way.

The method employed for obtaining preparations of the adults and embryos of these worms was the following:—Place adult male and female, after removing from faeces and washing, on a glass slide; add a few drops of Müller's fluid (K_2 Cr_2 07 2·5 parts, Nr_2 50_4 1 and distilled water 100 parts), just sufficient to produce capillarity when the cover glass is put on, then place in a moist closed chamber for two days; this moist chamber is produced by putting moist blotting paper at the bottom of a petri dish. Draw water through to wash out the Müller's fluid, then draw 20-25 per cent. alcohol under the cover glass and place in moist chamber for a day; now replace by 30 per cent., then 40 per cent. alcohol, each for a few hours; next draw a mixture of equal parts of 40 per cent. alcohol and glycerine through and leave exposed to air so that alcohol may evaporate off, from time to time adding more glycerine so that finally the specimens lie in pure glycerine. Now remove the cover glass, draw off the glycerine, and clean in neighbourhood of specimens with weak alcohol, and finally mount in glycerine gelatine.

In the case of the embryos, on account of their minute size, it is difficult to draw the various fluids under the cover glass without losing the embryos, and the process is better carried out by placing large numbers of the embryos in lens glasses, and removing the different fluid re-agents by slow decantation. The above method is employed because Nematodes cannot be mounted in Canada Balsam owing to the wrinkling and contraction of their cuticle in oils. Also Nematodes are very impermeable to stains on account of their cuticle.

The majority of the microphotographs are of specimens prepared in this way.

The first experiment was to find whether isolation of young lambs, except at feeding times, would secure freedom from infection. Several very young lambs, whose faeces on examination showed no egg capsules, were placed in a stable in which some trusses of grass were placed for warmth. They were supplied with fresh water regularly, and the stable was constantly cleaned. The only time they were allowed out of the stable was for short periods to go to the ewes for milk. On examination of the manure of these lambs daily it was found that, after a period of 1-2 weeks, egg capsules were present in the manure. These egg capsules rapidly increased in number after about 2 weeks. Sixty-three samples of manure were examined from these lambs and the result was always the same that the lambs soon became infected.

The next experiment was to find a sheep which was heavily infected and to try to get cultures of the embryos from the manure. Thirty samples of manure were examined from sheep, when one was

found which showed 40 egg capsules under an 18 mm. coverslip. This sheep was isolated and a large number of cultures of its manure set going. The cultures were prepared by placing the manure at the bottom of a large test tube and placing moist blotting paper at the mouth of the tube. This blotting paper must be kept moist as it is found that the embryos will not appear if the manure is completely dry. On the other hand if too much moisture is present and the manure is covered by water, the embryos will not appear either. At the end of 3-6 days, at average temperature, large numbers of embryos were seen in white bands on the sides of the test tube just above the manure. At colder temperature the embryos only appeared after a period of ten days or even longer.

Several of these culture test tubes were placed in an incubator at 37° C., but it was found that the embryos did not come up the sides of the test-tube.

These embryos remained on the sides of the test tubes in some of the cultures for over one month. They undergo moults in this position, and by examining the embryos every day, in one of these culture tubes, they were found to moult at the end of the 19th day after manure was placed in culture. This was probably very late on account of the low temperature at the time.

It is stated by Ransom and other authorities that these so-called "rhabditiform" embryos undergo 2 or 3 moults before they finally reach the infectious stage. In the cultures made the number of moults was not observed, but at the end of one month on examination it was found that on the sides of the test tube there were several embryos and numerous cast skins.

As far as the observations went the embryos were found to be of very simple structure throughout, with a straight uniform alimentary tract. At the end of over one month the embryos showed the same structure and no reproductive organs were seen to be developed. The only change observed was an increase in size of the embryos. Thus embryo, taken from a culture tube less than 1 week old, measured 534 x 17·3μ, while those taken from a culture tube over 3 weeks old, measured 624 x 21μ. (*Vide* Plate No. 48.)

At this stage it is necessary to point out a difficulty which arose. On examination of the faeces 2 kinds of egg capsules were seen, 1st a smaller one in which when laid an active embryos was usually present, and 2nd, a larger egg capsule which had reached the morula or blastula stage, or, more rarely, a stage where only 4 segments were present. Ten of each of these egg capsules were measured, and the average length for the small egg capsule was 56·2 x 32·2μ, while that of the larger was 81·3 x 46μ. According to some works, the measurements of the larger egg capsule would agree more closely with those of the egg capsules of Strongylus contortus, and the egg capsules are described as being laid in the segmenting condition. It was therefore concluded at first that the larger was that of Strongylus contortus. An examination of the contents of the abomasum taken at the post-mortems of several sheep showed the large egg capsules to be present, but in none of these cases was the worm found.

Hanging drop cultures of the larger egg capsule proved that an embryo escaped from the larger egg capsule, after a period of from 15 hours to 3 days or more according to temperature. These embryos

showed a bulb at the end of the oesophagus, which is a characteristic of the young embryos of some of the Strongylidae described by Ransom. This embryo measured 301·6 x 33·6μ.

However, it was proved that the smaller egg capsule was that of Strongylus contortus by the ovary having ruptured in one of the permanent preparations, and several egg capsules having escaped, all of which were of approximately the same size as the small egg capsule, and contained a fully formed embryo coiled up inside.

Possibly the larger egg capsules are those of a smaller strongylus, such as Strongylus gracilis, which is commonly present in the abomasum, and which could easily be overlooked in a macroscopic examination. Limit of time prevented an investigation of this point.

As the embryos were very plentiful on the sides of the culture test tubes made from manure of the isolated sheep, it was next desired to find, by drenching experiments, at what stage the embryos became infective, accordingly, examination was made of the faeces of several very young lambs until two were found which were free from egg capsules. These two lambs were then drenched, the first with a culture which was 27 days old, the second with embryo from a culture 15 days old. The manure from these two lambs was then examined every day. At the end of 10 days the first lamb showed 40 egg capsules under an 18 mm. coverslip, and the second showed 20. At the end of 13 days the first lamb showed 60 egg capsules, but this lamb did not show any increase in number, and later the number became less, but the second lamb at the 17th day showed 87 egg capsules, on the 18th 110, the 24th 220 and so a gradual increase until as many as 360 were counted. As a confirmation of severe infection or not, blood smears were constantly made from these two lambs, but, though slight anaemia was observed, revealed by slight anisocytosis, and poikilocytosis; a few corpuscles showed basophilia and polychromatophilia, the anaemia did not become more marked, nor were the other signs of severe worm infection observed, such as inappetence or wasting. The result of the post-mortem of this second lamb was contrary to expectation, as, on a search of the contents of the abomasum, only one Strongylus contortus was found. The other compartments of the stomach and also the intestines were searched, but no worms or nodules were observed. This was the case at the post-mortem of the isolated sheep also, and it was therefore desired to repeat these experiments, but as only $4\frac{1}{2}$ months were available for the work, the time was too short to do so.

In order to test whether sheep which had been dosed with Bluestone and Cooper's Dip were free of the worms, the faeces of a large number of sheep, which had received various amounts of the vermicid were examined up to two weeks after reciving the dose, but in all cases egg capsules were found to be present, proving that complete removal of the worms was not effected.

According to the researches of Ransom, the embryos, when they reach the infectious stage, if moisture is present, climb up to the tips of blades of grass. A large quantity of the faeces from the isolated sheep was placed at the base of grass planted in a pot, and this was kept constantly moist and observed for 8 weeks, but worms were not seen to come up the grass. This was contrary to expectation, as the embryos readily climbed up the sides of the culture test-tube. It is

possible that the embryos had not yet reached the infectious stage, or that as the temperature has been so low (often below 30° F), the embryos were killed before they had reached the infectious stage, as it is known that although the embryos, when they have reached the infectious stage, can withstand freezing or drying, before that stage they succumb to the effects of severe heat or cold, though against this is the fact that the embryos in the culture tubes survived on the sides of the test-tube for over a month under the same conditions of temperature.

Cultures were made from the manure of the 2nd lamb which showed such a large number of egg capsules, and after 6-10 days (the temperature was low) the embryos appeared on the sides of the test-tubes. Lambs which were free from egg capsules were again sought, and 2 were drenched, one with a culture 9 days old, and the other with a culture 8 days old. The manure of these lambs has been examined for over 2 weeks, but neither yet show signs of infection, as no marked increase has been observed in the number of egg capsules. Also manure from the 2nd lamb was placed at the foot of the grass in a pot. This was kept moist, and has been observed for 3 weeks, but no embryos have yet been seen on the grass.

It was desired to get a pure culture and keep it under observation for a long period to see if any change took place in the embryos, and also how long the embryos would remain on the sides of the test-tube if the blotting paper was kept constantly moist in the mouth of the test-tubes. Accordingly a collection of the manure from very young lambs was examined, and the manure free from egg capsules was placed at the bottom of test-tubes. Then embryos from several culture tubes were washed into these tubes. This experiment was not successful, as in no case did the embryos re-appear on the sides of the egg-free culture tubes. Blades of grass also were put in these test-tubes to observe whether the embryos would come up, but they were not seen to do so.

Summary.

(1) Isolation of young lambs in clean stables, except for short periods when they are allowed to go to the ewes, does not secure freedom from infection.

(2) Drenching of sheep with Cooper's Dip and Bluestone does not completely expel the worms, as egg capsules were found in the faeces after the animals had been drenched.

(3) Artificial cultures may be obtained of the embryos by placing the manure under suitable conditions of moisture and temperature, and the embryos which are thus produced, undergo moults and increase in size, but no marked change in structure was observed, nor was any trace of reproductive organs seen. There is no intermediate host, and the embryos die, unless taken into their specific host.

(4) 4 lambs drenched with these culture embryos (1) 27 days old, (2) 15 days old, (3) 9 days old, (4) 8 days old did not show signs of heavy infection. In the case of the lamb drenched with a culture 15 days old, a large number of egg capsules were seen in the faeces after 2 weeks, but the lamb did not show marked anaemia, inappetance, wasting, nor were worms found on post-mortem.

(5) The 2 experiments with manure placed at base of grass plants did not give the expected results. The embryos were not observed to climb up the grass blades, though they readily climb up the sides of the test-tubes.

(6) None of the sheep or lambs examined at post-mortem showed heavy infection. Only a few strongylus contortus were found in the abomasum. In a few cases fairly numerous Oesophagostomum columbianum were found in the caecum and colon.

Strongylus contortus: Cause of verminous gastritis found free on the mucosa of the abomasum. Body red or white (red if the intestine is full of blood sucked from the mucous membrane). Attenuated at the extremities, except in the case of male, which has the caudal bursa at posterior end. Mouth nude, has no external armature, but near the anterior end there are 2 small lateral papillae in form of teeth directed backwards, these are further forward in male than in female. Cuticle finely striated transversely, and also with longitudinal ridges.

Male: 13 mm. or $\frac{1}{2}''$ long. Conspicuous bursa at posterior end with 2 large lateral lobes, and a small asymmetrically situated dorsal lobe attached to left lateral lobe on its inner side near the base. Dorsal ray supporting dorsal lobe bifurcates 6 rays in each lateral lobe. There are, reading from, say, nearest the dorsal lobe and going round the lateral lobe, 1 ventro-ventral, 2 latero ventral, 3 extremo lateral, 4 redio lateral, 5 postero lateral, and 6 exterio dorsal.

Spicules long, with small knobbed tips. There is on each spicule a barb-like projection near its tip. The anus is situated at the posterior end in the median line between the lateral lobes of bursa. In a lateral view of the tail of male the gubernaculum is seen as a broad flat body at the base of the spinules, and extending posteriorly along their upper surface for about $\frac{1}{2}$ their length.

Female: 27 mm. or $1''$ long. The tail is slender, sharply pointed. The ovaries are spirally wound round the intestine, vulva about 1-5th length of body from posterior end, covered by a prominent backward projecting linguiform process. Anus about 500μ from tip of tail. Eggs, ovoviporous, $56{\cdot}2 \times 32{\cdot}2\mu$, containing a fully formed, active embryo usually.

Oesophagostomum columbianum: Usually found in caecum and colon of foot, causes verminous enteritis. The head does not show tapering as in the case of strongylus contortus, and is fairly broad at the anterior extremity. There are 2 crown of teeth projecting anteriorily from the buccal capsule. The cuticle surrounding the mouth is usually inflated to form a ring-like mouth collar, which is limited behind by an annular construction. There are 6 circum oval papillae. At about 400μ from anterior end, there is a transverse groove, which extends round the body, and the cuticle between the mouth collar and transverse groove is usually inflated. Just behind the transverse groove are 2 lateral cervical papillae. Prominent lateral membranous wings are present, beginning at the transverse groove, and extending backwards along the lateral lines.

Male: 13 mm. or $\frac{1}{2}''$ long. Bursa consists of 2 lateral lobes united by a smaller median lobe. In each lateral lobe 6 rays, viz., 2 ventral rays, 3 lateral and 1 exterio dorsal. Specules long and slender. In median view 2 lateral trebursal papillae are seen projecting into cuticle, a short distance in front of bursa.

Female: Head very similar to that of male, though usually slightly thicker, 16 mms. Pointed at tail end. The vulva is situated near the posterior end, and appears as a slit between 2 raised lips. The anus is a short distance nearer tip of tail. Laval stages occur in nodules in the intestinal wall.

A full description of Strongylus contortus and Oesophagostomum columbianum is found in Bulletin 127, 1911, U.S.A. Bureau of Animal Industry, in an article by B. H. Ransom on the Nematodes parasitic in the alimentary tract of cattle, sheep and other ruminants.

The Plates will be found to illustrate most of the points mentioned above.

WORKS OF REFERENCE.

Of the works of reference consulted, the most useful were found to be:—

(1) *Bureau of Animal Industry, U.S.A. Department of Agriculture.* Annual Reports 1900 and 1901 Articles by Stiles on Nematode parasites.
(2) Annual Reports 1906 and 1908 Articles by Ransom on Nematode parasite.
(3) Bulletin 127 of 1911. The Nematodes parasitic in the alimentary. tract of cattle, sheep, goats, and other ruminants by B. H. Ransom (this was the most useful).
(4) Bulletin 135, 1911. A comparative study of methods of examining faeces for evidences of faeces by M. C. Hall.
(5) Parasites and Parasitic Diseases of the Domestic Animals. Neumann, edited by Macqueen, 1905.
(6) Braun & Lühe's Handbook of Practical Parasitology, 1910.

Plate 46.—Adult female and male Strongylus contortus. Female 27½ mms., male 13 mms. The twisted form of the female may be seen, due to coiling of ovaries round the intestine. The belobate caudal pouch is distinctly seen.

Plate 47.—Egg capsule of Strongylus contortus, 56·2μ x 32·2μ. Ovoviparous: the eggs usually laid with an active embryo inside. In this plate the outline of the embryo is well seen, but the enveloping egg capsule is not well shown.

Plate 48.—Embryo of Strongylus contortus measuring 624μ x 21μ from a test-tube culture over 3 weeks old.

This mottled cuticle is an indication that a moulting stage is reached.

Plate 49.—Head of female Strongylus contortus. Note the pointed termination, and the absence of external armature in the head region. The 2 cervical spines or backward projections of the cuticle in each lateral line of the body, and the alimentary tract are well shown.

Plate 50.—Head of male Strongylus contortus. Similar to that of female, but the 2 lateral cervical spines are more anterior in position.

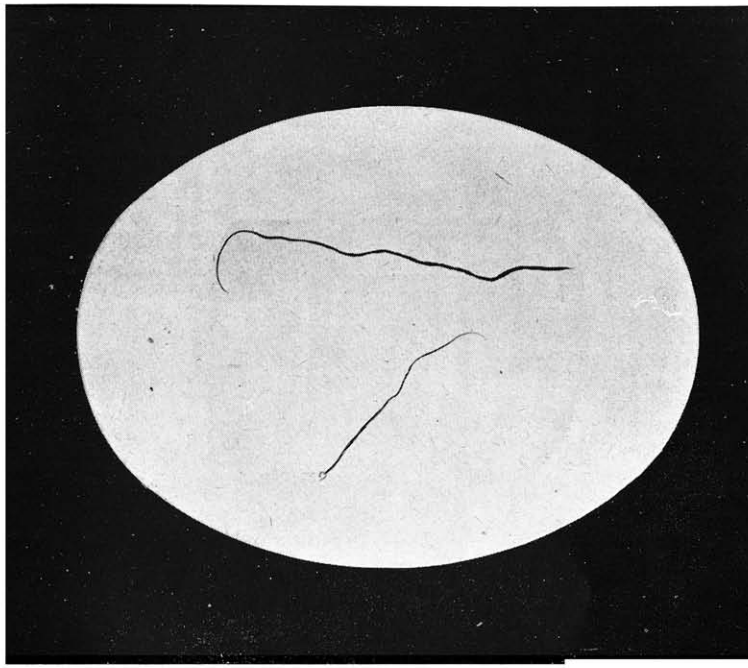

Plate No 46.] *Haemonchus contortus.* Male and Female.

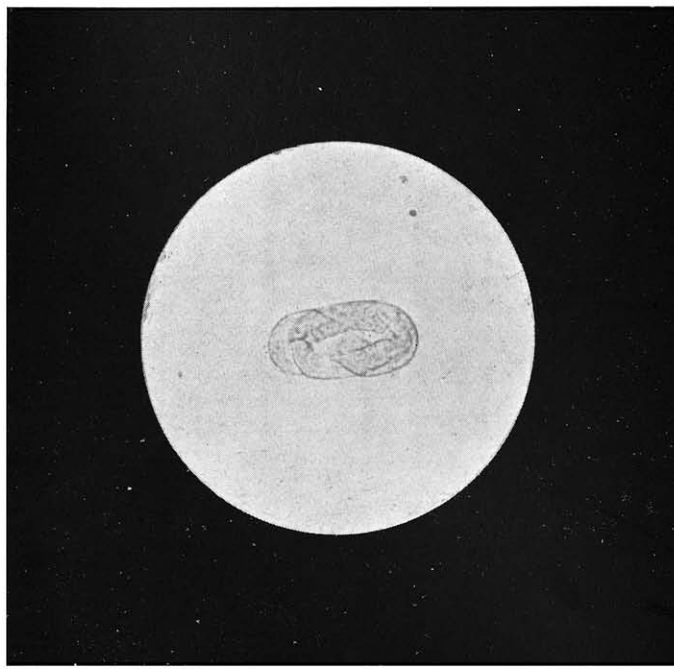

Plate No. 47] *Haemonchus contortus.* Egg.

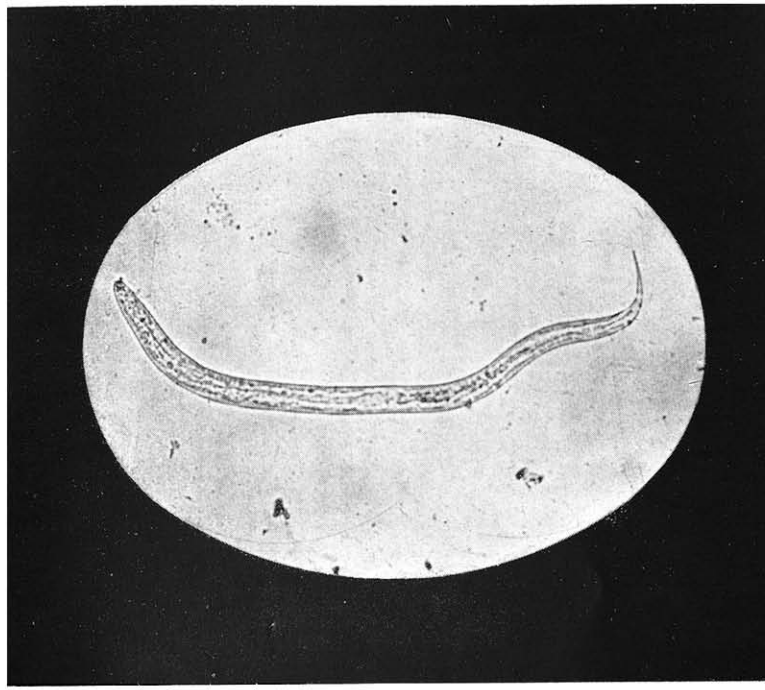

Plate No 48]

Haemonchus contortus. Embryo.

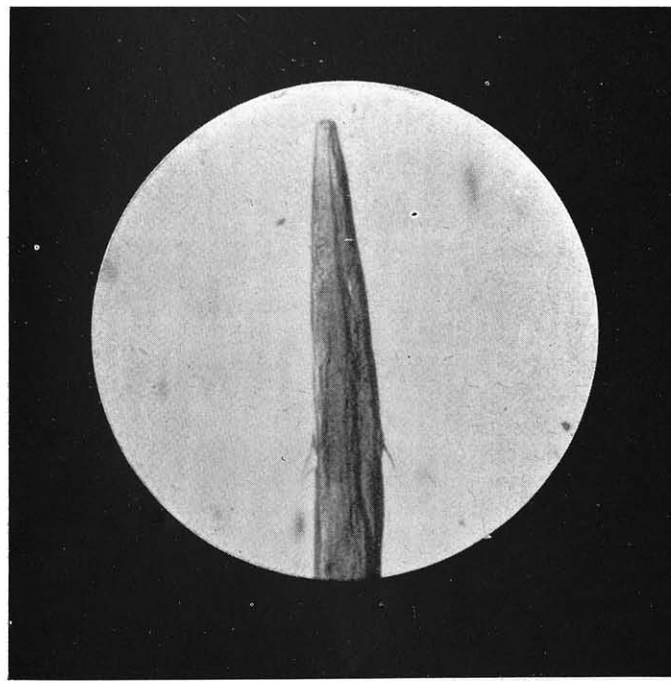

Plate No. 49.]

Haemonchus contortus. Head of Female.

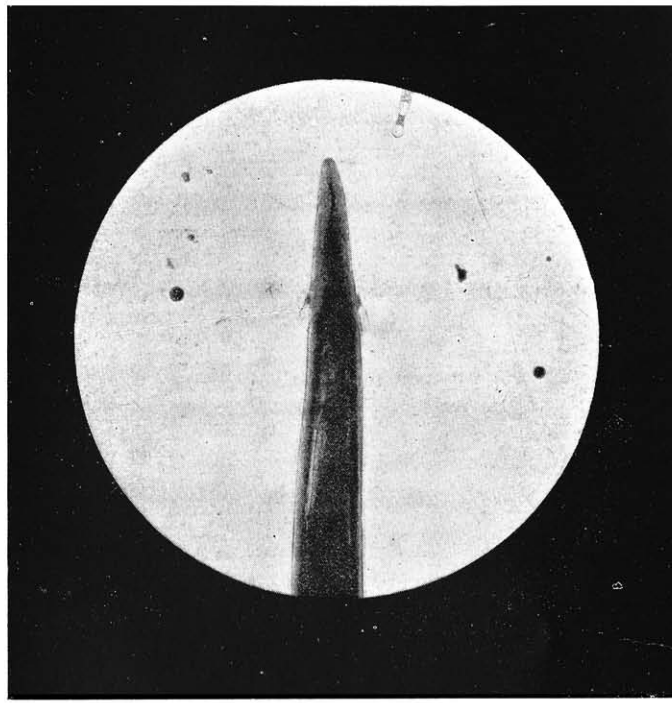

Plate No. 50.] *Haemonchus contortus.* Head of Male.

Plate No. 51.] *Haemonchus contortus.* Tail of Male.

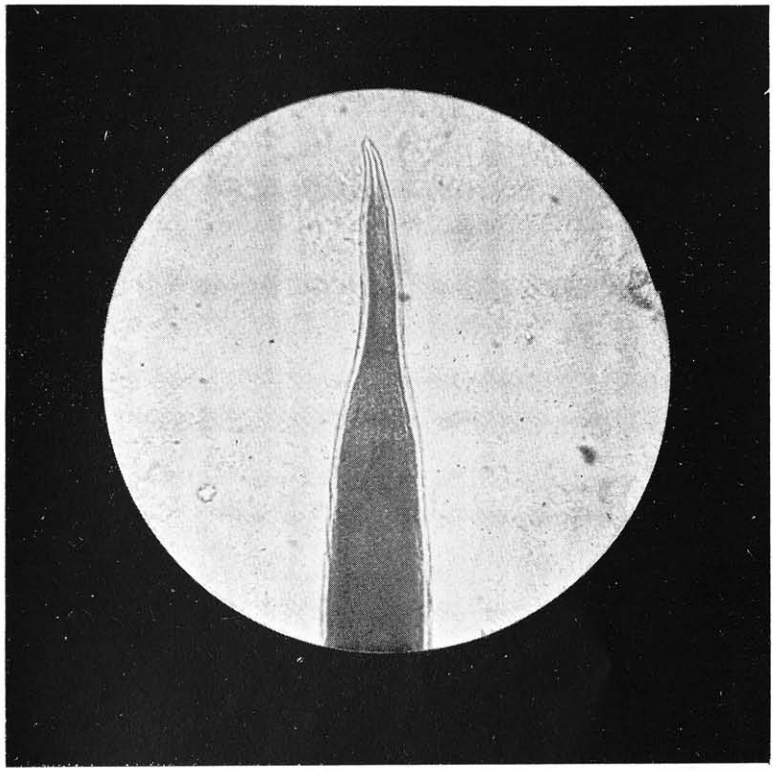

Plate No. 52.] *Haemonchus contortus.* Tail of Female.

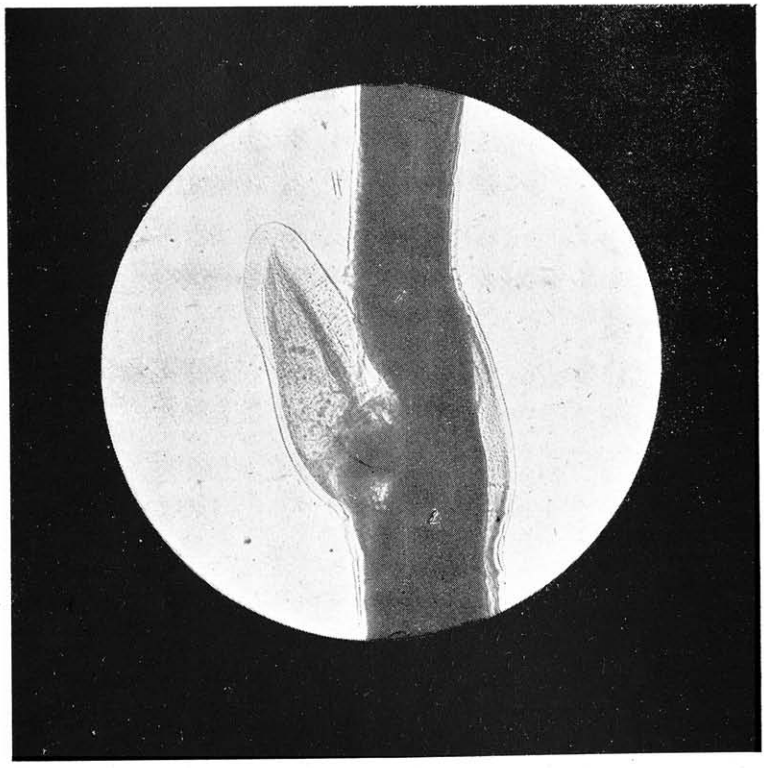

Plate No. 53.] *Haemonchus contortus.* Vulva.

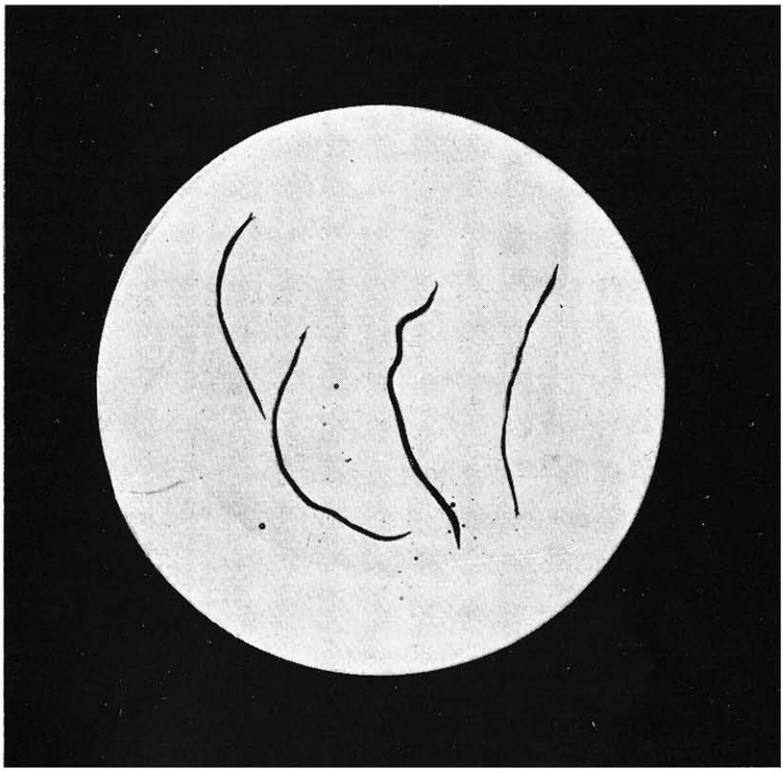

Plate No 54.]
Oesophagostomum columbianum. Male and Female.

Plate No. 55.]
Oesophagostomum columbianum. Head of Male.

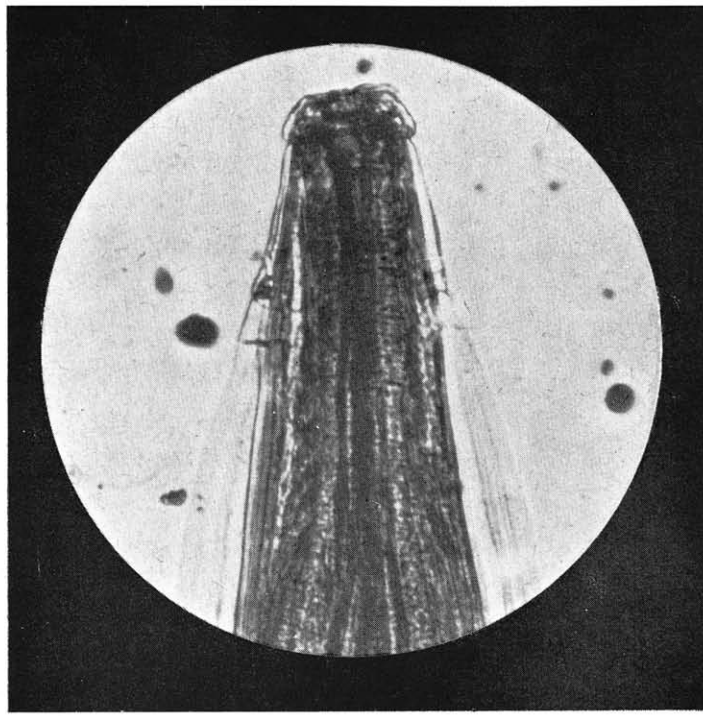

Plate No. 56.]
Oesophagostomum columbianum. Head of Male.

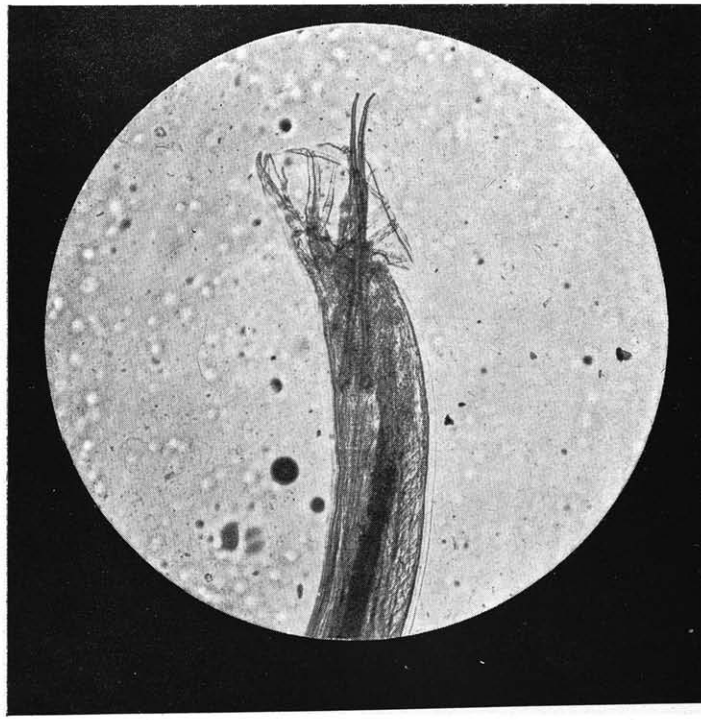

Plate No. 57.]
Oesophagostomum columbianum. Tail of Male.

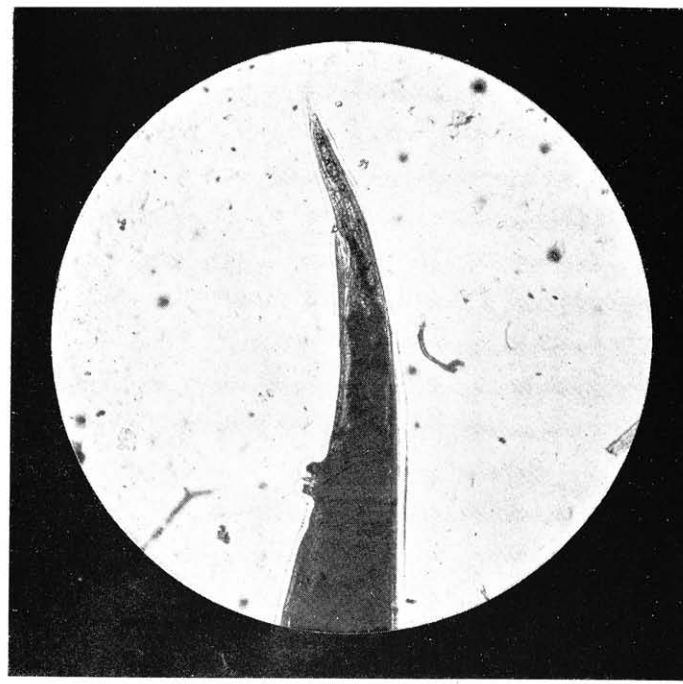

Plate No. 58.]
Oesophagostomum columbianum. Tail of Female

Plate 51.—Tail of male Strongylus contortus. Large lateral lobes of bursa; the asymmetrically situated dorsal lobe is attached to the left lateral lobe on the inner side near its base. Dorsal ray supporting dorsal lobe. Reading from ray nearest dorsal lobe, and going round the left lateral lobe, the rays are: 1 ventro ventral, 2 latero ventral, 3 externo dorsal, 4 medio lateral, 5 postero lateral, 6 externo dorsal. The spicules are seen projecting slightly in the median line between the lobes.

Plate 52.—Tail of female Haemonchus contortus.

Plate 53.—Haemonchus contortus—vulva.

Plate 54.—Adult male and female Oesophagostomum columbianum. 2 outer forms males, 2 inner females. Female seen to be shorter and thicker than female Strongylus contortus. Male about the same length as male Strongylus contortus, but thicker. Male 13 mm., female 16 mm. Caudal pouch can be seen on right of upper specimen and on left of lower specimen.

Plate 55.—Head of male Oesophagostomum columbianum. The cuticle surrounding the mouth is seen to be inflated to form a ring-like mouth collar. The 2 crowns of chiterious teeth, projecting from inside the buccal cavity, are well shown.

Plate 56.—Enlarged view of head of male Oesophagostomum columbianum. Cuticular expansion to form mouth collar which is limited behind by an annular construction, the crown of teeth, and 2 of the circumoral papillae in the mouth collar region are shown. The transverse groove, the lateral membranous wings and the cervical papillae are also shown. The oesophagus is clearly seen and its bulb-like expansion posteriorly is also well marked.

Plate 57.—Lateral view of tail of male Oesophagostomum columbianum. The bursa with its rays and the long, slender spicules are well marked. The alimentary tract is seen to terminate at posterior end, medio ventrally, beneath the spicules, between the 2 lateral lobes of the bursa. The rays starting ventrally and going round dorsally are:—

1. Ventro ventral. 2. Lateral and ventral.	Closely approximated.	These terminate ventral to the spicules.
3. Externo lateral. 4. Medio lateral. 5. Postero lateral. 6. Externo dorsal. 7. Dorsal.	Closely approximated.	These terminate dorsal to the spicules.

Plate 58.—Another view of tail of female Oesophagostomum columbianum showing vulva on ventral surface as a slit between 2 raised lips, also the alimentary canal terminating at the anus which is clearly seen in a notch on ventral surface 1″ from tip of tail.