ON SOME STRONGYLID LARVAE IN THE HORSE, ESPECIALLY THOSE OF *CYLICOSTOMUM*

BY

J. E. W. IHLE, D.Sc.

AND

G. J. VAN OORDT, D.Sc.

(*Zoological Laboratory of the Veterinary College, Utrecht, Holland*)

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The adult stage of species of *Cylicostomum* found in the large intestine of the horse has been extensively studied by Looss and, more recently, by Boulenger, and Yorke and Macfie (cf. Ihle, 1922), but very little is known as yet of the development of these species in the body of the horse.

The larvae of *Cylicostomum* are to be found in large numbers in the mucosa of the caecum and colon of the horse. They were first found by Dick (1836) and described by Knox (1836). By Diesing (1851, Vol. II, p. 332) they were mentioned under doubtful species as *Nematoideum equi caballi.* T. Spencer Cobbold (1874, p. 85) describes and figures *Cylicostomum* larvae as adult Nematodes under the name *Trichonema arcuata*, but the next year (1875, p. 241) he states that *Trichonema* is only the larval form of *Cylicostomum* (' *Strongylus tetracanthus* ').

Short descriptions and sketches of the larvae, encysted in the mucosa of the large intestine, are also to be found in Leuckart (1876, p. 445), Cobbold (1886, p. 288) and Giles (1892, p. 15, Pl. III, figs. 16, 18). The last-mentioned author thought he had found a free-living *Rhabditis* generation of *Cylicostomum.* This mistake, which has been made repeatedly and also recently, when the development of different Nematodes was traced, is due to the fact that the cultures of larvae were infected with free-living Nematodes.

An investigation of Cuillé, Marotel and Roquet (1913), dealing with our subject, is of more importance than the older publications above mentioned. These authors distinguished three types of larvae, living in the mucosa of the large intestine of the horse and considered as belonging to *Cylicostomum*:—(1) 'La larve oesophagostomiforme,' with mouth-capsule and dorsal tooth and having a length of 2 to 5 mm.; (2) 'la larve metastrongyliforme,' without mouth-capsule (length 800μ to 2 mm.); and (3) 'embryons,' without recognisable internal structure (length 300μ to 800μ). They showed that the 'larve oesophagostomiforme' passes over into the juvenile *Cylicostomum* by a moult.

Recently a part of the development of *Cylicostomum insigne* was shortly described by Boulenger (1921), who figures small larvae (6 to 7 mm. in length) and large larvae (up to 11 mm. in length), both agreeing with the 'larve oesophagostomiforme' of the French authors. In the larger larvae the adult mouth-capsule makes its appearance, which represents the preparation for the last ecdysis.

We ourselves have examined a large number of larvae, partly collected by the Commission appointed to inquire into Sclerostomiasis in Holland, and partly by ourselves in the horses dissected in the Anatomical Institute of the Veterinary College at Utrecht. All the larvae were found in the mucosa of the large intestine, though a small number were met with free in the lumen of the intestine.

The larvae examined by us can be divided into different types, to be described in subsequent pages. They belong for the greater part to *Cylicostomum*, a few perhaps to *Triodontophorus*; others were not identified. In addition we found a few very small larvae without recognisable internal structure. They agree with the so-called 'embryons' of Cuillé, Marotel and Roquet (1913, p. 8 of reprint), and were only obtained by us in a few cases by scratching the mucosa of the large intestine. We have not yet studied these forms in detail, but we do not think that these small worms (according to the French authors measuring 300μ to 800μ in length) must be considered to belong to the genus *Cylicostomum*, because it follows from the investigations of A. Albrecht (1909) and of De Blieck and Baudet (not yet published) that the larvae of *Cylicostomum* infecting the horse are much more differentiated.

CYLICOSTOMUM LARVAE

All *Cylicostomum* larvae, found by us in the mucosa or in the lumen of the intestine, show a cup-shaped larval mouth-capsule, which has been already described by Cobbold (1874, p. 86). In agreement with this author (1886, p. 288) we will call this larval stage *Trichonema* stage. The name 'larve oesophagostomiforme' must be rejected, as these larvae do not in any particular agree with *Oesophagostomum.*

The cuticle is ringed. The cuticle surrounding the circular mouth-opening may also be called mouth-collar here; this larval mouth-collar, however, is much less developed than the adult one. The mouth-opening is generally surrounded by six papillae. External and internal leaf-crown are absent. The mouth-capsule is either sharply marked off from the mouth-collar or passes gradually over into it. In the middle the mouth-capsule is mostly wider, and possesses a thicker wall than posteriorly and anteriorly. Especially near the mouth-opening, the wall is very thin. The anterior part of the mouth-capsule is mostly provided exteriorly with a collar, often strongly developed, and which we will call mouth-capsule collar. It is divided into six lobes, which have a crescent shape and are almost perpendicular to the outer surface of the mouth-capsule. Between every two lobes a head-papilla is to be found.

An oesophageal funnel, in which the three sectors of the oesophagus continue, is present. The dorsal sector is always provided with a tooth, more or less protruding into the lumen of the mouth-capsule. The cuticular lining of the anterior margin of the oesophageal funnel shows a circular thickening, adjacent to the mouth-capsule. We will call this thickening the funnel-ring; it is directed to the exterior.

The oesophagus is cylindrical in shape and somewhat swollen posteriorly. Where the oesophagus passes over into the mesenteron three valves protrude into the lumen of the intestine. A nerve-ring, surrounding about the middle of the oesophagus, is present.

The mesenteron is composed for the greater part of a dorsal and a ventral row of alternating, polynuclear cells, which are mostly pigmented. In the anterior part of the mesenteron the cells are always much flatter than in the posterior part. In the anterior part

the cell-limits run transversely or directed obliquely to the front; so that the lateral parts of the cell-limits are situated more anteriorly than the dorsal and ventral parts.

The very short rectum opens into the exterior through the anus, situated at a small distance from the sharp posterior extremity of the body.

Sometimes, but not always, the larvae living in the mucosa are red in colour. It appears that in the few cases examined by us the whole body, the pigmented intestine excepted, is red. When such a larva is pricked, a red fluid is emitted. The juvenile specimens, living in the lumen of the colon and caecum, may also show this colour, but, as in the case of the larva, the intestine was not red in the specimens examined by us. Prof. B. Sjollema and Miss J. E. van der Zande were so kind as to analyse microchemically and spectroscopically a few juvenile specimens of *Cylicostomum insigne* for us. The fluid appeared to be due to oxyhaemoglobin.

We assume that the larvae living in the mucosa feed on blood at least during a part of their life. Boulenger (1921, p. 324) found these larvae in cysts, filled with blood; this is not always the case, however. As mentioned above, the red colour was not observed in the cells of the intestine of the larvae. We suppose that the larvae had fed on blood in an earlier period; consequently the red colour must have disappeared already from the intestinal wall, but not yet from the rest of the body. Further, we are of opinion that the red colour of the adult worm is the consequence of the larvae having fed on blood, for the adult *Cylicostomum* feeds on the contents of the large intestine of the host and not on blood. After dissection these worms are never found attached to the mucosa of the host's intestine.

The larvae, which we consider to belong to the genus *Cylicostomum*, can be divided into two types, to be described below. Not much importance must be attached to the dimensions indicated, as larvae of numerous species are brought together which when adult differ strongly in size. We cannot state to which species of *Cylicostomum* these different types belong, because we have not at our disposal a large enough number of moulting specimens.

Cylicostomum Larva. Type A (fig. 1).

To this type the smallest larvae of the genus *Cylicostomum* are considered to belong, having a length of 3 to 4·5 mm. and a maximum thickness of 110μ to 200μ. The mouth-margin is smooth. Around the mouth-opening the cuticle is thick. Head-papillae could not be observed. A mouth-capsule collar was not found by us. The length of the mouth-capsule, including the mouth-collar, varies from 20μ to 28μ.

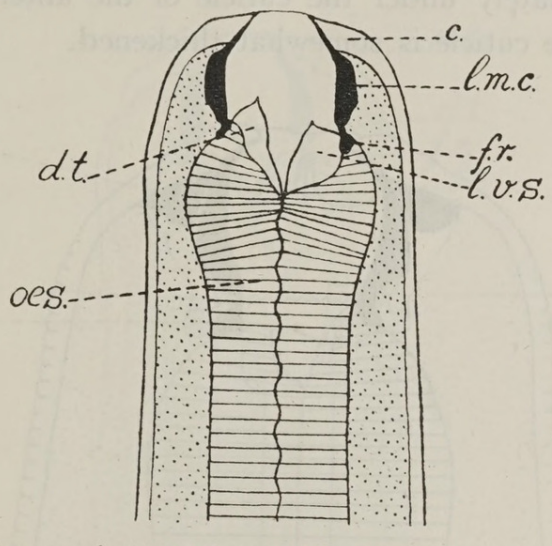

Fig. 1. Anterior extremity of a *Cylicostomum*-larva, type A, seen from right side. × 540 (× ¾). *d.t.*—Dorsal tooth; *oes.*—Oesophagus; *c.*—Cuticle; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-rings; *l.v.s.*—Latero-ventral sector of the oesophageal funnel.

In this type the oesophageal funnel also possesses three sectors. The dorsal sector always possesses a tooth, varying in size; the two latero-ventral sectors are rounded or bear an inconspicuous tooth, which never protrudes as far into the lumen of the oral capsule as the dorsal, large tooth. The length of the oesophagus varies from 250μ to 350μ. The distance from the anus to the posterior extremity of the body is 80μ to 130μ.

This type is very common.

Cylicostomum Larva. Type B (fig. 2).

Another type (B) is also of frequent occurrence. It differs from Type A in being of a larger size and in possessing a mouth-capsule collar. The length is 7·5 to 12·5 mm., the maximum thickness

420μ to 580μ. Six head-papillae are present, agreeing as to arrangement with those of the adult specimens; so there are two lateral and four sub-median papillae. The oral margin is mostly somewhat incised near the six papillae. Length of the mouth-capsule, including the mouth-collar 55μ to 65μ. At one-third of the length of the mouth-capsule from the posterior margin the wall of the oral capsule is thickest. The wall of the mouth-capsule becomes thinner anteriorly and passes gradually over into the mouth-collar. The mouth-capsule collar is very well developed and situated immediately under the cuticle of the anterior part of the body. Here the cuticle is somewhat thickened.

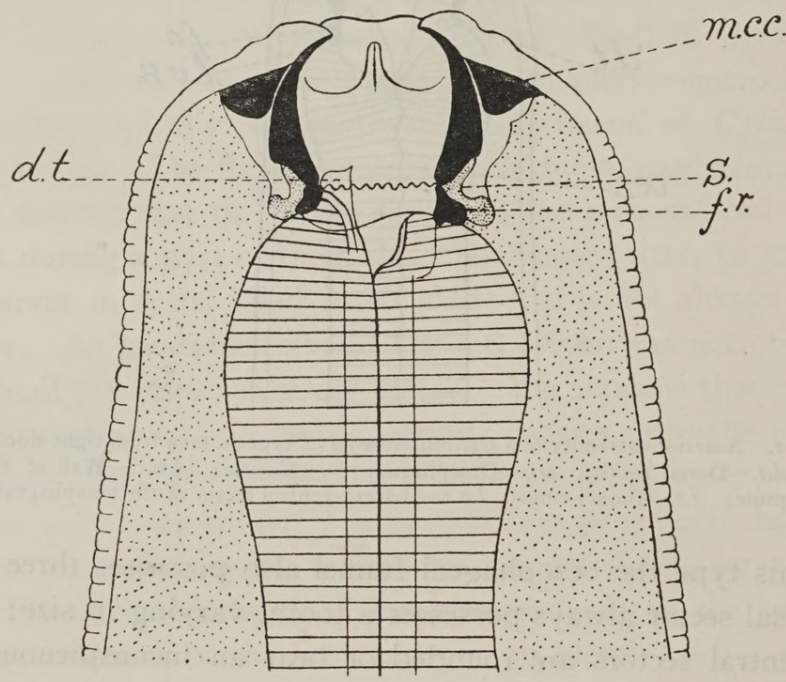

FIG. 2. *Cylicostomum*-larva, type B, seen from right side. × 435 (× $\frac{2}{3}$). *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *s.*—Septum; *f.r.*—Funnel-ring.

The oesophageal funnel is conspicuous and bears a cuticular lining of variable thickness. The funnel-ring is well developed. In some cases the border between mouth-capsule and funnel-ring is irregular or undulating. Sometimes the funnel-ring possesses a circular groove at its outer surface, so that in optical section it appears as a double ring. In this type, too, the dorsal sector of the oesophagus is continued as a tooth, protruding into the lumen of the oral capsule; this tooth is relatively not so large as in Type A.

The latero-ventral sectors are truncated anteriorly, or become lower and lower, to end at the funnel-ring. At the bottom of the grooves by which the sectors are separated the cuticle is thickened, just as in the adult worm. The oesophagus measures 550μ to 650μ in length; the distance from the anus to the extremity of the body is 190μ to 220μ.

We consider that the larvae belonging to Type B represent a more developed stage of Type A, because we have found several larvae with the rudiments of the mouth-capsule collar (fig. 3); these

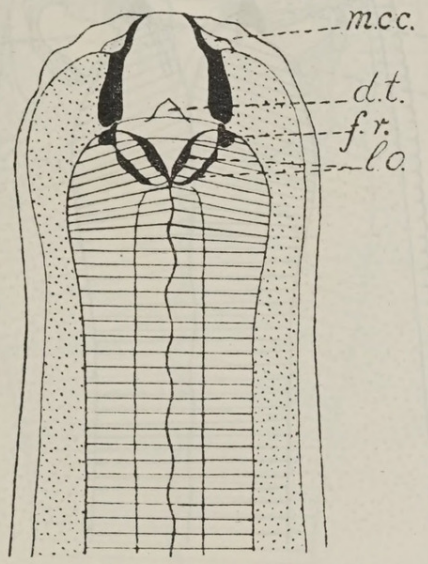

FIG. 3. *Cylicostomum*-larva, intermediate between types A and B, seen from dorsal side. × 540 (× $\frac{3}{4}$). *m.c.c.*—Mouth-capsule collar; *d.t.*—Dorsal tooth; *f.r.*—Funnel-ring; *l.o.*—Lining of the oesophageal funnel.

larvae are of a size intermediate between those of Types A and B. Length 5 mm. to 6·5 mm., maximum thickness 350μ; length of the mouth-capsule, including the mouth-collar, 42μ; oesophagus 540μ long.

The last ecdysis (figs. 4, 5).

The *Trichonema* stage passes over into the juvenile worm, living in the lumen of the large intestine, by a moult. In agreement with the development of other Nematodes, we assume that this moult is the fourth and last. The ecdysis itself takes place in the intestinal lumen.

The moult begins with the formation of a cavity around the larval mouth-capsule (fig. 2). We consider that one continuous cavity is

present from the beginning. Boulenger (1921, p. 325) mentions a series of cavities. However, according to Looss (1897, p. 925), two cavities (a dorsal and a ventral one) are formed in the larva of the fourth stage of *Ancylostoma*. Later on these cavities unite to form a circular lumen.

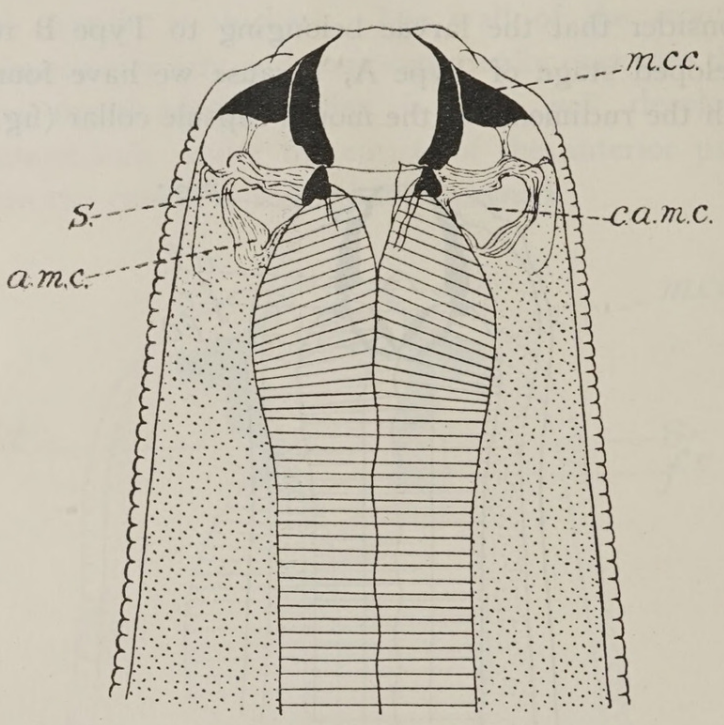

FIG. 4. *Cylicostomum*-larva, type B, seen from left side, with rudiment of the cavity of the adult mouth-capsule. × 290 (× ¾). *s.*—Septum; *a.m.c.*—Wall of the adult mouth-capsule; *m.c.c.*—Mouth-capsule collar; *c.a.m.c.*—Cavity of the adult mouth-capsule.

When the cavity makes its appearance, it is narrow at the front and a little wider backwards. Later on the anterior part of this cavity extends to the mouth-capsule collar. In the posterior part, which extends almost to the oesophagus, we see a granular substance, which seems to form a thin layer (fig. 2, *s.*) about at the level of the posterior margin of the mouth-capsule. This layer corresponds with the definitive anterior side of the mouth-capsule of the adult worm. This septum (fig. 4, *s.*) gradually becomes thicker, possibly formed by the granular substance mentioned above, while the cavity lying behind this septum, and in the beginning filled up with this substance, becomes empty and extends simultaneously backwards. This cavity, situated behind the septum, is the lumen of the adult mouth-capsule. At the periphery of the septum the definitive

mouth-collar and the definitive head-papillae develop (fig. 5). The cavity mentioned gradually widens and peripherally begins to form the wall of the adult oral capsule. Now this circular cavity surrounds the anterior part of the oesophagus (fig. 4).

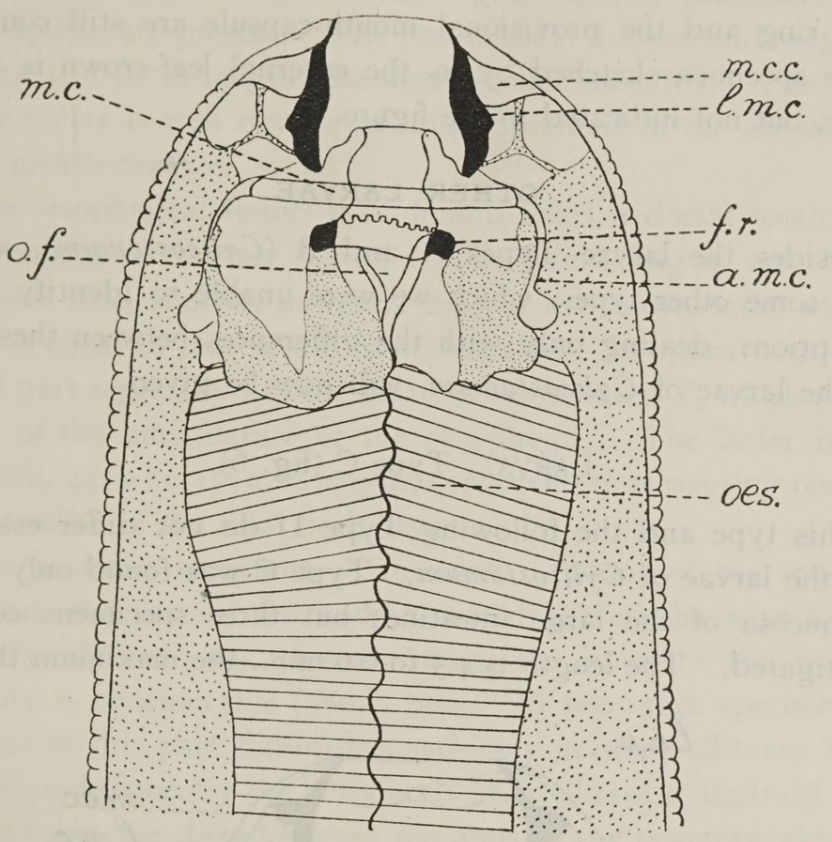

FIG. 5. *Cylicostomum*-larva, type B, moulting. × 290 (× ¾). *m.c.*—Mouth-collar; *o.f.*—Larval oesophageal funnel; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-ring; *a.m.c.*—Wall of the adult mouth-capsule; *oes.*—oesophagus.

Meanwhile the cuticle of the adult worm is formed below the provisional one. Before the ecdysis proper the oesophagus loosens itself from the cuticular lining of its funnel (fig. 5). In earlier stages the oesophagus tapers to the anterior extremity and ends at the funnel-ring (fig. 4). But at this stage it becomes truncated in front. Now the lumen of the mouth-capsule is situated before the oesophagus, whereas the anterior part of the latter was formerly surrounded by the adult mouth-capsule. Simultaneously the mouth-capsule and the funnel-ring, which remains connected with the cuticular lining of the provisional oesophageal funnel, separate. The posterior margin of the mouth-capsule and the anterior margin

of the funnel-ring remain connected by a thin membrane, of which the origin is difficult to trace. Boulenger (1921, fig. 5 *b*) also figures it, without describing it. In the moulting specimen sketched by Cuillé, Marotel and Roquet (1913, fig. 17, 7), in which the adult mouth-capsule and mouth-collar have developed completely, the funnel-ring and the provisional mouth-capsule are still connected. In the specimen sketched by us the external leaf-crown is already visible, but not indicated in the figure.

OTHER LARVAE

Besides the larvae Types A and B (*Cylicostomum*), we also found some other types, which we were unable to identify. Short descriptions, dealing only with the differences between these types and the larvae of *Cylicostomum*, will now be given.

Larva. Type C (fig. 6).

This type and the following Type D do not differ essentially from the larvae of *Cylicostomum*. Type C was found only once in the mucosa of the large intestine; but three specimens could be investigated. The length is 4·5 to 6·6 mm., the maximum thickness

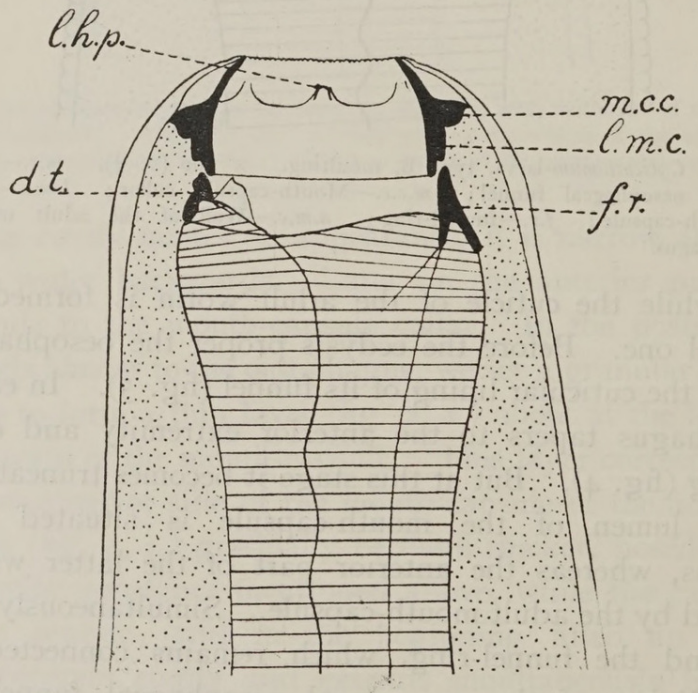

Fig. 6. Larva, type C, seen from right side. × 310 (× ¾). *l.h.p.*—Lateral head-papillae; *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

225μ to 380μ. The mouth-opening is circular, its margin is delicately denticulated. A mouth-collar is present, of which the side directed to the body-axis possesses a layer passing over into the anterior margin of the mouth-capsule. (In the figure the limit between mouth-collar and mouth-capsule is not indicated.) The head-papillae are present. The oral capsule is short and very wide; it is 52μ to 65μ in length, the mouth-collar included. The mouth-capsule collar is well developed and implanted in the anterior half of the mouth-capsule.

The oesophageal funnel is wide and bears a dorsal tooth. The latero-ventral sectors are smooth. The funnel-ring is very long at the dorsal side, shorter, however, than at the ventral side, where its length is equal to that of the mouth-capsule. In optical section this ventral part especially has the shape of an inverted Y, which encloses a part of the musculature of the oesophagus. The latter is short and thick, 435μ to 550μ in length, consequently measuring one-tenth to one-twelfth of the total body-length.

The posterior extremity is rounded. In one of the specimens examined, the part of the body situated behind the anus becomes gradually thinner; the distance from the anus to the posterior extremity is considerable (380μ) here. In two other specimens the thickness of this part diminishes suddenly at some distance behind the anus; consequently the body ends in an almost cylindrical point. In these cases the distance from the anus to the posterior extremity is 155μ to 180μ. Possibly these are sexual differences.

LARVA, Type D (fig. 7).

We found this type five times (only a few specimens) in the mucosa of the large intestine. We do not know whether this type and also the former (Type C) belong to *Cylicostomum*. Length 3·5 to 5·1 mm., maximum thickness 120μ to 190μ. The head-papillae are present. The cuticle is swollen around the mouth-opening; here the part of the cuticle directed to the body-axis possesses a particular layer, which passes over into the mouth-capsule. The latter is 25μ to 27μ long, the cuticle surrounding the mouth-opening included. Posteriorly its wall increases in thickness. A slightly developed mouth-capsule collar is present, lying immediately against the cuticle.

The dorsal sector of the oesophageal funnel bears a large tooth, protruding far into the lumen of the mouth-capsule. At the dorsal side its anterior margin possesses a small point and at the ventral side a large one. Each of the latero-ventral sectors bears a small tooth with one point. Moreover, the well-developed funnel-ring

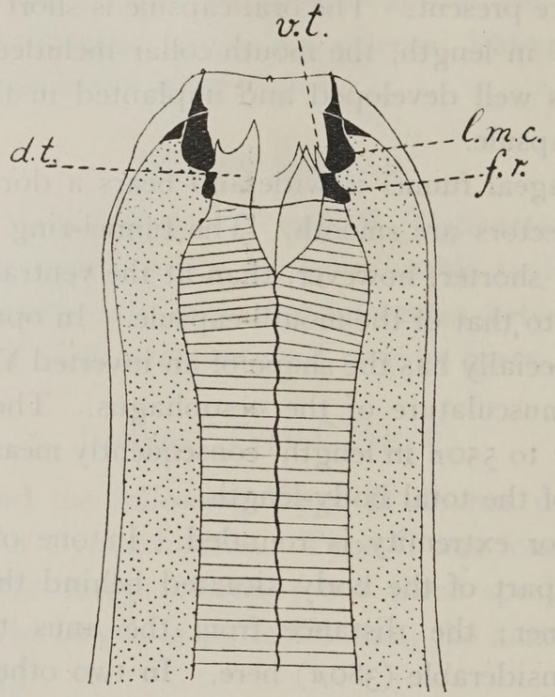

FIG. 7. Larva, type D, seen from right side. × 540 (× ¾). *d.t.*—Dorsal tooth; *v.t.*—Ventral tooth; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

possesses medio-ventrally a pointed tooth, being directed anteriorly (fig. 7, *v. t.*). Peripherally the funnel-ring does not protrude markedly. The oesophagus is long (400μ to 435μ), being one-ninth to one-twelfth part of the body-length. The mesenteron agrees with that of *Cylicostomum* larvae. The anus is situated 105μ to 115μ from the posterior extremity of the body.

LARVA. Type E (fig. 8).

We found this type only once in four specimens in the lumen of the large intestine. We consider that these larvae belong to *Triodontophorus*. It is, however, very remarkable that we found this type only once in our comprehensive material, as two *Triodontophorus* species are common in Holland, and sometimes inhabit one host in large quantities.

The length of these four larvae is 7·6 to 8·5 mm., the maximum thickness 310μ to 365μ. The mouth-opening is circular and surrounded by a thin mouth-collar, finely and longitudinally striated, and resembling an extremely little developed external leaf-crown. The six head-papillae are distinctly visible. The length of the mouth-capsule (including the mouth-collar) is 65μ to 82μ. The mouth-capsule is wide, cup- or barrel-shaped, and sharply marked

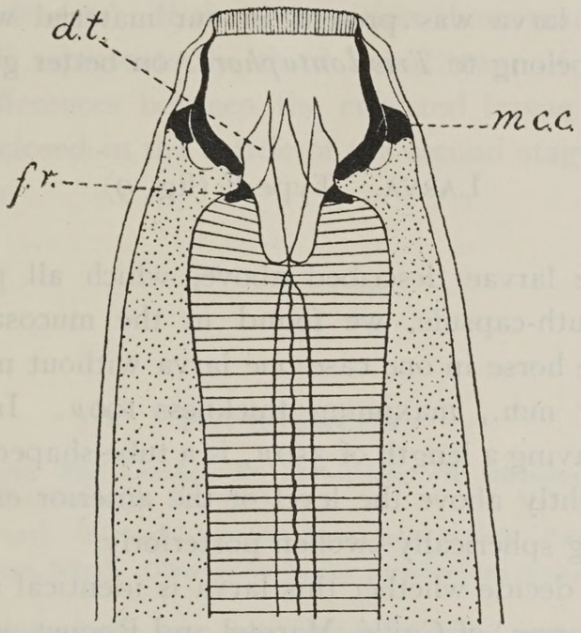

Fig. 8. Larva, type E, seen from right side. × 335. (× ¾). *d.t.*—Dorsal tooth; *f.r.*—Funnel-ring; *m.c.c.*—Mouth-capsule collar.

off from the mouth-collar. In some of the specimens it lies immediately against the cuticle. (The space between cuticle and mouth-capsule in the specimen figured is possibly due to the preservation.) The mouth-capsule collar reaches very far posteriorly beyond the equator of the mouth-capsule. Around the posterior part of the latter a circular cavity is already visible: the rudiments of the lumen of the adult mouth-capsule.

The oesophagus is long (750μ to 820μ), being about one-tenth of the body-length. The oesophageal funnel is well developed. It bears three large, pointed teeth, which protrude far into the lumen of the mouth-capsule. The dorsal tooth is a little larger than both the latero-ventral teeth. A funnel-ring is present. The distances from the anus to the rounded posterior extremity of the body is in

two specimens respectively 100μ and 110μ, in both other specimens respectively 220μ and 240μ; possibly these are sexual differences.

We suppose that these larvae belong to *Triodontophorus*, because the oesophageal funnel bears three large teeth protruding into the lumen of the mouth-capsule, this characteristic being present among the adult Strongylids of the horse, in *Triodontophorus* only. Moreover, the great length of the oesophagus of this type agrees with the long oesophagus in *T. intermedius* and *T. brevicauda*. For the rest, no other larva was present in our material which could be considered to belong to *Triodontophorus* on better grounds.

Larva. Type F (fig. 9).

Besides the larvae described above, which all possess a well-developed mouth-capsule, we found in the mucosa of the large intestine of the horse in one case one larva without mouth-capsule.

Length 3·7 mm., maximum thickness 190μ. In front of the oesophagus, having a length of 350μ, is a tube-shaped mouth-cavity, projecting slightly above the level of the anterior extremity of the body and being spherically swollen posteriorly.

We cannot decide whether this larva is identical with the 'larve metastrongyliforme' of Cuillé, Marotel and Roquet, which, however, is shorter (length 800μ to 2 mm.) than the specimen found by us.

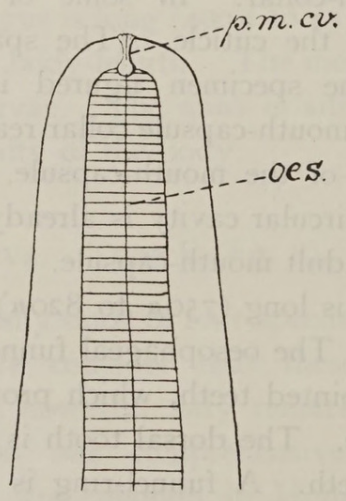

Fig. 9. Larva, type F. × 340 (× $\frac{3}{4}$). *p.m.cv.*—Provisional Mouth-cavity; *oes.*—Oesophagus.

Possibly both the larvae observed by the French authors and the specimen found by us are identical with a larva encountered by Leuckart (1876, p. 446) in the mucosa, being 1 mm. long and differing 'durch die Abwesenheit des Mundbechers, dessen Stelle durch einen schlanken und dünnhäutigen Chitincylinder vertreten war, wie bei den ersten parasitischen Jugendzuständen des *Dochmius trigonocephalus*. Die Umwandlung in die Form mit Mundbecher geschieht durch eine Häutung, die schon bei Exemplaren von 1·5 mm. vollendet ist.' If the supposition above made proves to be correct, Type F represents the third larval stage of *Cylicostomum*, though the differences between the encysted larvae (larvae of the third stage, enclosed in the cuticle of the second stage) and Type F are conspicuous.

REFERENCES

Albrecht, A. (1909). Zur Kenntnis der Entwicklung der Sklerostomen beim Pferde. *Zeitschr. f. Veterinärkunde*, Vol. XXI.

Boulenger, C. L. (1921). Strongylid parasites of horses in the Punjab. *Parasitology*, Vol. XIII.

Cobbold, T. Spencer, (1874). Observations on rare parasites from the horse. *Veterinarian*, Vol. XLVII.

——— (1875). Epizoöty in the horse, more especially in relation to the ravages, produced by the four-spined Strongyle (*Strongylus tetracanthus*). *Veterinarian*, Vol. XLVIII.

——— (1886). Description of *Strongylus arnfieldi* (Cobb.), with observations on *Strongylus tetracanthus* (Mehl.). *Journ. Linn. Soc.* (Zool.), Vol. XIX.

Cuillé, Marotel et Roquet (1913). Nouvelle et grave entérite vermineuse du cheval : la cylicostomose larvaire. *Bull. Mém. Soc. Sc. Vétérinaire*, Lyon.

Diesing, C. M. (1851). Systema Helminthum, Vol. II.

Giles, G. M. J. (1892). Some observations on the life-history of *Sclerostomum tetracanthum* Diesing and on Sclerostomiasis in equine animals, in connection with a so-called outbreak of 'Surra' at Shillong. *Scient. Mem. by Med. Officers of the Army of India.* Pt. 7.

Ihle, J. E. W. (1922). The adult Strongylids (Sclerostomes), inhabiting the large intestine of the horse. *Report of the Commission appointed to inquire into the Sclerostomiasis in Holland.* I. Zoological part, Vol. I.

Knox (1836). Remarks on the lately discovered Entozoa infesting the muscles of the human body ; with some observations on a similar animal found beneath the intestinal membrane of the horse. *Edinburgh Med. and Surg. Journ.*, Vol. XLVI.

Leuckart, R. (1876). Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten, Vol. II.

Looss, A. (1897). Notizen zur Helminthologie Egyptens. II. 3. Die Lebensgeschichte des *Anchylostomum duodenale* (Dub.). *Centralbl. Bakt. Parasitenk.* I Abt., Vol. XXI.