

Zoology. —“*On the development of the larva of the fourth stage of Strongylus vulgaris (Looss)*”. By Prof. J. E. W. INLE and Dr. G. J. VAN OORDT. (Communicated by Prof. C. PH. SLUITER).

(Communicated at the meeting of March 29, 1924).

The investigations of MAUPAS, LOOSS, SEURAT and others showed that many Nematodes undergo 4 moults during their larval development; consequently 5 stages can be distinguished in the post-embryonic life. During the 5th (imaginal) stage no changes occur in the body-structure; the worm gradually becomes adult.

Though the development of *Strongylus vulgaris* (Looss), syn. *Sclerostomum bidentatum* Sticker, a common parasite inhabiting the large intestine of the horse, has been investigated repeatedly, the number of moults occurring in this species, is not precisely known. The investigations of DE BLIECK (1923) and BAUDET showed that the free-living larva undergoes two moults, so that the horse infects it self *per os* with larvae of the third stage. As is often the case in Nematodes, this larva is enveloped by the cuticle, which was not shed during the second ecdysis (encysted larva). Moreover DE BLIECK and BAUDET stated that this larva, penetrating the wall of the intestine, sheds its cuticle. It is not known what happens to the larvae after the horse is infected. It is supposed that they are transmitted by the blood to their definitive seat in the arteries, especially in the Arteria mesenterica cranialis, where they cause the aneurysmata, so often mentioned in literature. This supposition is supported by the fact that several investigators found in liver and lungs small knobs of the size of the head of a pin, in which Nematode-larvae, some of which were calcinated, were found. These are considered to be larvae of *Strongylus vulgaris* (OLT, 1895, pp. 363, 364, ADELMANN, 1908, p. 16), which stay in liver or lungs and die there. However, we could not find any description about their structure in literature.

It is certain that the larvae living in the aneurysmata undergo one more moult, which takes place during their presence in the aneurysmata. For this reason we may consider these larvae to belong to the 4th stage. So it is not known how the larva of the 3^d stage,

which infects the horse, passes over into the larva of the 4th stage, which lives in the aneurysma and where the 3^d moult takes place.

In the following pages some particulars about the structure of the larva of the 4th stage and of the 4th ecdysis will be mentioned; we especially paid attention to the imaginal mouth-capsule, which develops in this larva, but which does not function till after the last moult.

The numerous larvae investigated, found in aneurysmata, were collected by the commission, appointed to inquire into the Sclerostomiasis in Holland. They were fixed in hot alcohol and rendered transparent in creosote afterwards.

Though larvae of the 4th stage are repeatedly mentioned in veterinary literature, the structure of them has not been completely investigated from a zoological point of view; cf. LEUCKART (1876, pp. 447—454) and STICKER (1901*a*). LEUCKART, especially traces the development of the mouth-capsule. STICKER investigated the development rather circumstantially. Besides the free-living stages he distinguishes a young and an old larval stage (p. 211). According to STICKER the "young larva" with mouth-rosette passes over into the old larva by means of a moult and the old larva into the adult worm by means of another ecdysis. However, we never observed the ecdysis last-mentioned, nor did we find any proof of it in the publication of STICKER. Consequently the "older larva" of STICKER must be the young worm, not yet sexually mature.

The smallest (male) larva of the 4th stage found by us in an aneurysma measures 3.8 mm. in length; so it is somewhat smaller than the smallest larvae (4 mm. in length), mentioned by STICKER (1901*b*, p. 54). Female larvae are larger than male ones of the same age, the former measuring 16—17 mm., the latter 11.5—13 mm. in length, just before moulting.

This larva is characterized by the possession of a round mouth-opening, surrounded by a six-lobed rosette, of which the margin is delicately denticulated (cf. STICKER, 1901*a*, p. 193; MARTIN, 1900, p. 22 (thesis), fig. III). This rosette is connected with the cuticle of the mouth-margin, but peripherally it is separated from it by a thin layer, as is visible in the optic section. The short funnel-shaped mouth-cavity conducts directly to the oesophagus. We did not observe an independent mouth-vesicle, mentioned by STICKER (1901*a*, p. 193), nor did we see a provisional mouth-cup, as LEUCKART (1876, pp. 450, 451) did. The oesophagus is cylindrical in shape and measures about 1.2 mm. in length in the largest specimens. The anterior pointed extremity of the oesophagus is surrounded by a

cuticular collar, originating from the wall of the mouth-cavity. The posterior margin of this oesophagus-collar is thicker than the anterior one. This collar is called by STICKER the "tiefere Schicht" of the "transparente Gebilde" (1901a, p. 193, in his figures indicated by c).

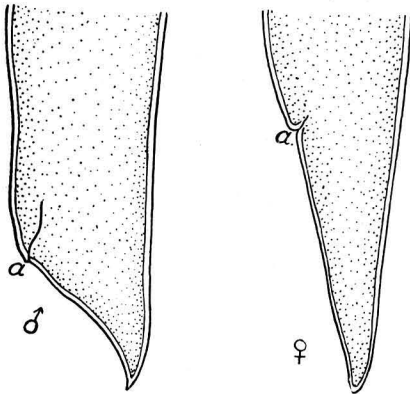


Fig. 1. Posterior end of a young male larva (long 5.6 mm.) and of a young female larva (long 5.1 mm.) of *Strongylus vulgaris* ($\times 183$).

Male and female larvae differ in the structure of the posterior end. In the σ the distance from the anus to the posterior extremity is much shorter than in the φ . Hence the posterior end of the σ is more obtuse than that of the φ (fig. 1).

The first changes, connected with the formation of the imaginal mouth-capsule, are found already in very young larvae. Peripherally of the oesophagus-collar, close behind the mouth-rosette, a circular slit-shaped cavity arises (fig. 2). Between the oesophagus-collar and this cavity is situated a fine granular substance, called by STICKER "oberflächliche Schicht" of the "transparente Gebilde" (STICKER 1901a, figs. 13, 14, 15, (c₁)). Gradually this cavity increases in size by

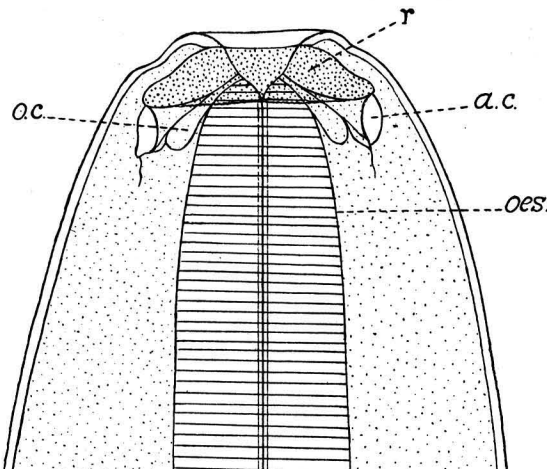


Fig. 2. Anterior end of a female larva of *Strongylus vulgaris*, long 9.1 mm. ($\times 275$).

extension in caudal direction. Then a second cavity develops, directly behind the anterior one, separated from it by a thin membrane (fig. 3). This septum is situated at the level of the posterior margin of the

oesophagus-collar. This posterior cavity is the rudiment of the definitive mouthcapsule-cavity, while the mouth-collar will develop

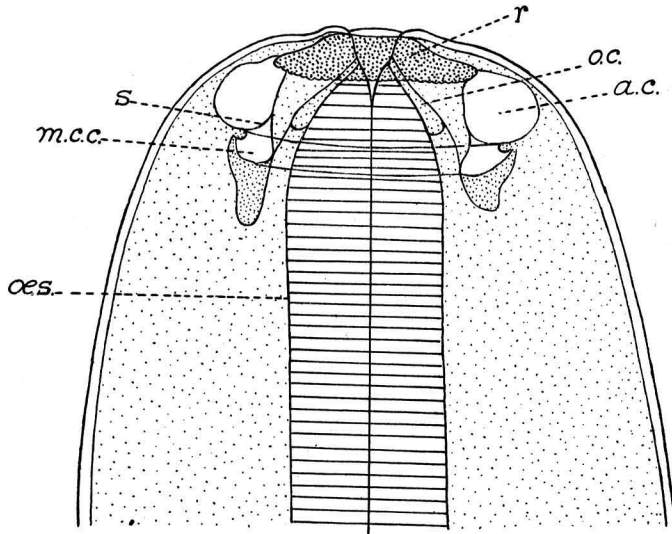


Fig. 3. Anterior end of a female larva of *Strongylus vulgaris*, long 16 mm. ($\times 210$). The rudiment of the definitive mouthcapsule-cavity is visible.

peripherally at the level of the septum. At the posterior side of this second cavity a granular substance, possibly the material from which the imaginal mouthcapsule-wall will develop, is found. In older larvae (fig. 4) the rudiment of the mouthcapsule-wall and of the

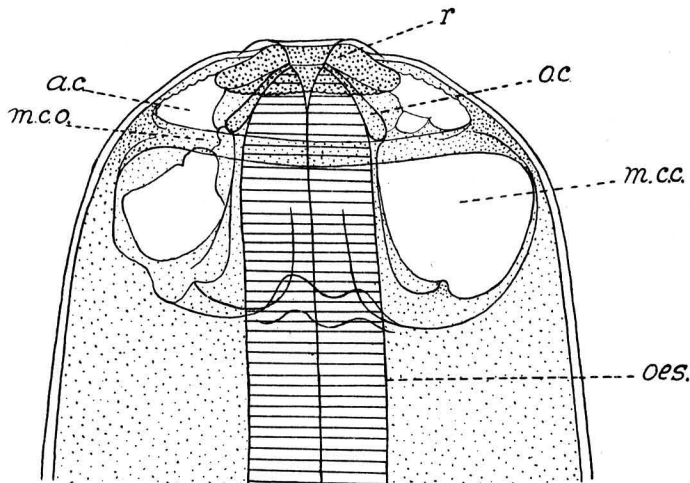


Fig. 4. Anterior end of an older female larva of *Strongylus vulgaris*, long 15.5 mm. ($\times 170$). The definitive mouth-capsule cavity and the mouth-collar are clearly perceptible.

mouth-collar are clearly perceptible. The septum, above mentioned, situated at the level of the mouth-collar, still separates the lumen of the definitive mouth-capsule from the anterior cavity.

We direct the attention to the agreement in the development of the mouth-capsule in *Strongylus* and in *Cylicostomum*: in the latter we also found (1923) this anterior cavity situated in front of the adult mouth-capsule.

From the above it follows that we cannot agree with the opinion of STICKER on the development of the mouth-capsule. This investigator (1901a, p. 194) derives the exterior leafcrown (STICKER'S "Wimperkranz") from the "oberflächliche Schicht" of the "transparente Gebilde" (the fine granular substance around the oesophagus-collar) and the rudiment of the mouth-capsule from the "tiefere Schicht" of the "transparente Gebilde" (oesophagus-collar).

As we said before, LEUCKART also traces the development of the mouth-capsule. He communicates (1876, p. 451) that a circular slit-shaped cavity occurs of which the posterior part becomes the lumen of the mouth-capsule and of which the anterior part corresponds to our "anterior cavity". A septum is not mentioned by LEUCKART.

Before the ecdysis itself takes place, the definitive mouth-capsule

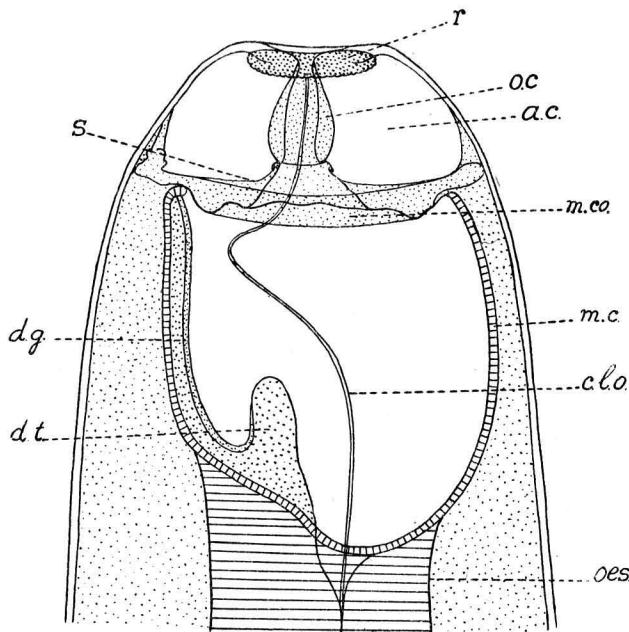


Fig. 5. Anterior end of an old female larva of *Strongylus vulgaris*, long 17 mm. ($\times 123$). The definitive mouth-capsule is present; the larval mouth-rosette and the oesophagus-collar have separated from the oesophagus.

is already completely formed (fig. 5). Then the dorsal gutter and the double tooth at the posterior end of the gutter are also present. Still a septum divides the anterior cavity from the cavity of the mouth-capsule. However, the anterior extremity of the oesophagus has loosened itself from the mouth-rosette and the oesophagus-collar: it has moved backward and forms the continuation of the mouth-capsule. Now this collar encloses a small cylindrical space; at the anterior side it is still connected with the mouth-rosette and at the posterior side with the septum. The internal cuticular lining of the oesophagus of the larva does not participate in the backward movement above mentioned and connects the mouth-rosette with the anterior extremity of the oesophagus.

Moulting specimens have been described several times. The young animal does not completely fill up the shed cuticle, which projects a long way at both ends of the worm (cf. LEUCKART, 1876, fig. 258; FIEBIGER, 1912, fig. 200). At this moment the cuticular lining of the oesophagus of the larva is still connected with the old cuticle, which is still provided with the mouth-rosette and the oesophagus-collar, now swollen.

Finally we remark that contrary to M. SCHLEGEL (1907, p. 52), OLT (1900, p. 393) and F. ADELMANN (1908, p. 26) we agree with the current opinion, which holds that the passage of the larvae of *Strongylus vulgaris* necessarily goes through the bloodstream. Hence the larvae, which are found in the aneurysma are by no means stray specimens. This is evident from the observation that larvae provided with a rosette, are exclusively found in the aneurysmata and not anywhere else in the body of the horse. If the passage through the bloodstream were not necessary, the larvae of the 4th stage would be met with in other places as well and this is never the case, a fact we can establish after having investigated an extensive material.

Utrecht, March 1924. *Zoological Laboratory, School of
Veterinary Medicine.*

LITERATURE CITED.

- ADELMANN, F. 1908. Das Aneurysma verminosum equi vom pathologisch-anatomischen, statistischen, klinischen und zoologischen Standpunkte. Thesis Giessen, also Arch. f. wiss. u. prakt. Tierheilkunde. Bd. 34.
- DE BLIECK, L. 1923. Infectie en prophylaxis bij strongylosis van het paard. Hand. XIXe Ned. Natuur- en Geneesk. Congres.
- FIEBIGER, J. 1912. Die tierischen Parasiten der Haus- und Nutztiere. Erste Auflage. Wien und Leipzig.

- IHLE, J. E. W. and VAN OORDT, G. J. 1923. On some Strongylid Larvae in the Horse, especially those of *Cylicostomum*. Annals of trop. Med. and Parasitology. Vol. 17.
- LEUCKART, R. 1876. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten. Bd. 2. Leipzig und Heidelberg.
- MARTIN, O. 1910. Beiträge zur Kenntnis der Verbreitung und Entwicklung des *Sklerostomum edentatum* Looss. Thesis Bern, also Arch. f. wiss. prakt. Tierheilkunde. Bd. 37.
- OLT, 1895. Die kalkig-fibrösen Knötchen in den Lungen und der Leber des Pferdes. Arch. f. wiss. u. prakt. Tierheilkunde. Bd. 21.
- OLT, 1900. Die Wanderungen des *Strongylus armatus* und Folgen seines Schmarotzerthums. Deutsche Tierärztliche Wochenschrift. Bd. 8.
- SCHLEGEL, M. 1907. Die Sklerostomenseuche (Sklerostomiasis) des Pferdes. Berliner tierärztliche Wochenschr. Jahrgang 1907.
- STICKER, A. 1901a. Untersuchungen über den Bau und die Lebensgeschichte des *Sclerostomum armatum*. Arch. f. wiss. u. prakt. Tierheilkunde. Bd. 27.
- STICKER, A. 1901b. Der Aufenthalt von *Sclerostomum armatum* in der Wand des Dickdarmes. Deutsche tierärztliche Wochenschrift. Bd. 9.

ABBREVIATIONS.

- a.* = anus.
a.c. = anterior cavity.
c.l.o. = cuticular lining of the oesophagus.
d.g. = dorsal gutter.
d.t. = dorsal tooth.
m.c. = mouth-capsule.
m.co. = mouth-collar.
o.c. = oesophagus-collar.
oes. = oesophagus.
r. = mouth-rosette.
s. = septum.
-