

# Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae

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**ABSTRACT:** Migration pattern of third-stage *Anguillicola crassus* larvae, and pathogenesis of the lesions induced by third-stage larvae, was investigated in European eel *Anguilla anguilla* L. Young elvers (1 g) were fed infected *Paracyclops fimbriatus* (Copepoda). Eel samples were collected and examined histologically at varying intervals during the 6 mo post-infection period. Third-stage larvae (L-III) migrated directly through the intestinal wall and body cavity to the swimbladder within 17h post-infection. L-IV larvae were detected 3 mo post-infection, and immature adults were detected within 4 mo post-infection. The parasites occasionally showed aberrant migration paths. Pathological effects caused by the parasite were less severe after experimentally induced infections than those detected in some natural infections.

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## INTRODUCTION

The parasitic nematode *Anguillicola crassus* (Kuwahara et al. 1974, Moravec & Taraschewski 1988) originates from eastern Asia where it infects the Japanese eel *Anguilla japonica*, but does not cause serious pathological changes (Egusa 1979). In contrast to Japanese eels, European eels *Anguilla anguilla* L. develop pathological effects from *A. crassus* infections (Egusa 1979, Liewes & Schaminee-Main 1987, van Banning & Haenen 1989).

The life cycle of *Anguillicola* spp. in Japanese eels has been described by Egusa (1979) and Puqin & Yuru (1980). The adult resides in the swimbladder lumen of the eel. After the female has copulated, the fertilized eggs are released through the vulva and, according to Egusa (1979), also by rupture of the female parasite. First-stage larvae (L-I) moult into second-stage larvae (L-II) while still within the egg. The eggs pass via the pneumatic duct through the digestive tract and out of the eel into the water. After hatching, the L-II larvae are eaten by copepods which serve as intermediate hosts. Inside the copepod the L-II larvae migrate to the haemocoel and moult into L-III larvae in 10d. When these copepods are eaten by eels the L-III larvae migrate through the lumen of the digestive tract to the

swimbladder lumen, where, according to Puqin & Yuru they moult into L-IV larvae 4 to 5 mo later. Immature adult and adult nematodes reside in the swimbladder lumen and feed actively on eel blood. The total life cycle of *Anguillicola* spp. in the Japanese eel has been estimated at 1yr (Egusa 1979, Puqin & Yuru 1980).

The life cycle of *Anguillicola crassus* in European eels was studied recently by De Charleroy et al. (1989), who demonstrated that, under optimal conditions, the life cycle of *A. crassus* in European eels takes less than 2 mo.

In 1980 Puqin & Yuru proposed a direct migration route of third-stage larvae of *Anguillicola globiceps* through the intestinal lumen and body cavity into the swimbladder lumen of Japanese eel *Anguilla japonica*.

This report describes the migration of L-III larvae of *Anguillicola crassus* in the European eel and the pathological effects induced by these parasites in an experimentally induced infection.

## MATERIALS AND METHODS

Eggs of *Anguillicola crassus*, containing L-II larvae, were collected from the swimbladder fluid of an infected eel. These eggs were released into fresh water

at 20 °C, where they hatch within a few hours (De Charleroy et al. 1989). The intermediate host, the copepod *Paracyclops fimbriatus*, was cultured at 20 °C in the laboratory and fed with the newly hatched L-II larvae (estimated equal numbers of larvae and copepods). The mean infection level of the copepods, after 9 d infection, was 1.2 larvae copepod<sup>-1</sup>. Three hundred unparasitised European eelers, each weighing ca 1g, were fed with the infected copepods (1 exposure of about 4 times as many copepods as eels). Afterwards, the eelers were kept in water at 20 °C and fed with commercial pellet food, at a rate of 2 % of body weight per day.

After this single infection, 60 % of the eels were found to be infected with *Anguillicola crassus* larvae (infection level varying from 1 up to more than 20 larvae; dependent on the individual feeding behaviour of the eels).

At 28 different time intervals, samples of 10 eels were collected, anaesthetized and fixed in Bouin Hollande

for histological examination (t = 0 and 1h post-infection [p.i.]; every 4 h during the first 3 d; at 4, 7 and 8 d p.i.; and at 1, 2, 3, 4 and 6 mo p.i.). Histological sections of 4 µm were stained with hematoxylin and eosin or trichrome, according to the method of Pollack (1944).

## RESULTS

One hour after feeding the eels with the infected copepods, L-III larvae of *Anguillicola crassus* were detected in the stomachs of the eels. The swimbladder was still uninfected (Fig. 1). At 1h p.i., L-III larvae were detected in the different layers of the digestive tract, especially in the submucosa, and in the body cavity. Tunnels were detected in the wall of the digestive tract (Fig. 2), as well as haemorrhages with numerous mononuclear phagocytes (Fig. 3). Until 17h p.i. this situation did not change. At 17h p.i. L-III larvae were detected for the first time in the swimbladder wall (Fig. 4); they

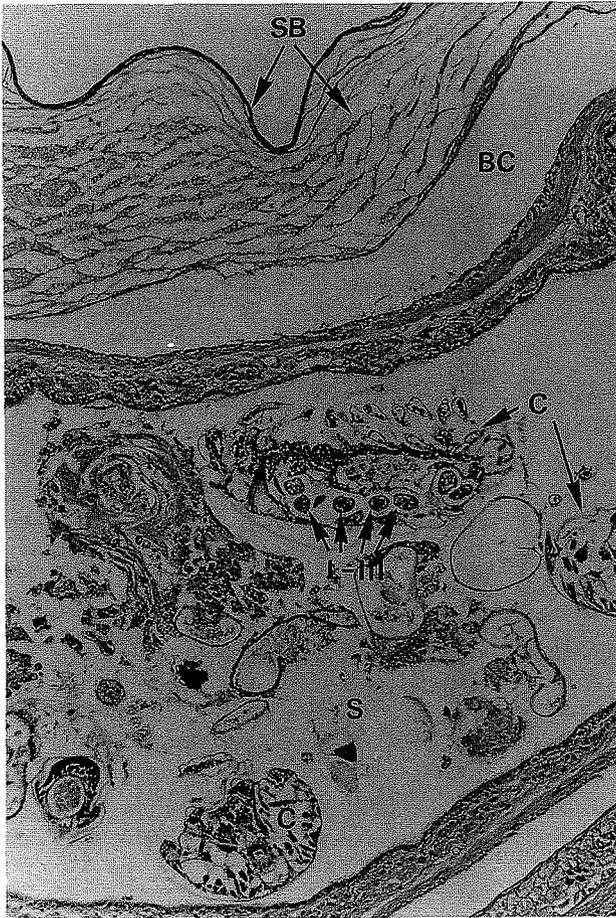


Fig. 1. *Anguilla anguilla*. Copepods (C) containing L-III larvae of *Anguillicola crassus* in the stomach (S) of eel, 1h p.i. The swimbladder (SB) is not yet infected. BC: body cavity. H&E, 100 x, cross section

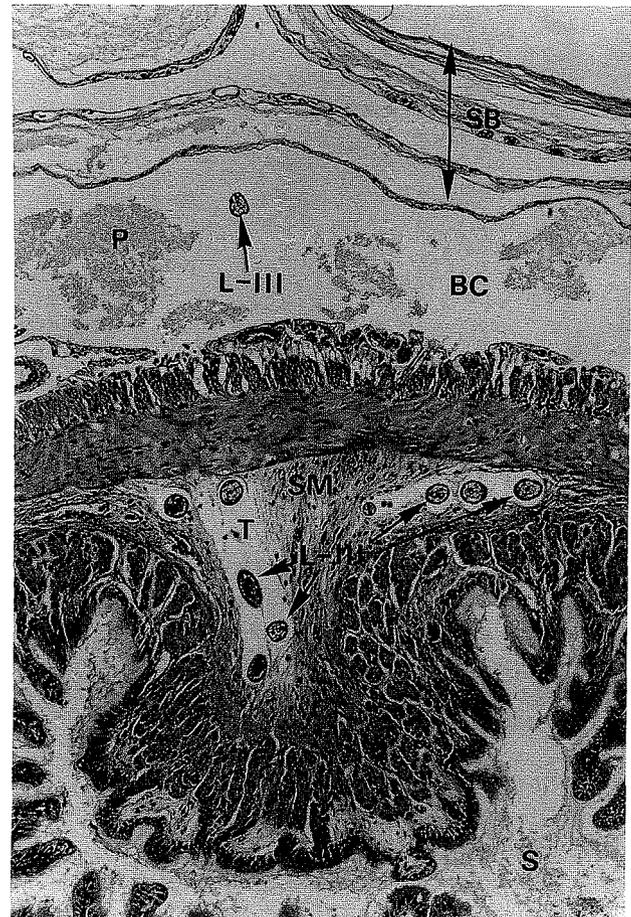


Fig. 2. *Anguilla anguilla*. L-III larvae of *Anguillicola crassus* migrating directly through the submucosa (SM) of the stomach (S) to the body cavity (BC) and caudal lunnets (T), 5 h p.i. Protein (P), a sign of haemorrhages, is visible in the body cavity. The swimbladder (SB) is not yet infected. Trichrome, 100 x, cross section

were situated in the subserosa and had not yet fed on eel erythrocytes. This situation remained unchanged until 3 mo p.i. At 3 mo p.i. L-IV larvae were detected for the first time, in the swimbladder wall; eel erythrocytes were detected within the parasite's intestine (Fig. 5). At 4 mo p.i. immature adults, full of eel erythrocytes and developing gonads, were detected within the swimbladder lumen (Fig. 6). At 6 mo p.i. this was again seen. No adult parasites were found at all. A summary of the results is given in Table 1. No pathological effects, such as inflammations or fibrosis of the swimbladder, were detected.

Occasionally, L-III larvae were detected migrating aberrantly, for instance in the ventral musculature (Fig. 7).

### DISCUSSION

In this study the artificially induced infection of *Anguilla anguilla* with *Anguillicola crassus* was successful. We could detect the migration patterns of the parasitic larvae.

The 17 h period between feeding with infected copepods and the first appearance of L-III larvae in the swimbladder wall is remarkably short. The L-III larvae were detected mostly in the submucosa of the digestive tract, where they apparently reside some time before passing the denser muscularis. What attracts the larvae to the swimbladder is not known. Some larvae migrated aberrantly, but most of the L-III larvae migrated to the swimbladder.

L-IV larvae were detected in the swimbladder wall at 3 mo p.i., earlier than was reported for Japanese eels

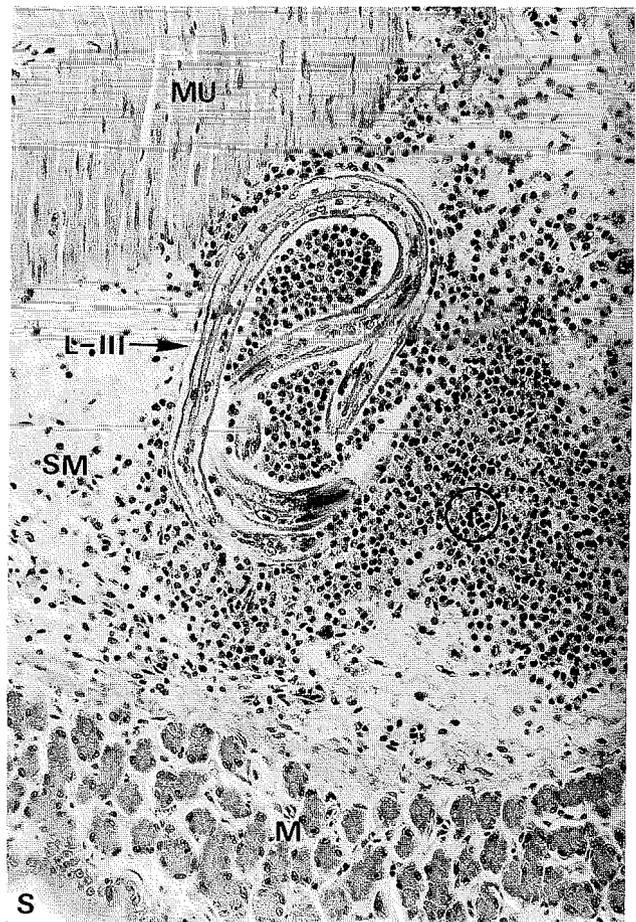


Fig. 3. *Anguilla anguilla*. Longitudinal section of L-III larvae of *Anguillicola crassus* situated in the submucosa (SM) of the eel stomach (S). Inflammatory cells (I) (mainly mononuclear phagocytes and erythrocytes) are visible. M: mucosa; MU: muscularis. Trichrome, 200 x

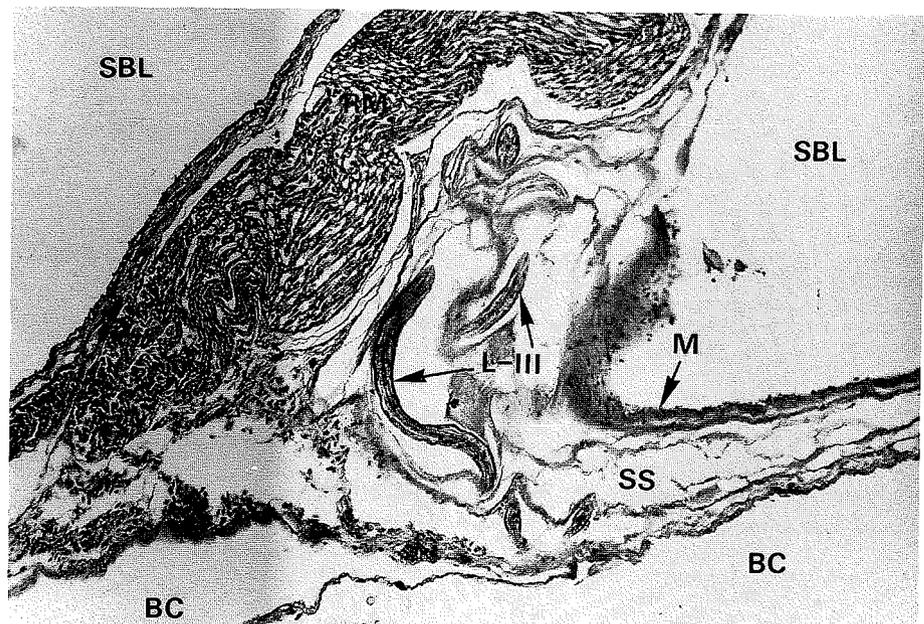


Fig. 4. *Anguilla anguilla*. Longitudinal section of eel showing L-III larvae of *Anguillicola crassus* situated in the swimbladder subserosa (SS), 17h p.i. M: mucosa; RM: rete mirabile, gas organ; SBL: swimbladder lumen; BC: body cavity. Trichrome, 100 x

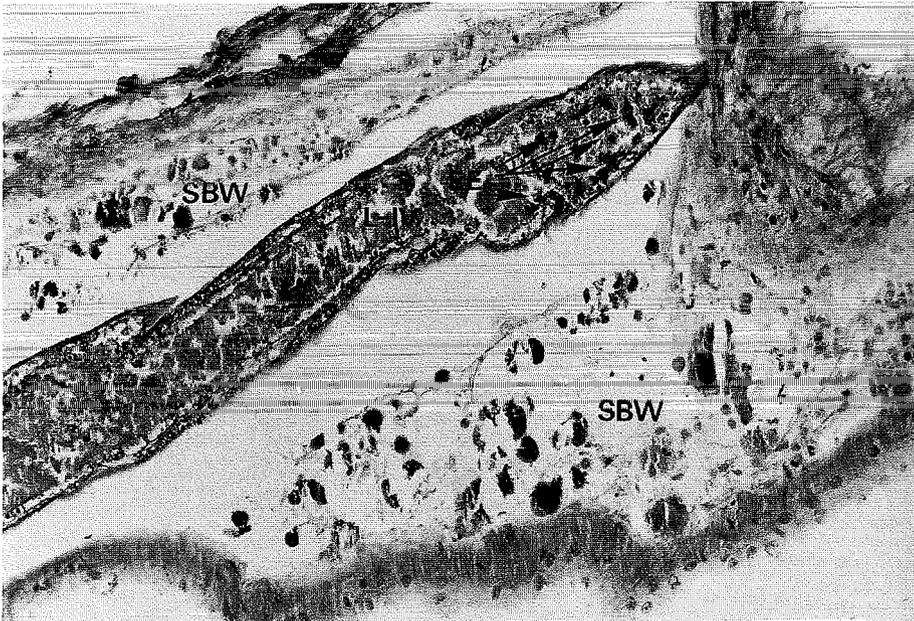


Fig. 5. *Anguilla anguilla*. Detail of L-IV larva of *Anguillicola crassus* situated in the swimbladder wall (SBW), 3mo p.i. Eel erythrocytes (EE) are visible within the parasite. Trichrome, 200 x

(Puqin & Yuru 1980). These larvae had already been feeding on eel erythrocytes.

Since immature adults were detected in the swimbladder at 4 mo p.i." the life cycle of the nematode in European eels is considerably shorter than that described for Japanese eels (1 yr) (Egusa 1979, Puqin & Yuru 1980). The life cycle seems longer however, than that reported for European eel by De Charleroy et al. (1989), of 2 mo. This is probably related to the different detection methods used in the experiments. De Charleroy et al. examined whole swimbladders of fresh eels for *Anguillicola crassus*, whereas we only examined a few histological sections. Therefore, eels in our experi-

ments may actually have contained older larval stages at earlier times, which we missed.

Although we are aware of no reports that *Anguillicola crassus* migrates aberrantly in the Japanese eel, our study revealed that the nematode does on occasion migrate aberrantly in the European eel.

In a previous study of naturally occurring infections (van Banning & Haenen 1989), we demonstrated that *Anguillicola crassus* caused pathological changes in eels. In contrast, the infections experimentally induced in the present study did not cause severe pathological changes. Eels living under natural conditions may be continuously exposed to nematode infections and thus

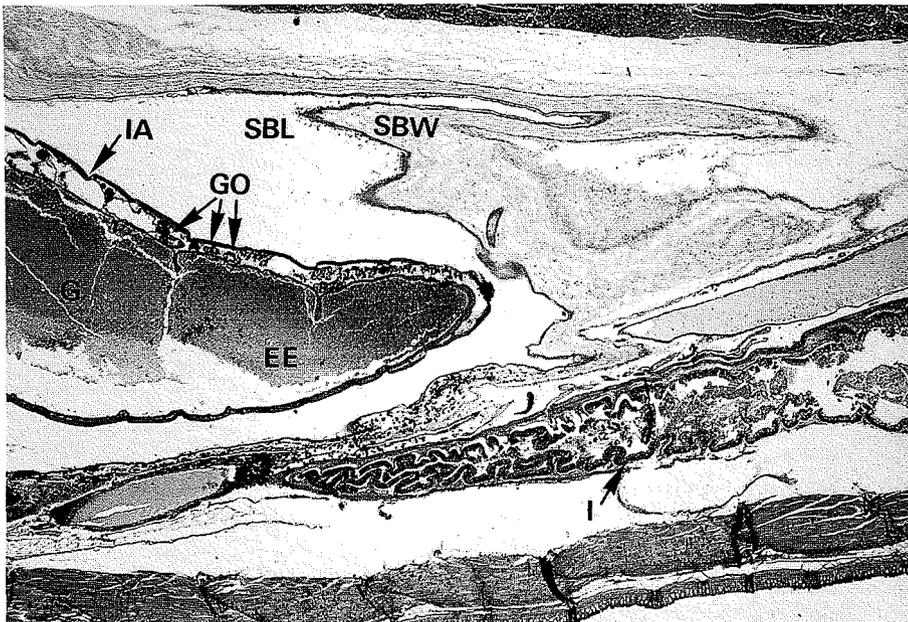


Fig. 6. *Anguilla anguilla*. Immature adults (IA) of *Anguillicola crassus* situated in the swimbladder lumen (SBL) of the eel, 4 mo p.i. The gut (G) of the parasite is filled with eel erythrocytes (EE). GO: Early development stage of gonads; SBW: swimbladder wall; I: intestine. Trichrome, 20 x

Table 1. *Anguillicola crassus* in *Anguilla anguilla*. Location of parasite larvae in the European eel after experimentally induced infection

Location	Time p.i.				
	1 h	5 h	17 h	3 mo	4 mo
Intestinal lumen	L-III	L-III	L-III	L-III	L-III
Intestinal wall		L-III	L-III	L-III	L-III
Body cavity		L-III	L-III	L-III	L-III
Swimbladder wall			L-TT	L-III & L-IV	L-III & L-IV
Swimbladder lumen					IA
IA: immature adult					

may suffer more severe lesions than the experimentally infected eels, which were exposed only once to the parasites. Therefore, future studies on the pathological changes induced by *A. crassus* on European eels should include repeated experimentally induced infections.

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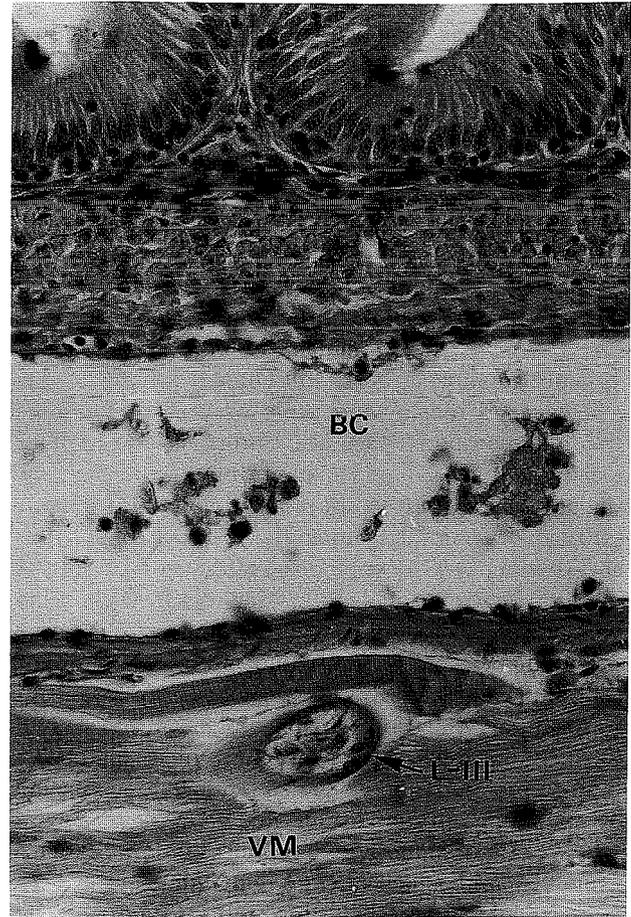


Fig. 7. *Anguilla anguilla*. L-III larva of *Anguillicola crassus* aberrantly migrating through the ventral musculature (VM) of the eel. BC: body cavity. H&E, 400 x

Responsible Subject Editor: Professor W. Körting, Hannover, P.R. Germany