Larval production of cats infected and re-infected with Aelurostrongylus abstrusus (Nematoda: Protostrongylidae)

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SUMMARY

Production of larvae by Aelurostrongylus abstrusus following infection and re-infection was studied by infecting 17 cats with 800 third stage larvae each and by re-infecting eight animals after 390 days. Peak larval production occurred 60-120 days after infection. Two infected cats continued to produce A. abstrusus larvae for over one year. Fifty-six percent of the animals began to produce larvae 90 days after re-infection then again from 150 days onwards. The pre-patent period for re-infected animals was thus generally longer than that for those infected only once. One animal that produced larvae immediately after re-infection ceased production 30 days later, demonstrating immunity until the end of the study. The larval output persisted for two animals till the 660th day, when the experiment was terminated.

KEY-WORDS: Aelurostrongylus abstrusus - cats - Baermann method.

Introduction

Aelurostrongylus abstrusus (Railliet, 1898) is a protostrongylid nematode of worldwide distribution [2, 10, 13] that parasitizes the lung parenchyma, pulmonary artery and its branches in domestic and wild cats. Infection frequently leads to pneumonia, pleural effusion, pyothorax and even death in the absence of therapeutic intervention [3, 8, 11].

The life cycle of A. abstrusus is complex, oviposition taking place in lung parenchyma and small blood vessels where the first stage larvae develop, passing along the digestive tract before being expelled in the feces. The intermediate hosts are snails, either terrestrial (Helix aspersa, Mesodon thyroideus and Triodopsis albolabris) or aquatic (Biomphalaria glabrata), infected through invasion or ingestion of first stage larvae. The nematode develops in the snail until it reaches the infective third larval stage [1, 4, 11, 12].

Infection of the cat results from ingestion of the infective intermediate host or paratenic hosts such as frogs, lizards, snakes, birds, mice and rats [1, 10]. The infective larvae penetrate the mucosae of the cat digestive system and travel via the lymphatic system to the lungs, where they mature to adult worms.

The objective of this study was to evaluate the kinetics and larval production of cats infected and re-infected experimentally with A. abstrusus.

Materials and methods

A) INFECTIO N AND RE-INFECTION

Twenty-two mixed breed female cats (Felis domesticus) of mean age 18.2 months were used in the study (Table I). The
animals were reared in the laboratory or donated by breeders
and were free of infection by \textit{A. abstrusus}. They were caged
individually and supplied with commercial cat food (Hill’s
Feline Maintenance) and water \textit{ad libitum}.

Infective larvae (L3) were recovered from experimentally
infected \textit{B. glabrata}. In order to infect these snails, groups of
10 were placed in Petri dishes containing 400 L1 larvae in 10
ml dechlorinated water, heated by a 70W lamp placed at a
distance of 40 cm for 24 h. After this period the snails were
transferred to a plastic tank 20 cm wide and 40 cm long
containing dechlorinated water. Thirty days after infection
the snails were sacrificed, wrapped in surgical gauze and pla-
ced in a Baermann apparatus containing an 1 \% aqueous pep-
sin solution for 24 h, to recover the infective (L3) larvae. The
L3 thus harvested were counted using the 25X objective of a
stereoscopic and aliquots of 800 larvae were stored in hemo-
lysis tubes containing dechlorinated water, until used to
infect the cats.

During the infection and re-infection procedures, the cats
were anesthetized with atropine sulfate (0.044 mg/kg sc),
then after 15 min given intramuscular injections of acepro-
mazine maleate (0.1 mg/kg) and ketamine hydrochloride
(5 mg/kg) in the same syringe. Seventeen animals in the
infection experiment and eight in the re-infection experiment
were inoculated individually with 800 infective larvae (L3)
of \textit{A. abstrusus} diluted in 1.5 ml of 0.9 \% saline solution
administered via a stomach tube. Five animals submitted to
the same anesthetic procedure received only 1.5 ml of saline
solution and were kept as uninfected controls. Re-infection
was performed on eight randomly selected animals 390 days
after infection. In the infection all animals were observed for
24 h after inoculation. Animals which vomited during this
period were withdrawn from the experiment to guarantee that
all those included in the study had received the correct infec-
tive dose. Re-infected animals which vomited were not reti-
red from the study.

B) LARVAL PRODUCTION

Fecal samples were collected from the cage of each animal
on the day of infection and re-infection, as well as on three
consecutive days, to determine the direct passage of infective
larvae.

Fecal samples were examined daily from 20 days post-
infection (dpi) and re-infection (dpr) onwards to determine
the pre-patent period (PPP). Thereafter, fecal samples were
examined once per month, during a total observation period
of 660 days.

The Baermann method was used to examine all fecal
samples, which contained 100 g of material.

Results

A) INFECTION AND RE-INFECTION

Clinical observations are summarized in Table II. All ani-
imals presented abdominal movements within 5 min of infec-
tion or re-infection. After infection (ai), three (18 \%) cats
vomited within 300 min and were withdrawn from the study,
while eight (47 \%) showed symptoms of nausea until 240
min ai. One animal (13 \%) vomited 120 min after re-infection
and seven (87 \%) demonstrated nausea during 40 min.

L3 larvae were not present in the feces of any of the ani-
mals within three days of infection or re-infection.

The animals in the uninfected control group did not show
any changes during infection and re-infection.

B) LARVAL PRODUCTION

The mean pre-patent periods (PPP) for cats experimentally
infected and re-infected with \textit{A. abstrusus} are presented in
Table III. Values for the first infection ranged from 35 - 48
days, with a mean of 38 days.
LARVAL PRODUCTION OF CATS INFECTED AND RE-INFECTED WITH *AEUROSTRONGYLUS ABSTRUSUS*

Table II. — Clinical symptoms in cats after experimental infection and re-infection by *Aelurostrongylus abstrusus*.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Infection</th>
<th>Re-infection</th>
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<tbody>
<tr>
<td>1</td>
<td>Abdominal movements 15 and 30 seconds&lt;br&gt;Nausea — 120, 180 and 240 minutes</td>
<td>Abdominal movements 30 seconds and 1 minute&lt;br&gt;Nausea — 5 minutes&lt;br&gt;Vomiting — 120 minutes</td>
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<td>2</td>
<td>Abdominal movements 20 seconds</td>
<td>Abdominal movements 30 seconds&lt;br&gt;Nausea — 5 and 15 minutes</td>
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<tr>
<td>3</td>
<td>Abdominal movements 20 and 40 seconds&lt;br&gt;Vomiting — 15 and 300 minutes</td>
<td>Abdominal movements 1 and 3 minutes&lt;br&gt;Nausea — 5, 8 and 20 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Abdominal movements 20 seconds, 1 and 3 minutes&lt;br&gt;Vomiting — 45 and 60 minutes</td>
<td>Abdominal movements 15 and 30 seconds, 2 and 5 minutes&lt;br&gt;Nausea — 3, 10 and 40 minutes</td>
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<tr>
<td>5</td>
<td>Abdominal movements 30 and 50 seconds</td>
<td>Abdominal movements 3 minutes&lt;br&gt;Nausea — 5, 8, 30 and 40 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Abdominal movements 10, 30 and 50 seconds, 1 minute&lt;br&gt;Vomiting — 20 minutes</td>
<td>Abdominal movements 40 seconds, 2 and 3 minutes&lt;br&gt;Nausea — 9 and 20 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Abdominal movements 5, 20, 30 and 45 seconds</td>
<td>Abdominal movements 10, 20 and 50 seconds&lt;br&gt;Nausea — 3 minutes</td>
</tr>
<tr>
<td>8</td>
<td>Abdominal movements 30 and 50 seconds, 2 and 3 minutes</td>
<td>Abdominal movements 10 and 50 seconds, 2,3 and 5 minutes&lt;br&gt;Nausea — 1, 5, 20 and 40 minutes</td>
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<tr>
<td>9</td>
<td>Abdominal movements 20 and 40 seconds&lt;br&gt;Nausea — 15 and 30 minutes</td>
<td>Abdominal movements 20 and 40 seconds&lt;br&gt;Nausea — 1, 4, 7, 10, 15, 20 and 25 minutes</td>
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<td>10</td>
<td>Abdominal movements 15 and 40 seconds, 2 minutes</td>
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<td>11</td>
<td>Abdominal movements 30 seconds&lt;br&gt;Nausea — 30 and 60 minutes</td>
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<td>12</td>
<td>Abdominal movements 20 seconds&lt;br&gt;Nausea — 15 minutes</td>
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<td>13</td>
<td>Abdominal movements 30 and 55 seconds</td>
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<td>14</td>
<td>Abdominal movements 40 seconds and 2 minutes&lt;br&gt;Nausea — 10 minutes</td>
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<td>15</td>
<td>Abdominal movements 20 seconds and 3 minutes&lt;br&gt;Nausea — 5 minutes</td>
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<td>16</td>
<td>Abdominal movements 10 and 30 seconds</td>
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<tr>
<td>17</td>
<td>Abdominal movements 20 seconds, 2 and 3 minutes&lt;br&gt;Nausea — 20 minutes</td>
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Table III. — Individual and mean values for pre-patent periods of cats experimentally infected and re-infected with *Aelurostrongylus abstrusus*.

<table>
<thead>
<tr>
<th>Cat</th>
<th>PPP</th>
<th>Cat</th>
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<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>1</td>
<td>82</td>
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<td>2</td>
<td>90</td>
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<td>36</td>
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<td>11</td>
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<td>12</td>
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<td>17</td>
<td>48</td>
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</table>

Mean 38 Mean 88
Table IV. — Individual larval production / 100g feces in cats infected with *Aelurostrongylus abstrusus*.
Five re-infected animals (63%) began to produce larvae again 80 - 100 days after re-infection. Three (37%) did not present larvae in the feces. The mean PPP among animals which resumed larval production was 88 days.

The individual larval output/g of feces during the entire 660-day observation period is shown in Table IV. Peak larval production occurred was achieved between 60 and 120 dpi.

Although there was generally a gradual reduction in larval output after 120 dpi, two cats maintained considerable levels of production until the end of the study.

Peaks of larval output were seen for three (21%) animals at 60 dpi, for nine (64%) at 90 dpi and for one each (8%) at 180 dpi and 360 dpi.

One cat produced large numbers of larvae from the day of re-infection until 30 dpr, when they disappeared from the feces until the end of the experiment. At 90 dpr, larvae were found in the feces of five animals: three of these ceased to produce larvae in the feces at this point, while two maintained L1 output until the end of experiment. The other three animals (37%) were free of larvae in the feces during the entire observation period.

The mean larval output per month of cats experimentally infected and re-infected with A. abstrusus, expressed as logarithmic values [log (Lpg + 1)], are shown in Figs. 1 and 2.

Two cats died naturally during the experiment; one at 90 dpi, when the animal concerned showed the greatest number of L1 larvae in the feces, and the other at 15 dpr. Necropsy of the first animal revealed lesions only in the lungs, containing numerous abscesses in various stages of development. Collateral congestion was seen in the caudal lobe, with probable intra-alveolar hemorrhage. Chronic productive pleurisy was observed with adherence of the visceral and parietal pleura to the right cranial lobe. In the second animal, pyothorax was observed, originating from a fistulated pulmonary abscess with purulent and fetid material spilling into the pleural space. The pulmonary surface presented diffuse nodular abscesses, in some regions these coalesced into larger abscesses, causing it to appear torn. Necrosis was observed in the visceral and parietal pleura, as well as the pericardium and the mediastinal tissue, accompanied by severe purulent pleurisy was also observed.

![Figure 1](image1.png)

**FIGURE 1.** — Production of L1 larvae of *Aelurostrongylus abstrusus* in cat feces following infection.

![Figure 2](image2.png)

**FIGURE 2.** — Production of L1 larvae of *Aelurostrongylus abstrusus* in cat feces following re-infection 390 days after infection.
Discussion

The strong abdominal movements observed following infection and re-infection suggest that the inoculated material, which also contained residues of snail tissue, produced acute irritation of the gastric mucosae. Although PENNISI et al. [10] stated that this irritation is due only by the snail tissues, larval activity may also irritate the mucosae enough to produce abdominal movements, nausea and vomiting.

The mean PPP value of 38 days observed for the first infection was shorter than that found by HAMILTON & MacCaw [7]. The shortest PPP observed (35 days) was longer than the 30 days reported by HAMILTON [6] whereas the longest value (48 days) was considerably shorter the 63 days observed by HAMILTON & MacCaw [7]. Fifty-six percent of the re-infected animals, resumed production of larvae in the feces, although with a longer PPP than for the first infection. This resumption of larval output after re-infection does not support the conclusions of HAMILTON [5] who reported that this led to immunity without further production of larvae. However, this immunity was significant for those animals which did not produce larvae in the feces following re-infection.

The longest period of larval output after first infection ranged from 60 - 120 days, shorter than the interval of 90 - 210 days by SOULSBY [12] who found the highest larval output ranged from 90 to 210 days, but similar to that observed by HAMILTON [6].

With regard to the two fatalities, one occurred at the point of highest larval output in the feces (90 dpi), due to pneumonia and multiple lung abscesses. The other death took place 15 days after re-infection, when larvae penetrate the lung parenchyma and there is no larval output in the feces, although with a longer PPP than for the first infection. Although both deaths were directly related to A. abstrusus infection, the pathology of infection occurred in different situations, the first fatality being due to marked egg-laying activity, hatching of eggs and larval migration through the lung parenchyma, while the second was due to penetration and maturity of infective larvae in the pulmonary and pleural tissues. Both pathologies are characteristic of aelurostrongylosis in cats.

At 150 dpi, 64 % of the animals showed larvae in the feces with a daily maximum output of 10 larvae/g. After 180 dpi only two cats (14 %) produced more than 10 larvae/g of feces. These animals maintained this output until 390 dpi, although one cat produced less than 10 larvae/g at 300 dpi, production then recovering to the higher level until 390 dpi. At 390 dpi, one of these animals was re-infected but remained free of larvae in the feces till the end of this experiment. The other cat continued to produce larvae until 450 dpi. These two animals thus showed patencies of infection superior to those reported by HAMILTON [6] and LOSONSKY et al. [9].

At 90 dpr five animals (56 %) showed larvae in the feces; however only one (11 %) produced more than 10 larvae/g. At 120 dpr no animals showed larvae in the feces, then from 180 - 240 dpr only one animal showed more than 10 larvae/g of feces. At 270 dpr (660 days after the first infection), two animals produced larvae in the feces, albeit less than 10 larvae/g. Although the larval output in the feces following re-infection is sufficient to present a risk of transmission the amount of L1 larvae observed was lower than that produced as the result of first infections.

References