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The cockroach *Periplaneta americana* as a potential paratenic host of the lungworm *Aelurostrongylus abstrusus*

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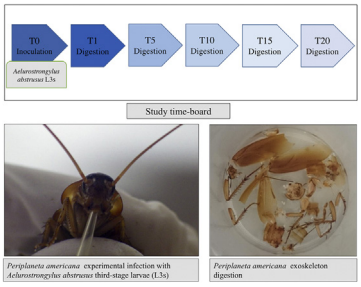
HIGHLIGHTS

- Spectrum of *Aelurostrongylus abstrusus* paratenic hosts is not yet completely defined.
- *Aelurostrongylus abstrusus* lifecycle involves several species of paratenic hosts.
- Cat lungworms larvae found alive in the cockroach 20 days after inoculation.
- *Periplaneta americana* is proposed as a further lungworm source for domestic cats.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: *Aelurostrongylus abstrusus* is a well-known nematode affecting the respiratory system of felids worldwide. Snails and slugs act as intermediate hosts of this parasite, whereas rodents, birds and reptiles may serve as paratenic hosts. *Periplaneta americana*, the American brown cockroach, shares the same habitat and ecological features (e.g. nocturnal activity) with both snails and cats. The aim of this study was to evaluate the capability of *P. americana* to maintain alive *A. abstrusus* third stage larvae (L3s) after artificial inoculation.
Material and methods: Twenty-five specimens of *P. americana* were infected with 100 *A. abstrusus* L3s collected from experimentally infected *Cornu aspersum* snails, whereas five specimens were used as control group. After the infection, cockroaches were maintained in individual plastic boxes until dissection for the presence of L3s at 1 (T1), 5 (T5), 10 (T10), 15 (T15), and 20 (T20) days post-infection.
Results: Except for T15, alive *A. abstrusus* L3s (n = 63) were found at all time-points, being 26, 19, 16 and 2 L3s retrieved at T1, T5, T10 and T20, respectively. Eleven (17.4%) L3s were found within the digestive tract, 10 (15.9%) in other-than-digestive organs and 42 (66.7%) in the exoskeleton and associated tissues. Nine out of the twenty-five experimentally inoculated cockroaches (36%) died soon after the artificial infection (T1), while in the control group, two out of the five (40%) died before the end of the study (T15) with no difference in the mortality rate between groups.
Discussion: Results of this study suggest that *P. americana* could act as a paratenic host of *A. abstrusus*. *Periplaneta americana* cockroaches, have a ubiquitous distribution and may be preyed by cats, representing a potential source of infection to cats living in endemic areas.

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1. Introduction

The lungworm *Aelurostrongylus abstrusus* (Strongylida, Angiostrongylidae) is a well-known and widely distributed (Giannelli et al., 2017) nematode species affecting the respiratory system of domestic cats (*Felis silvestris catus*). Adult females localize in terminal bronchi, alveolar ducts and pulmonary alveoli, where they lay eggs, from which first-stage larvae (L1s) hatch, eventually reaching the pharynx where they are swallowed and shed within host faeces (Anderson, 2000; Gerichter, 1949). In the environment, L1s of *A. abstrusus* can survive for up to 60 days being infective to snails and slugs intermediate hosts (Gökpinar and Yildiz, 2010; Gerichter, 1949). In gastropods L1s develop to third-stage larvae (L3s), and the life cycle continues when gastropods harbouring infective L3s, are ingested by cats. Alternative routes of transmission of metastrongyloids to domestic cats include the shedding of L3s in the environment by dead or alive gastropods (Giannelli et al., 2015). Additionally, snail-to-snail transmission of *A. abstrusus* L3s (also known as *intermediesis*) has been proposed as a potential way for the establishment of endemic foci of infected gastropods in a given area (Colella et al., 2015). Nonetheless, infected snails alone may be not sufficient to account for the high prevalence of feline aelurostrongylosis detected in cat populations. Accordingly, rodents, birds and reptiles may serve as paratenic hosts, playing a role in the transmission of L3s to the definitive hosts (Anderson, 2000; Gerichter, 1949; Hobmaier and Hobmaier, 1935).

Periplaneta americana (Dictyoptera, Blattoidae), the American brown cockroach, is one of the most common scavenger insects (Bell and Adiyodi, 1981). This insect species originated in tropical Africa and became globally distributed, favoured by the trade of goods (Bell and Adiyodi, 1981). This cockroach species generally live in moist areas, but can survive in dry habitat where a source of water is available. In addition, American brown cockroaches share the same habitat and ecological features (e.g. nocturnal activity) with snails (Bailey, 1981). *Periplaneta americana* feed on mammalian faeces, garbage and sewage (Rust et al., 1991) and they can assume and disseminate bacteria, such as *Escherichia coli*, *Streptococcus* Group D, *Bacillus* spp., *Klebsiella pneumoniae*, and *Proteus vulgaris* (Zarchi and Vatani, 2009) as well as nematodes, such as *Toxocara canis* (Sasmal et al., 2008). The aim of this study was to assess the capability of *P. americana* to maintain alive *A. abstrusus* L3s after oral inoculation.

2. Material and methods

2.1. Nematode collection

Aelurostrongylus abstrusus L1s were obtained from the faeces of a naturally infected cat using the Baermann technique (Hendrix, 1998). Larvae were concentrated by centrifugation at 1678 x *g* for 5 min, identified at species level using morphometrical keys (Gerichter, 1949; Brianti et al., 2014), suspended in sterile saline solution and divided in infective doses of 0.1 mL containing 250 L1s each. *Cornu aspersum* (Eupulmonata, Helicidae) specimens (n = 60) were infected by injecting the infective dose in the muscular foot (Napoli et al., 2016). In order to rule out the presence of natural nematode infections, 5 snails were digested 5 days before the beginning of the study. After the infection the snails were maintained in a plastic vivarium (45 × 30 × 10 cm), kept at room temperature (20 °C ± 3 °C, 80% ± 5% R.H. and 16:8 light:dark cycle) and fed *ad libitum* with fresh vegetables. Snails were sacrificed and processed through a hydrochloridric acid/pepsin artificial digestion to recover *A. abstrusus* L3s (Colella et al., 2015), 20 days after the infection.

Larvae were collected under a light microscope and stored in tubes, each containing a dose of 100 L3s, suspended in saline solution to a final volume of 0.2 mL.

2.2. Cockroach rearing and inoculation procedure

Periplaneta americana (n = 100) were bought from an insect farm in Germany (Jöerg Bernhardt, Halsbrücke, Germany) and maintained in a soft net box in dark condition, providing water soaked cotton wads, bakery products, fresh fruits and vegetables. Five days before the larval inoculation, *P. americana* (n = 5) were dissected and digested to detect if any other nematode species was present. Two days before artificial infection, 30 cockroaches were placed in individual plastic boxes (15 × 10 × 10 cm). Infective doses of 100 *A. abstrusus* L3s were concentrated by centrifugation (1678 x *g* for 5 min) to a final volume of 30 μL and administered by a gentle introduction of the tip between the buccal mandibular appendices, in order to allow the cockroaches (n = 25) to ingest the larvae (Fig. 1, Video 1). An equal volume of saline solution was administered to the remaining 5 specimens (control group). After the inoculation procedure, the cockroaches were monitored for 30 min and kept individually in the plastic boxes until dissection. Five cockroaches were randomly dissected and analysed (see below) at 1 (T1), 5 (T5), 10 (T10), 15 (T15), and 20 (T20) days post-infection.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.exppara.2017.09.023.

2.3. Insect dissection

Dissection of cockroaches was performed after rinsing the insects with saline solution to remove larvae potentially trapped during the infection procedures. Cockroaches were sacrificed by decapitation in a Petri dish and the whole digestive system from the oral part of the oesophagus to the anus (herein after referred as to the “alimentary canal”) was isolated from the remaining body (Cameron, 1961). The alimentary canal and other-than-digestive

Fig. 1. Experimental inoculation of *Periplaneta americana* with *Aelurostrongylus abstrusus* third-stage larvae (100 larvae/30 μL).