Larval development of the cat lungworm *Aelurostrongylus abstrusus* in the tropical freshwater snail *Biomphalaria glabrata*

EVA-MARIA ZOTTLER *and* MANUELA SCHNYDER*

University of Zurich, Vetsuisse Faculty, Institute of Parasitology, Winterthurerstrasse 266a, 8057 Zurich, Zurich, Switzerland

SUMMARY

Aelurostrongylus abstrusus is a worldwide occurring lungworm affecting felids. This metastrongyloid nematode has an indirect lifecycle relying on slugs and snails as intermediate hosts. In the present study the development of first-stage (L1) to third-stage larvae (L3) in the tropical freshwater pulmonate snail *Biomphalaria glabrata* was assessed. A total of 306 snails were individually exposed to 300 *A. abstrusus* L1, which were obtained from a naturally infected stray cat. The species was confirmed by biomolecular analysis. Second stage larvae (L2) and L3 were first isolated by artificial digestion of snails in the second and fourth week post exposure (wpe), respectively. From 8 wpe onwards, all larvae had developed into L3. Snails remained infected for up to 26 wpe. Only 0.4% of the L1 had pursued their development into L3, indicating low suitability of this artificial intermediate host for production of infective *A. abstrusus* L3.

Key words: *Aelurostrongylus abstrusus*, lungworm, cats, *Biomphalaria glabrata*, snails, larval development, intermediate host, experimental infection.

INTRODUCTION

The nematode *Aelurostrongylus abstrusus* is a worldwide occurring cat lungworm with prevalences varying between 0.5 and 39.7% in Europe (Barutzki and Schaper, 2011; Knaus *et al.* 2014), Australia (Palmer *et al.* 2008; Lacorcia *et al.* 2009), North and South America (Lucio-Forster and Bowman, 2011; De Souza Ramos *et al.* 2013). Infected cats mostly show respiratory signs (Grandi *et al.* 2005; Traversa *et al.* 2008), but non-pathognomic signs (Genchi *et al.* 2014; Schnyder *et al.* 2014) or asymptomatic cats are also commonly observed (Genchi *et al.* 2014). In some cases, an infection with *A. abstrusus* may result in death (Gerdin *et al.* 2011; Dirven *et al.* 2012; Philbey *et al.* 2014). *Aelurostrongylus abstrusus* has an indirect lifecycle in which gastropods serve as intermediate hosts (Hobmaier and Hobmaier, 1935; Gerichter, 1949) and various animals, such as rodents, reptiles and birds (Hamilton and McCaw, 1967; Scott, 1973; Jezewski *et al.* 2013) can serve as paratenic hosts. Since Hobmaier and Hobmaier's studies (1935), different molluscs have been used as intermediate hosts and the development of *A. abstrusus* from first (L1) to third stage (L3) larvae has been described (Gerichter, 1949; Wallace and Rosen,

1970; Ash, 1970; Lopez *et al.* 2005; Di Cesare *et al.* 2013; Giannelli *et al.* 2014).

Biomphalaria glabrata is a neotropical, freshwater pulmonate snail. Because of its essential role as a natural intermediate host for *Schistosoma mansoni*, *B. glabrata* has been studied and maintained in laboratories for a long time. Consequently, suitable conditions for breeding and maintenance of this very rapidly reproducing snail are well known (DeJong *et al.* 2001; Pointier *et al.* 2005). *B. glabrata* has previously been used as an intermediate host for *A. abstrusus* (Ash, 1970; Wallace and Rosen, 1970; Jefferies *et al.* 2010). However, descriptions of the chronological and morphometric development of *A. abstrusus* L1 to L3 in this snail and the success rate of snail infections, which are key factors for the identification and recovery of infective larval stage for further experimental studies, were missing to date.

The aims of this study were to describe and assess the development rate from L1 to the infective and relevant L3 stage of *A. abstrusus* in the snail *B. glabrata*, as well as to evaluate the suitability of this species as an experimental intermediate host.

MATERIAL AND METHODS

The *A. abstrusus* L1 used for the infection of 306 *B. glabrata* snails (sized 0.8–1.5 cm in diameter) were obtained from feces from a 2 to 3 months old naturally infected female stray cat with high-grade dyspnoea presented at the Animal Hospital of the Vetsuisse Faculty of the University of Zurich. L1

* Corresponding author. Institute of Parasitology, University of Zurich, Vetsuisse Faculty, Winterthurerstrasse 266a, 8057 Zürich, Switzerland. Tel.: ++41 (0)44 635 85 25. Fax: ++41 (0)44 635 8907. E-mail: manuela.schnyder@uzh.ch

doi:10.1017/pao.2016.3

were isolated using the Baermann–Wetzel technique (Deplazes *et al*. 2013) and analysed with a duplex-polymerase chain reaction (PCR) (Annoscia *et al*. 2014) to verify their species identity and to exclude a co-infection with the lungworm *Troglostrongylus brevior*.

The snails were bred at the Institute of Parasitology at the Vetsuisse Faculty of the University of Zurich and thus, had no previous contact with other parasites. Prior to infection, the snails were fasted for 24 h in order to increase the ingestion rate, and placed individually into the wells of a 24 well polystyrene cell-culture plate (Nunc™, Roskilde, Denmark) with 2–3 mL of tank water, each containing 300 L1 of *A*. *abstrusus*. After 24 h the wells were checked for remaining L1 with a stereomicroscope (Leica® MS 5, Leica Microsystems GmbH, Wetzlar, Germany) and the snails placed into two large tanks. They were maintained under standardized conditions at constant temperature (24–26 °C) and fed on lettuce, in accordance with the accepted principles of animal welfare for invertebrates. Every week, five snails were randomly chosen, humanely euthanized by prompt crushing and digested individually for 1 h in 50 mL H_2O, 1% HCl containing 0.12 g Pepsin (800–2500 U mg^{-1}, Sigma P700, Sigma-Aldrich, Missouri, USA) in a water bath rotary shaker (Aquatron®, Infors AG, Basel, Switzerland) at 45 °C. After digestion, the tubes containing the material were centrifuged at $500 \times \boldsymbol{g}$ for 3 min. Each pellet was re-suspended in tap water, centrifuged again and the sediment examined under the stereomicroscope. Larvae were stained with Lugol, photographed, measured by using a digital image processing system (Leica® DM 100 LED, Leica®

DFC 420, Leica® LAS 4, Leica Microsystems GmbH, Wetzlar, Germany) and morphologically identified based on previously described developmental stage characteristics (Gerichter, 1949; Di Cesare *et al*. 2013). Starting from the day when all isolated larvae had developed into L3, the time interval between digestions was extended to every second week until the end of the trial (28 weeks post exposure, wpe). In the eighth and ninth wpe, 200 snails were removed to extract the L3 for other purposes.

Microsoft Excel 2010 for Windows (Microsoft Corporation, Redmond, USA) was used to calculate means and standard deviations (SD). Statistical analysis was performed using Windows IBM® SPSS ® Statistics (Version 22). The Mann–Whitney *U*-Test was used for comparing the number of larvae per snail across time points. Differences with $P < 0.05$ were considered statistically significant.

RESULTS

As confirmed by PCR, *A*. *abstrusus* was the only parasite present in the cat feces from which L1 were isolated. Twenty-four hours after snail exposure to L1, a mean of 10.1 L1 (SD: 14.02 L1) per well were counted, indicating a penetration rate of 96.6%. Morphological features are presented in Fig. 1A–C. L1 presented the typical notched S-shaped tail with a dorsal dent, L2 displayed a characteristic outer sheath, and L3 showed a round knob at the tip of the tail.

L1 were present until 6 wpe. In the first wpe a high number of L1 in the snails were damaged. The first L2 were obtained in the second wpe, while the first L3 occurred in the fourth wpe. After

Fig. 1. *Aelurostrongylus abstrusus* larvae: (A) first-stage larva and detail of the tail end; (B) second-stage larva; (C) third-stage larva and detail of the tail end (stained with Lugol).
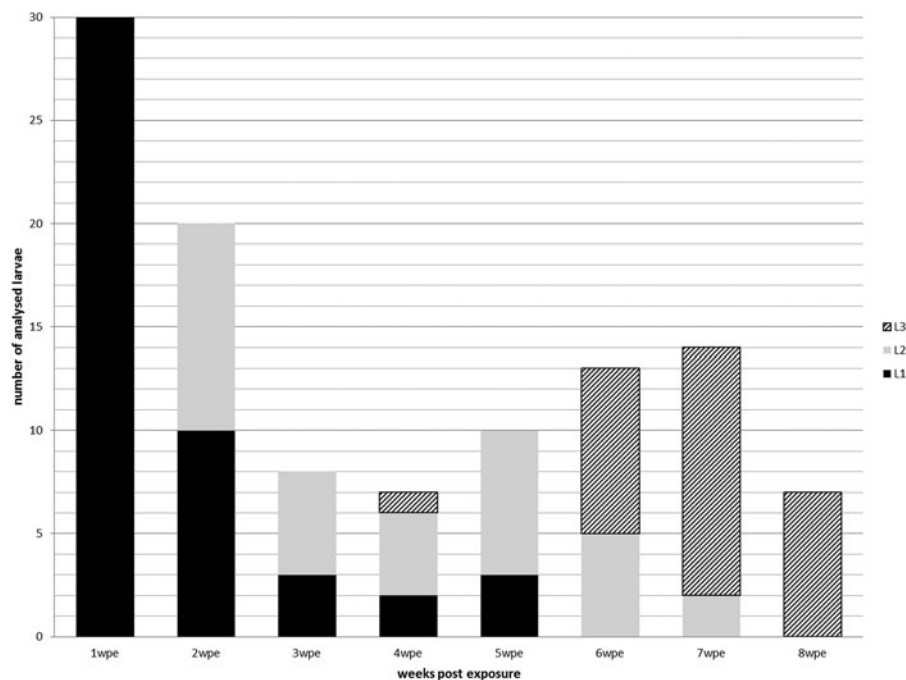
Fig. 2. Occurrence of first (L1), second (L2) and third-stage larvae (L3) of *Aelurostrongylus abstrusus* in *Biomphalaria glabrata* snails in weeks post exposure (=wpe).

8 wpe, all larvae had reached the L3 stage (Fig. 2). Except for one dead L3 observed after artificial digestion, all recovered L3 were alive and motile throughout the experiment. Larval sizes (length and width) are summarized in Table 1 and are presented with results from previously published studies. L3 were isolated from snails until 26 wpe, while at 28 wpe, the remaining last five snails were all found exempt of L3. Development times for the different larval stages of *A. abstrusus* are shown in Table 2, besides findings from other studies where different snail species were used.

From the first wpe (mean: 21.2, SD: 14.3) to the second wpe (mean: 4.4, SD: 8.3), we observed a significant reduction of larvae per snail ($P < 0.05$). Afterwards, the number of larvae per snail remained constant. From the second to the seventh wpe, a mean of 4.7 (SD: 0.5) larvae per snail were recovered (Fig. 3). After 8 wpe, a further non-significant decrease in the number of recovered larvae was noted, down to a mean of 0.6 (SD: 0.3; $P > 0.05$) larvae per snail. In total, only 0.4% of the L1 used for inoculation of the snails completely developed into L3 in the course of the study.

DISCUSSION

Our data confirm the feasibility of experimental infections with the tropical freshwater snail *B. glabrata* as a potential intermediate host for the felid lungworm *A. abstrusus*. However, there are indications that this non-terrestrial snail species is not an adequate intermediate host for mass production of *A. abstrusus* L3.

Our measurements of total length and width of L1, L2 and L3 are in line with previously published numbers (Table 1). However, we obtained a broader range of length and width within each developmental stage than what others reported, while in our hands, L3 tended to be longer in size than previously described for L3 recovered from the same snail species (Ash, 1970). As suggested elsewhere, the size of snails as well as the duration of the life cycle within the gastropods may influence the size of the larvae (Di Cesare *et al*. 2015). Furthermore, also the fixative and brightening agents can influence the measured dimensions of larvae (Lopez *et al*. 2005).

Comparing the larval development time to the L3 stage with prior studies (Table 2), the occurrence of L3 appears delayed in the present work (4 wpe) than in investigations carried out with the terrestrial snails *Helix aspersa* (15 and 21 dpi) and *Cernuella virgata* (18 dpi). Nonetheless, the time point when all larvae had reached the L3 stage (8 wpe) was consistent with the findings of the other authors.

As many as 96.6% of L1 had invaded the snails after 24 h, representing a higher penetration rate than described by Lopez *et al*. (2005), with 65.5% in experimentally infected *C. virgata*. The mechanisms for infecting aquatic and terrestrial snails are different, which may explain discrepancies in terms of penetration and development rate. Nematodes infect aquatic snails more often passively, through accidental ingestion by the snail, and less commonly by direct penetration of the snail tegument. In contrast, in terrestrial snails direct penetration, mostly through the foot, is a very common mechanism of

Table 1. Morphometric data of the developmental stages (first stage (L1), second stage (L2) and third stage (L3)) of *Aelurostrongylus abstrusus* larvae in intermediate hosts obtained from the present work and from reports published elsewhere (measurements in µm: L: body length, W: body width). n.m. = not mentioned

	Present study[a]	Gerichter (1949)[b]	Lopez et al. (2005)[c]	Giannelli et al. (2014)[c]	Di Cesare et al. (2013)[a]	Ash (1970)[a]	Thiengo et al. (2008)[a]	Jezewski et al. (2013)[a]
L1	L: 409.7 (367.4–458.6) W: 17.7 (13.8–27.6)	L: 360–390 W: 18–19	L: 400 ± 21.3	L: 384.5 ± 33.4 W: 17.7 ± 2.6	L: 397.4			
L2	L: 453.3 (384.9–532.8) W:27.6 (19.8–37)	L: 450–480 W: 31–34	L: 490 ± 38.9	L: 479.4 ± 53.6 W: 27.6 ± 4.5	L: 533.6 (470–570)		L: 454 (424–519)	
L3	L: 541.2 (423.7–673.4) W: 30.3 (21.1–40.9)	L: 460–510 W: 26–28	L: 608 ± 31.7	L: 538.9 ± 51.8 W: 26.7 ± 1.9	L: 586.7 (550–670)	L: 519 (459–568) W: 29 (27–31)	L: 583 (546–602)	L: 533 (461–566) W: 29 (25–38)
Intermediate host	Biomphalaria glabrata	11 different land snails	Cernuella virgata	Helix aspersa	Helix aspersa	Biomphalaria glabrata	Achatina fulica	Arion lusitanicus
	Aquatic snail	Terrestrial snails and slugs	Terrestrial snail	Terrestrial snail	Terrestrial snail	Aquatic snail	Terrestrial snail	Terrestrial slug
Temperature	24–26 °C	n.m.	20 ± 2 °C	20 ± 1 °C	18.8–29.5 °C 6.7–11.4 °C	n.m.	n.m.	n.m.
Type of infection	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental	Natural	Natural

[a] Mean and range in brackets.
[b] Range.
[c] Mean and standard deviation.

infection (Morley, 2010). In the study by Lopez *et al.* (2005) nearly 5% of the L1 developed into L3. Di Cesare *et al.* (2013) obtained an even higher development rate, with up to 50% developing in *H. aspersa*. In the present study the percentage of larvae completing their development into L3 was limited to 0.4%. Therefore, a high ingestion/penetration rate is not necessarily an indication for a high development rate. The reasons behind this low development rate in the present study remain unclear. The snail species, size and age, the L1 isolate, its age and the infective dose, as well as the environmental conditions may all influence the development rate (Di Cesare *et al.* 2015). Environmental conditions were described to have an important impact on larval development in intermediate hosts: the maturation is faster and more larvae start and complete their development at higher temperatures, respectively, in summer, i.e. in *H. aspersa* snails infected with *A. abstrusus* (Di Cesare *et al.* 2013; Giannelli *et al.* 2014). As we kept the environmental factors constant at the indicated temperatures for *B. glabrata* under established experimental conditions, other reasons for this low development rate have to be considered. In the past, we used to have a higher development rate of *A. abstrusus* L3 in *B. glabrata* at our institute (data not shown). Since the L1 originated from different cats, a diverging infectivity of the isolate cannot be excluded. Concerning the infective dose per snail, Wallace and Rosen (1970) infected *B. glabrata* with 100–800 *A. abstrusus* L1, obtaining a higher development rate. Since we had minor loss of snails over 28 wpe ($n = 6$), we conclude that the number of L1 ($n = 300$) used in our study should have been well tolerated by the snails.

Previous studies showed that after snails are infected with nematode larvae, their defence mechanisms are activated (Sauerländer, 1976; Pereira *et al.* 2006; Barcante *et al.* 2012). The difference between i.e. a penetration rate of 65.5% and a development rate of 9.96% into later stages suggested that a majority of L1 are destroyed during or shortly after penetration (López *et al.* 2005). In the present study we observed a significant drop of L1 per snail from the first to the second wpe. Furthermore, in the first wpe a high number of L1 in the snails were damaged. This strengthens the concept that a high number of incorporated larvae may not start their development and are destroyed by the immune system of the snails. Other reasons for the reduction of larval numbers within snails over time are possible. The last *A. abstrusus* positive snails in this study were identified at 26 wpe, while it has been shown in *H. aspersa* that snails can remain infected for up to 2 years (Hamilton, 1969). This might additionally suggest that in the most suitable intermediate hosts larvae may survive as long as the snails live. On the other hand, we cannot rule out the possibility that the absence of larvae at 28 wpe in *B. glabrata*

Table 2. Key time points in the development from first- to third-stage *Aelurostrongylus abstrusus* larvae in different gastropods in days post exposure (dpe) from the present work and from previous reports

	Present study (dpe)	Gerichter (1949) (dpe)	Lopez *et al.* (2005) (dpe)	Di Cesare *et al.* (2013) (dpe)	Giannelli *et al.* (2014) (dpe)
First L2	14	11	12	S: 15 W: 15	6
First L3	28	18	18	S: 21 W: 37	11
Only L3	56 dpe	n.e.	52 dpe	S: 58 dpe	n.e.
Snail species	*Biomphalaria glabrata*	Different terrestrial snails	*Cernuella virgata*	*Helix aspersa*	*Helix aspersa*

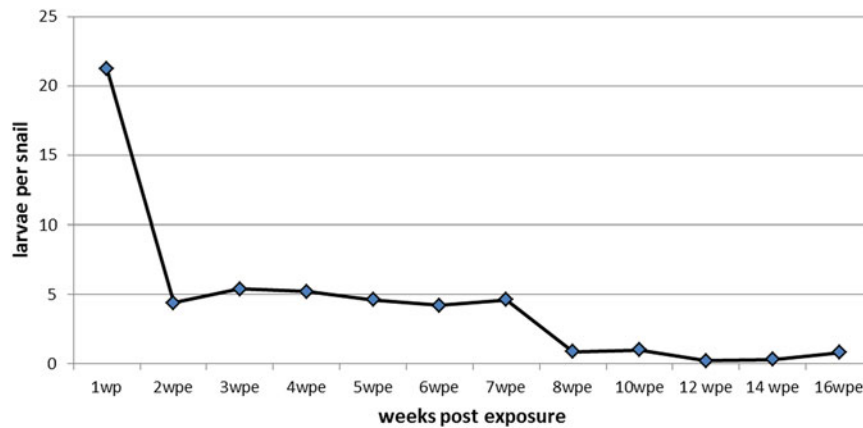S, summer; W, winter; n.e., not evaluated.

Fig. 3. Mean number of *Aelurostrongylus abstrusus* larvae (first, second and third stage) per dissected *Biomphalaria glabrata* snail over 16 weeks post exposure (wpe).

was due to spontaneous departure of L3 from the snails, as it has been demonstrated for the same snail species infected with *Angiostrongylus vasorum*, another metastrongyloid nematode (Barcante *et al.* 2003), or in *H. aspersa* infected with *A. abstrusus* (Giannelli *et al.* 2015).

Due to its high fertility and easy maintenance, *Biomphalaria glabrata* is not only a naturally occurring intermediate host but also a perfect experimental intermediate host for *S. mansoni* (DeJong *et al.* 2001; Pointier *et al.* 2005), and proved its suitability for experimental infections also with other metastrongylid parasites such as *A. vasorum* (Ash, 1970; Barcante *et al.* 2012). The findings presented here describe in detail the development of *A. abstrusus* from L1 to L3 and the development rate in *B. glabrata*. In particular, this study provides new insights into the chronological development of *A. abstrusus* in the aquatic snail. Knowledge of the time needed for all larvae to reach the infectious L3 stage and awareness of the potentially very low development rate are critical for the planning of subsequent experimental studies with metastrongyloid parasites.

CONFLICT OF INTEREST

None.

ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the use of invertebrate animals.

REFERENCES

Annoscia, G., Latrofa, M. S., Campbell, B. E., Giannelli, A., Ramos, R. A., Dantas-Torres, F., Brianti, E. and Otranto, D. (2014). Simultaneous detection of the feline lungworms *Troglostrongylus brevior* and *Aelurostrongylus abstrusus* by a newly developed duplex-PCR. *Veterinary Parasitology* **199**, 172–178.

Ash, L. R. (1970). Diagnostic morphology of the third-stage larvae of *Angiostrongylus cantonensis*, *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus*, and *Anafilaroides rostratus* (Nematoda: Metastrongyloidea). *Journal of Parasitology* **56**, 249–253.

Barcante, T. A., Barcante, J. M. P., Dias, S. R. C. and Lima, W. S. (2003). *Angiostrongylus vasorum* (Baillet, 1866) Kamensky, 1905:

emergence of third-stage larvae from infected *Biomphalaria glabrata* snails. *Parasitology Research* **91**, 471–475.

Barcante, T. A., Barcante, J. M., Fujiwara, R. T. and Lima, W. S. (2012). Analysis of circulating haemocytes from *Biomphalaria glabrata* following *Angiostrongylus vasorum* infection using flow cytometry. *Journal of Parasitology Research*, ID 314723. doi: 10.1155/2012/314723.

Barutzki, D. and Schaper, R. (2011). Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. *Parasitology Research* **109** (Suppl.), S45–S60.

DeJong, R. J., Morgan, J. A., Paraense, W. L., Pointier, J.-P., Amarista, M., Ayeh-Kumi, P. F., Babiker, A., Barbosa, C. S., Brémond, P. and Canese, A. P. (2001). Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. *Molecular Biology and Evolution* **18**, 2225–2239.

Deplazes, P., Eckert, J., Von Samson-Himmelstjerna, G. and Zahner, H. (2013). Diagnostische Methoden. In *Lehrbuch der Parasitologie für die Tiermedizin* (ed. Deplazes, P. and Eckert, J.), pp. 508–524. Enke press, Stuttgart, Germany.

De Souza Ramos, D. G., Alves da Cruz Scheremeta, R. G., Soares de Oliveira, A. C., Sinkoc, A. L. and de Campos Pacheco, R. (2013). Survey of helminth parasites of cats from the metropolitan area of Cuiaba, Mato Grosso, Brazil. *Revue Brasiliana Parasitologica Veterinaria* **22**, 201–206.

Di Cesare, A., Crisi, P. E., Di Giulio, E., Veronesi, F., Frangipane di Regalbono, A., Talone, T. and Traversa, D. (2013). Larval development of the feline lungworm *Aelurostrongylus abstrusus* in *Helix aspersa*. *Parasitology Research* **112**, 3101–3108.

Di Cesare, A., Crisi, P. E., Bartolini, R., Iorio, R., Talone, T., Filippi, L. and Traversa, D. (2015). Larval development of *Angiostrongylus vasorum* in the land snail *Helix aspersa*. *Parasitology Research* **114**, 3649–3655.

Dirven, M., Szatmári, V., Van den Ingh, T. and Nijsse, R. (2012). Reversible pulmonary hypertension associated with lungworm infection in a young cat. *Journal of Veterinary Cardiology* **14**, 465–474.

Genchi, M., Ferrari, N., Fonti, P., De Francesco, I., Piazza, C. and Viglietti, A. (2014). Relation between *Aelurostrongylus abstrusus* larvae excretion, respiratory and radiographic signs in naturally infected cats. *Veterinary Parasitology* **206**, 182–187.

Gerdin, J. A., Slater, M. R., Makolinski, K. V., Looney, A. L., Appel, L. D., Martin, N. M. and McDonough, S. P. (2011). Postmortem findings in 54 cases of anesthetic associated death in cats from two spay-neuter programs in New York State. *Journal of Feline Medicine and Surgery* **13**, 959–966.

Gerichter, C. B. (1949). Studies on the nematodes parasitic in the lungs of Felidae in Palestine. *Parasitology* **39**, 251–262.

Giannelli, A., Ramos, R. A., Annoscia, G., Di Cesare, A., Colella, V., Brianti, E., Dantas-Torres, F., Mutafchiev, Y. and Otranto, D. (2014). Development of the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in *Helix aspersa* snails. *Parasitology* **141**, 563–569.

Giannelli, A., Colella, V., Abramo, F., do Nascimento Ramos, R. A., Falsone, L., Brianti, E., Varcasia, A., Dantas-Torres, F., Knaus, M., Fox, M. T. and Otranto, D. (2015). Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways. *PLoS Neglected Tropical Diseases* **9**, e0003722.

Grandi, G., Calvi, L. E., Venco, L., Paratici, C., Genchi, C., Memmi, D. and Kramer, L. H. (2005). *Aelurostrongylus abstrusus* (cat lungworm) infection in five cats from Italy. *Veterinary Parasitology* **134**, 177–182.

Hamilton, J. M. (1969). On the migration, distribution, longevity and pathogenicity of larvae of *Aelurostrongylus abstrusus* in the snail *Helix aspersa*. *Journal of Helminthology* **43**, 319–325.

Hamilton, J. M. and McCaw, A. W. (1967). The role of the mouse in the life cycle of *Aelurostrongylus abstrusus*. *Journal of Helminthology* **41**, 309–312.

Hobmaier, M. and Hobmaier, A. (1935). Intermediate hosts of *Aelurostrongylus abstrusus* of the cat. *Proceedings of the Society for Experimental Biology and Medicine* **32**, 1641–1647.

Jefferies, R., Vrhovec, M. G., Wallner, N. and Catalan, D. R. (2010). *Aelurostrongylus abstrusus* and *Troglostrongylus* sp. (Nematoda: Metastrongyloidea) infections in cats inhabiting Ibiza, Spain. *Veterinary Parasitology* **173**, 344–348.

Jezewski, W., Bunkowska-Gawlik, K., Hildebrand, J., Perec-Matysiak, A. and Laskowski, Z. (2013). Intermediate and paratenic hosts in the life cycle of *Aelurostrongylus abstrusus* in natural environment. *Veterinary Parasitology* **198**, 401–405.

Knaus, M., Rapti, D., Shukullari, E., Kusi, I., Postoli, R., Xhaxhiu, D., Silaghi, C., Hamel, D., Visser, M., Winter, R. and Rehbein, S. (2014). Characterisation of ecto- and endoparasites in domestic cats from Tirana, Albania. *Parasitology Research* **113**, 3361–3371.

Lacorcia, L., Gasser, R. B., Anderson, G. A. and Beveridge, I. (2009). Comparison of bronchoalveolar lavage fluid examination and other diagnostic techniques with the Baermann technique for detection of naturally occurring *Aelurostrongylus abstrusus* infection in cats. *Journal of the American Veterinary Medical Association* **235**, 43–49.

Lopez, C., Panadero, R., Paz, A., Sanchez-Andrade, R., Diaz, P., Diez-Banos, P. and Morrondo, P. (2005). Larval development of *Aelurostrongylus abstrusus* (Nematoda, Angiostrongylidae) in experimentally infected *Cernuella* (*Cernuella*) *virgata* (Mollusca, Helicidae). *Parasitology Research* **95**, 13–16.

Lucio-Forster, A. and Bowman, D. D. (2011). Prevalence of fecal-borne parasites detected by centrifugal flotation in feline samples from two shelters in upstate New York. *Journal of Feline Medicine and Surgery* **13**, 300–303.

Morley, N. J. (2010). Aquatic molluscs as auxiliary hosts for terrestrial nematode parasites: implications for pathogen transmission in a changing climate. *Parasitology* **137**, 1041–1056.

Palmer, C. S., Thompson, R. C., Traub, R. J., Rees, R. and Robertson, I. D. (2008). National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology* **151**, 181–190.

Pereira, C. A., Martins-Souza, R. L., Coelho, P. M., Lima, W. S. and Negrao-Correa, D. (2006). Effect of *Angiostrongylus vasorum* infection on *Biomphalaria tenagophila* susceptibility to *Schistosoma mansoni*. *Acta Tropica* **98**, 224–233.

Philbey, A. W., Krause, S. and Jefferies, R. (2014). Verminous pneumonia and enteritis due to hyperinfection with *Aelurostrongylus abstrusus* in a kitten. *Journal of Comparative Pathology* **150**, 357–360.

Pointier, J. P., David, P. and Jarne, P. (2005). Biological invasions: the case of planorbid snails. *Journal of Helminthology* **79**, 249–256.

Sauerländer, R. (1976). Histological studies of the African giant snail (*Achatina fulica*) experimentally infected with *Angiostrongylus vasorum* or *Angiostrongylus cantonensis* (in German). *Zeitschrift für Parasitenkunde* **49**, 263–280.

Schnyder, M., Di Cesare, A., Basso, W., Guscetti, F., Riond, B., Glaus, T., Crisi, P. and Deplazes, P. (2014). Clinical, laboratory and pathological findings in cats experimentally infected with *Aelurostrongylus abstrusus*. *Parasitology Research* **113**, 1425–1433.

Scott, D. W. (1973) Current knowledge of aelurostrongylosis in the cat. Literature review and case reports. *Cornell Veterinarian* **63**, 483–500.

Thiengo, S. C., Fernandez, M. A., Torres, E. J., Coelho, P. M. and Lanfredi, R. M. (2008). First record of a nematode Metastrongyloidea (*Aelurostrongylus abstrusus* larvae) in *Achatina* (*Lissachatina*) *fulica* (Mollusca, Achatinidae) in Brazil. *Journal of Invertebrate Pathology* **98**, 34–39.

Traversa, D., Di Cesare, A., Milillo, P., Iorio, R. and Otranto, D. (2008). *Aelurostrongylus abstrusus* in a feline colony from central Italy: clinical features, diagnostic procedures and molecular characterization. *Parasitology Research* **103**, 1191–1196.

Wallace, G. D. and Rosen, L. (1970). Maintenance of two feline lungworms in aquatic snails (*Biomphalaria glabrata*). *American Journal of Veterinary Research* **31**, 809–810.