

## HISTOPATHOLOGY ASSOCIATED WITH ANGIOSTRONGYLOSIS IN AKODON SPECIES (RODENTIA: SIGMODONTINAE) FROM SIERRA DE LA VENTANA, BUENOS AIRES, ARGENTINA

María del Rosario Robles, Carlos Perfumo\*, John M. Kinsella†, and Graciela T. Navone

Centro de Estudios Parasitológicos y de Vectores CEPAVE (CCT-CONICET-La Plata), UNLP, Calle 2 #584, La Plata (1900), Buenos Aires, Argentina. e-mail: rosario@cepave.edu.ar

- 21** ABSTRACT: *Angiostrongylus* is a metastrongylid nematode that includes species found in carnivores, rodents, and occasionally primates (including humans). About 100 species of wild sigmodontine rodents occur in Argentina, 16 of which are species of *Akodon*. The lungs of *Akodon azarae* and *Akodon dolores* from Sierra de la Ventana, Buenos Aires, Argentina, were examined, and 2 of 10 *A. dolores* specimens were positive for angiostrongylosis, showing macroscopic lesions of verminous pneumonia. Adults found were identified as *Angiostrongylus morerae*. Histopathology of the lungs revealed multiple nodules in the interstitium, alveoli, and vessels, resulting in interstitial fibrosis and the destruction of small capillaries and arterioles. Since extensive pathology in the lung was noted here from only 2 adults, it is probable that heavier infections may cause mortality in their hosts. This is the first record of this nematode species from *A. dolores*, expanding its geographic distribution to the southwest of Buenos Aires Province, and the first description of the histopathology of larval angiostrongylosis in a wild rodent from South America.

*Angiostrongylus* Kamensky, 1905, is a metastrongylid nematode genus that includes species found in carnivores, rodents, and occasionally, primates, both nonhuman and human. Three species have been described from carnivores, i.e., *Angiostrongylus vasorum* (Baillet, 1866) (syn. *Angiostrongylus raillieti* (Travassos, 1927)), *Angiostrongylus gubernaculatus* Dougherty, 1946, and *Angiostrongylus chabaudi* Biocca, 1957. Fourteen species have been described from rodents (see, e.g., Kinsella, 1971; Anderson, 1978, 2000; Costa et al., 2003; Miller et al., 2006; Robles, Navone, and Kinsella, 2008). With the exception of *Angiostrongylus costaricensis* and *Angiostrongylus siamensis*, which are found in the mesenteric arteries of their hosts, all other species occur in the pulmonary arteries, lungs, and the right ventricle of the heart (Anderson, 2000) (Table I).

Currently, there are approximately 340 species of wild sigmodontine rodents distributed in South America, with 100 in Argentina. Of these, 16 are species of *Akodon* (Pardiñas et al., 2003, 2006; Musser and Carleton, 2005). *Akodon* spp. deserve special attention, because they can act as hantavirus reservoirs (Suzuki et al., 2004) and as hosts of human parasites, i.e., *Schistosoma mansoni* (Rodrigues-Silva et al., 1991), *Trypanosoma cruzi* (Herrera et al., 2005), and *Leishmania* spp. (Morales et al., 2012). Moreover, these rodents have been recorded as hosts of several other nematode species (see Quentin et al., 1968; Sutton, 1984; Suriano and Navone, 1994; Notarnicola, 2005; Robles and Navone, 2007; Miño, 2008; Robles, Carballo, and Navone, 2008; Robles, 2011; Miño et al., 2012).

In addition, 3 species of *Angiostrongylus* have been recorded in these hosts, i.e., *Angiostrongylus morerae* from *Akodon azarae* (Fischer, 1829) in Argentina, *Angiostrongylus lenzii* from *Akodon montensis* Thomas, 1913 (Souza et al., 2009), and *Angiostrongylus* sp. from *Akodon cursor* (Winge, 1887) and *Akodon montensis* Thomas, 1913, in Brazil (Robles, Navone, and Kinsella, 2008; Simões et al., 2011). A related genus and species, *Akodonema*

*luzsarmientae* n. g. n. sp. Morales, Ubelaker, and Gardner, 2011, has recently been described from *Akodon mollis* Thomas, 1894, in Peru (Morales et al., 2012) (Table II).

Two species of *Akodon* are distributed in Sierra de la Ventana, Buenos Aires Province, Argentina, i.e., *Akodon dolores* 1916 (syn. *Akodon molinae* Contreras, 1968; see Wittouck et al., 1995; Braun et al., 2008; Pardiñas, 2009), and *A. azarae*. *Akodon dolores* occurs from Tucumán and Santiago del Estero Province south to southwestern Buenos Aires, reaching the hilly system of Ventania, and northeastern Chubut Province in Patagonia. The western boundary of its distribution roughly coincides with the Monte-Huayquerías limit in Mendoza Province (Braun et al., 2008; Pardiñas, 2009; Nabte et al., 2009; Jayat et al., 2011). *Akodon azarae* occurs in northeastern and east-central Argentina, Paraguay, Uruguay, and extreme southern Brazil (Musser and Carleton, 2005; Pardiñas et al., 2004, 2006).

The present study examines the gross and histopathologic changes in the lungs of *Akodon* species due to angiostrongylosis. In addition, 2 other sympatric species of rodents were investigated to explore the limits of host range.

### MATERIAL AND METHODS

Twenty-seven *Akodon* spp. specimens (17 *A. azarae* and 10 *A. dolores* individuals) were trapped in May 2011 from Parque Provincial E. Tornquist (PPET; 38°04'39.1"S, 62°00'24.8"W) and adjacent areas of Sierra de la Ventana, Buenos Aires Province, Argentina. Two sympatric species of Tribe Akodontini were also examined, i.e., 12 *Necomys lasiurus* (Lund, 1840) (including *Necomys benefactus*; see D'Elia et al., 2008) and 7 *Oxymycterus rufus* (Fischer, 1814) individuals.

All rodents were killed with ether and necropsied, and lesions in the lungs were recorded. Lungs were fixed whole in 10% buffered formalin. Each of the 5 lobes of the lungs was trimmed in the subterminal transversal part, processed, sectioned at 5 μm (± 25 sections per slide), stained with hematoxylin and eosin (H&E), and examined microscopically. Pulmonary arteries and veins were opened and observed for adult worms using a stereoscopic microscope.

The prevalence was calculated according to Bush et al. (1997). An estimate was made of the intensity, i.e., quantity of larvae by area (μm<sup>2</sup> × 5 μm thickness), in each lobe.

Adult specimens and H&E-stained sections (slides) of lung were deposited in the Helminthological Collection of the Museo de La Plata (CHMLP, no. 6547 and 6548, respectively), and the hosts were deposited in the Mammal Collections of the Museo de La Plata (MLP, no. 2035, 2037), La Plata, Buenos Aires, Argentina.

Received 28 February 2012; revised 15 May 2012; accepted 4 June 2012.

\* Cátedra de Patología Especial, Instituto de Patología Dr. B. Epstein, Facultad de Ciencias Veterinarias, UNLP, CC 296, B 1900 AVW, Buenos Aires, Argentina.

† HelmWest Laboratory, 2108 Hilda Avenue, Missoula, Montana 59801.

DOI: 10.1645/GE-3128.1

TABLE I List of *Angiostrongylus* species.

Parasite species	Author	Host order	Site infection
<i>Angiostrongylus cantonensis</i>	(Chen, 1935)	Rodentia (Primates)	Lungs and heart (central nervous system)
<i>Angiostrongylus chabaudi</i>	Biocca, 1957	Carnivora	Lungs and heart
<i>Angiostrongylus costaricensis</i>	Morera and Céspedes, 1971	Rodentia Carnivora (Primates)	Mesenteric arteries (intestinal wall)
<i>Angiostrongylus dujardini</i>	Drozd and Doby, 1970	Rodentia	Lungs and heart
<i>Angiostrongylus gubernaculatus</i>	Dougherty, 1946	Carnivora	Lungs and heart
<i>Angiostrongylus lenzii</i>	Souza, Simoes, Thiengo, Lima, Mota, Rodrigues-Silva, Lanfredi, and Maldonado, 2009	Rodentia	Lungs and heart
<i>Angiostrongylus mackerrasae</i>	Bhaibulaya, 1968	Rodentia	Lungs and heart
<i>Angiostrongylus malaysiensis</i>	Bhaibulaya and Cross, 1971	Rodentia	Lungs and heart
<i>Angiostrongylus morerai</i>	Robles, Navone, and Kinsella, 2008	Rodentia	Lungs and heart
<i>Angiostrongylus petrowi</i>	(Tarjmanova and Tschertkova, 1969)	Rodentia	Lungs and heart
<i>Angiostrongylus ryjikovi</i>	(Jushkov, 1971)	Rodentia	Lungs and heart
<i>Angiostrongylus sandarsae</i>	Alicata, 1968	Rodentia	Lungs and heart
<i>Angiostrongylus schmidti</i>	Kinsella, 1971	Rodentia	Lungs and heart
<i>Angiostrongylus sciuri</i>	Merdevenci, 1964	Rodentia	Lungs and heart
<i>Angiostrongylus siamensis</i>	Ohbayashi, Kamiya, and Bhaibulaya, 1979	Rodentia	Mesenteric arteries
<i>Angiostrongylus tateronae</i>	(Baylis, 1928)	Rodentia	Lungs and heart
<i>Angiostrongylus vasorum</i>	(Baillet, 1866)	Carnivora (Primates)	Lungs and heart (central nervous system)

**RESULTS**

None of the 17 *A. azarae* and 2 of the 10 *A. dolores* individuals examined ( $P = 20\%$ ) were parasitized with *Angiostrongylus* sp.; no lung infections were found in 12 *N. lasiurus* or 7 *O. rufus* individuals from the same locality.

In 1 of 2 infected hosts, a thrombosis in a pulmonary artery was observed, containing 2 intact mature specimens (1 male and 1 female) and resulting in the complete obliteration of the lumen. No parasites were present in heart chambers. The specimens collected were identified on the basis of the morphology of the bursa, spicules, and several measurements as *An. morerai*.

The 2 infected rodents showed macroscopic lesions (firm nodules) of verminous pneumonia in the 5 lobes. Each lung lobe contained multiple small yellowish nodules scattered throughout the parenchyma (Figs. 1, 2). The appearance of infected lungs was similar to that described for *A. cantonensis* by MacKerras and Sandars (1955) and *A. sandarsae* by Alicata (1968).

Additionally, histopathology examination of tissue fragments showed multiple nodules in the vessels, interstitium, and alveoli (Figs. 3–8). Nodules were formed by larvae surrounded by an elevated number of granulocyte and mononuclear cells (Figs. 3, 4). The vessels, interstitium, and alveoli contained nematode

larvae with mild to moderate interstitial fibrosis (Fig. 3). Larvae were approximately 60–200  $\mu\text{m}$  long and contained numerous discrete basophilic and eosinophilic granules (Figs. 5, 6).

Numerous nodules (set of larvae) surrounded by granulomatous reactions were situated under the pleural surface (Fig. 7). Several damaged capillaries and small arterioles were observed (Fig. 8).

The lobe with the greatest intensity of larvae proportionally was the left upper (Fig. 3), followed by the right lower and right medium lobes (Figs. 7, 8); the right upper and left lower lobes had similar, but smaller, intensities of infection.

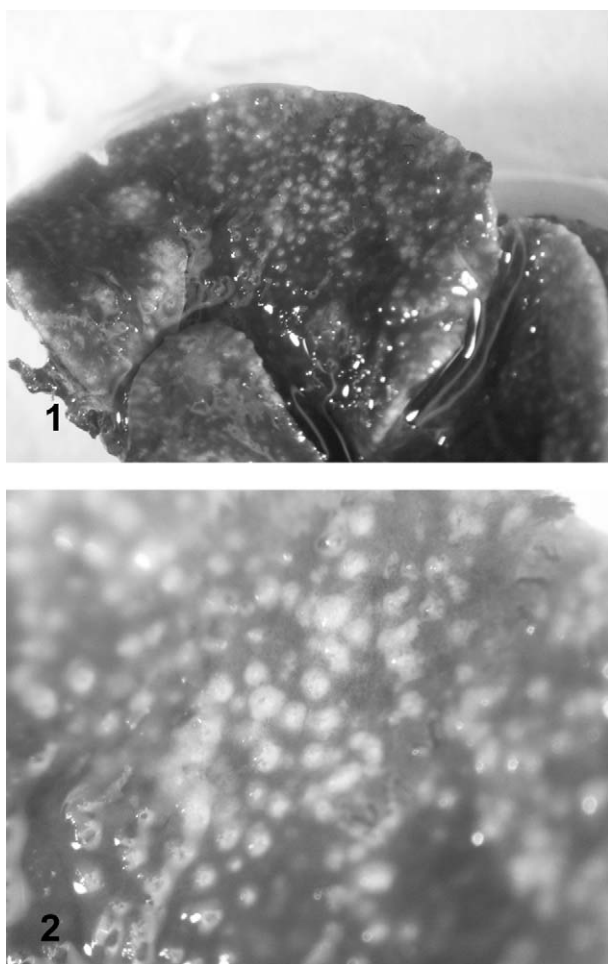
As an estimation, about 1–5 larvae per 200  $\mu\text{m}^2 \times 5 \mu\text{m}$  thickness could be observed in the left upper lobe. In right lower and right medium lobes, each nodule contained approximately the same quantity of larvae as in the left upper lobe, although these were more scattered (less by area). In the last 2 lobes, the nodules mostly contained 1–3 larvae, and these were more scattered than in the other lobes.

**DISCUSSION**

This is the first record of *An. morerai* from *A. dolores*, expanding its geographic distribution to the southwest of Buenos

TABLE II Distribution of metastrongyloid species in *Akodon* species.

Host species	Parasite species	Geographic distribution	References
<i>Akodon azarae</i>	<i>Angiostrongylus morerai</i>	Buenos Aires, Argentina	Robles, Navone, and Kinsella (2008)
<i>Akodon cursor</i>	<i>Angiostrongylus</i> sp.	Rio de Janeiro, Brazil	Simões et al. (2011)
<i>Akodon molinae</i>	<i>Angiostrongylus morerai</i>	Buenos Aires, Argentina	This paper
<i>Akodon mollis</i>	<i>Akodonema luzsarmientae</i>	Ancash, Perú	Morales et al. (2012)
<i>Akodon montensis</i>	<i>Angiostrongylus lenzii</i> / <i>Angiostrongylus</i> sp.	Rio de Janeiro, Brazil/Misiones, Argentina	Souza et al. (2009); Simões et al. (2011) ; unpubl. obs.



FIGURES 1, 2 Lung lobes of *Akodon dolores*. (1) Macroscopic lesions (firm nodules) of verminous pneumonia. Lung contains multiple small yellowish nodules scattered throughout the parenchyma. (2) Detail of macroscopic lesions of verminous pneumonia.

Aires Province, and the first description of histopathology studies of larval angiostrongylosis in a wild rodent from South America.

*Akodon* spp. seem to be the most frequent hosts of metastrongyloid nematodes as compared to other sigmodontine rodents in South America, since surveys of sympatric species of rodents such as *N. lasiurus*, *O. rufus*, *Phyllotis bonariensis* Crespo, 1964, *Oligoryzomys flavescens* (Waterhouse, 1837), *Ol. nigripes* (Olfers, 1818), and *Thaptomys nigrata* (Lichtenstein, 1830) have all been negative for angiostrongylosis (Simões et al., 2011; this study; unpubl. obs.) (Table II). Current records show the presence of specimens of metastrongyloid nematodes in 5 species of *Akodon*, including 3 species of *Angiostrongylus* and 1 species of *Akodonema*. Studies on the genetics of these parasites would likely prove that they are closely related, and may show some are synonyms, or alternatively reveal the existence of cryptic species. Such phylogenetic-coevolutionary studies could explain the peculiar distribution of a different metastrongyloid (*Akodonema*) in a group of host species parasitized by *Angiostrongylus* spp.

The majority of *Angiostrongylus* species reside in the pulmonary arteries and right heart of their hosts, and their unsegmented eggs are carried by the blood stream to the lungs, where they

lodge as emboli in the small blood vessels and hatch to first-stage larvae within 5 to 6 days (Anderson, 2000). After passage out of the respiratory system and gastrointestinal tract, larvae of *Angiostrongylus* spp. pass in the feces and are infective to both terrestrial and aquatic gastropods. Infection of definitive hosts is normally by ingestion of gastropods, although larvae of *A. cantonensis* are also capable of exiting live molluscs and contaminating vegetation (Heyneman and Lim, 1967; Slom et al., 2002). Presumably, *An. morerai* has a similar life cycle. In addition, other sympatric host species may not be infected because they do not eat gastropods and/or putative paratenic hosts.

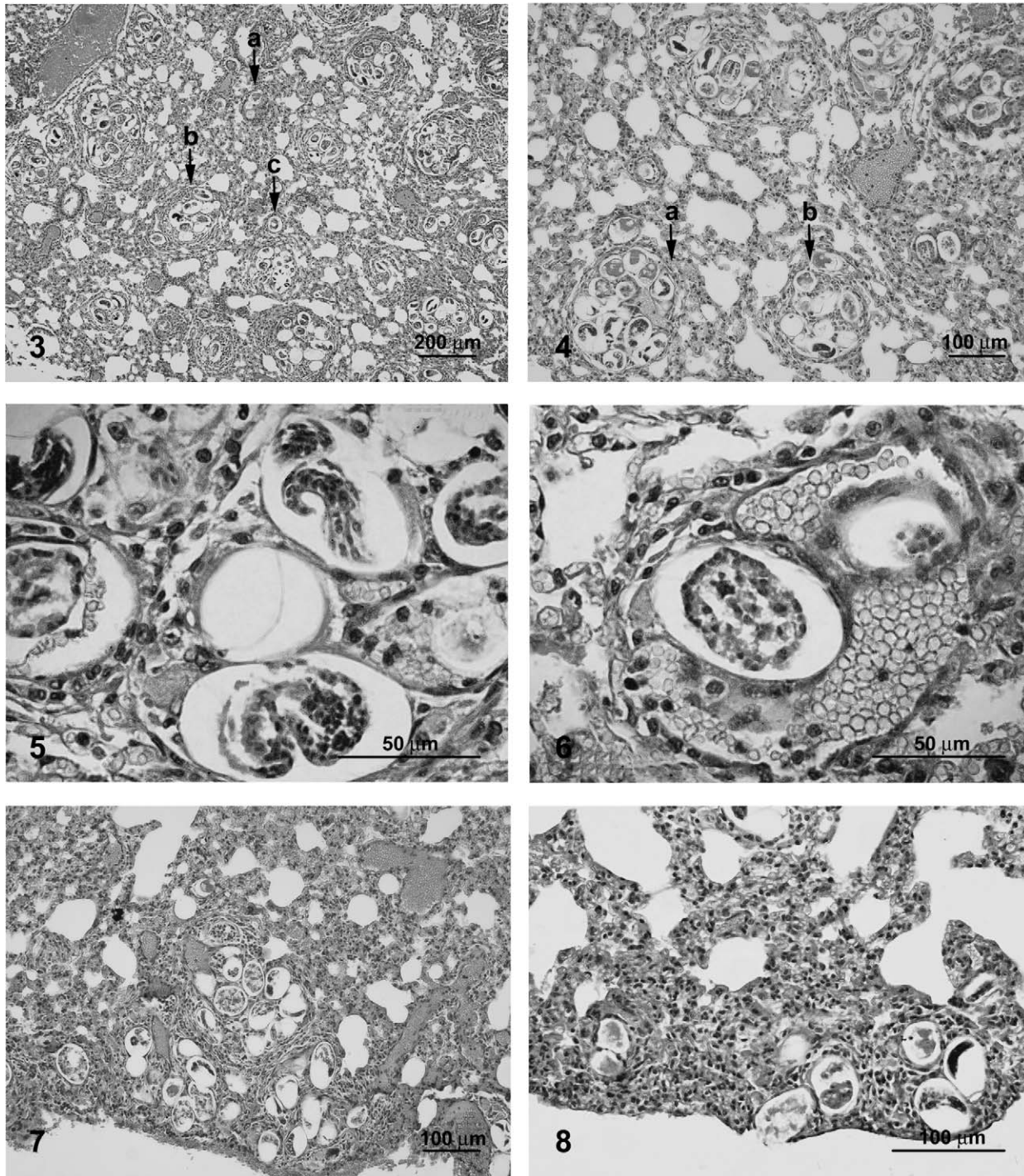
As demonstrated here in *An. morerai*, the resulting immune reaction can cause interstitial fibrosis and the destruction of small capillaries and arterioles. In 1 case, extensive lesions were apparently caused by a single male and female. Experimental studies by Kinsella (1987) on *A. schmidtii*, a parasite of rice rats, *Oryzomys palustris*, showed little evidence of host specificity. Rice rats and cotton rats (Sigmodontinae), cotton mice (Neotominae), white rats and white mice (Murinae), hamsters (Cricetinae), and gerbils (Gerbillinae) were all successfully infected. As a result, many of the smaller rodents (white mice, hamsters, gerbils) died from the infections. In addition, morbidity and mortality appeared to be dose related, with as few as 4 adults being fatal to 3-wk-old hamsters. Since extensive pathology in the lung was noted here from only 2 adults in the present study, it is distinctly possible that heavier infections, interfering with the normal function of this vital organ, may cause mortality in *Akodon* spp., as well as in other genera of rodents. Accordingly, the absence of signs of angiostrongylosis or the low prevalence of infection found here may underestimate the consequences of infection by this parasite.

Further studies are needed to determine the factors that influence the presence or absence of natural infections in these rodents, i.e., such as a different level of omnivory, anatomical limitations, segregation of trophic niches (possible explanation for the absence here of *An. morerai* in *A. azarae* [Robles, Navone, and Kinsella, 2008]), and immune defenses, etc.

Some studies indicate that the frequency of infection of *Angiostrongylus* spp. appears to be increasing during recent years (Chikweto et al., 2009; Taubert et al., 2009; Morgan et al., 2010) and expanding into new hosts and localities (Kim et al., 2002; Chikweto et al., 2009). For example, *A. cantonensis*, a parasite of *Rattus* spp. and occasionally humans, has been reported in several wildlife species, such as wood rats (*Neotoma floridanus*), cotton rats (*Sigmodon hispidus*), and marsupials (*Didelphis virginiana*) in North and Central America (Tesh et al., 1973; Kim et al., 2002). The dispersal of a possible intermediate host, *Achatina fulica* (Bowdich, 1822), into South America, the presence of definitive host species susceptible to angiostrongylosis in many areas, and the low host specificity of *Angiostrongylus* species (Kinsella, 1987) combine for a troubling epidemiological scenario.

#### ACKNOWLEDGMENTS

We offer thanks to Julio Idiart for his assistance in the histopathology study, to Emilio Topa for his help in the preparation of the tissue fragments (slides), to Ulyses Pardiñas, Carlos Galliari, Marcela Lareschi, Pablo Teta, and Agustín Abba for their essential help and cooperation in the field tasks and host collections, to Ulyses Pardiñas, Carlos Galliari, and Pablo Teta for the identification of the hosts, to Luis Giambelluca for



FIGURES 3–8 Histopathological examination of *A. dolores* lungs infected with *Angiostrongylus moreyai* (hematoxylin and eosin); vascular changes in medium-size vessels, capillaries, and alveoli. Section of left upper lobe of lung of *A. dolores*: (3) vessels (a), interstitium (b), and alveoli (c) contained nematode larvae ( $\times 10$ ). (4) Granulomatous inflammatory reactions, vessel (a) and interstitium (b) contained nematode larvae ( $\times 20$ ). (5) Detail of granulomatous inflammatory reactions surrounding each set of larvae ( $\times 100$ ). (6) Detail of the interior of a blood vessel containing nematode larvae ( $\times 100$ ). Section of right lower lobe of lung of *A. dolores*. (7) Interstitium, alveoli, and vessels contained nematode eggs and larvae ( $\times 20$ ). Section of right medium lobe of lung of *A. dolores*. (8) Superficial interstitium and alveoli containing nematode larvae. Granulomatous inflammatory reactions surround each set of larvae ( $\times 40$ ).

the assistance with the photographs, to Carlos Galliari and Ulyses Pardiñas for critical reading, to Mariano Mastropaolo for his advice, to Ricardo Cañete, Daniel Novoa, Raquel Stefanici (OPDS), Monica Casciaro (Dirección de Flora y Fauna, Province Buenos Aires), and the

rangers from Cerro Bahía Blanca, Sierra de la Ventana, for their help. This study was funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT 0924- Director GN and PICT 0547-Director UP). M.R.R. and G.N. are members of CONICET, and publication was

approved by Organismo Provincial para el Desarrollo Sostenible (OPDS), Province Buenos Aires, Disposición 0132011, and Dirección de Fauna y Flora, Ministerio de Asuntos Agrarios, Province Buenos Aires, Disposición 141.

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