LIFE HISTORY AND PATHOGENESIS OF *GALLEGOSTRONGYLUS AUSTRALIS* (NEMATODA: ANGIOSTRONGYLIDAE) IN MURIDAE

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Summary :

Gallegostrongylus australis Spratt, Haycock & Walter, 2001 (Nematoda: Angiostrongylidae) developed in *Deroceras panormitanum, Lehmannia nyctelia, L. flava* and *Milax gigates* (Gastropoda). The first moult occurred at 18-19 days after infection (DAI) and the second moult at 28 DAI. Larvae were infective to experimental murid definitive hosts at 35 DAI. In experimentally infected *Rattus fuscipes* larvae moulted L_{3-4} at 3 DAI and L_{4-5} at 6-7 DAI. Patency in *R.fuscipes, R. lutreolus, R. norvegicus* and *R. rattus* occurred 27-64 DAI and duration varied from 7-392 days. Histopathological changes in the lungs of *R. lutreolus* and development of debilitating clinical signs, in contrast to *R. fuscipes*, suggests that the former host-parasite relationship may be the more recent one but other traits suggest the opposite. Patent infections were established in some wild *R. rattus* and some laboratory *R. norvegicus* but not in wild *M. domesticus*, laboratory *M. musculus*, rabbit, *Oryctolagus cuniculus*, and marsupial bandicoot, *Isoodon macrourus*.

KEY WORDS : *Gallegostrongylus australis*, Nematoda, Angiostrongylidae, Muridae, Gastropoda, life-cycle, pathology.

Résumé : DÉVELOPPEMENT ET PATHOGÉNIE DE *GALLEGOSTRONGYLUS AUSTRALIS* (NEMATODA : ANGIOSTRONGYLIDAE) CHEZ LES MURIDÉS

Gallegostrongylus australis *Spratt, Haycock & Walter, 2001 (Nematoda : Angiostrongylidae) se développe chez* Deroceras panormitanum, Lehmannia nyctelia, L. flava *et* Milax gigates *(Gastropoda). La première mue s'effectue 18-19 jours après l'infection et la seconde au 28ème jour. Les larves sont infestantes pour le Muridé hôte définitif expérimental au 35ème jour. Chez* Rattus fuscipes *infesté expérimentalement, la mue des larves L 3-4 se produit au 3ème jour après infestation, et celle des L 4-5 aux 6-7èmes jours. Une infection patente chez* R. Fuscipes, R. lutreolus, R. norvegicus *et* R. rattus *apparaît 27 à 64 jours après l'infestation et dure de 7 à 392 jours. Les modifications histologiques observées au niveau des poumons de* R. lutreolus *et le développement de signes cliniques, contrairement à* R. fuscipes, *suggèrent que la relation hôte-parasite précédente peut être la plus récente, mais d'autres caractéristiques laissent penser le contraire. Une infection patente est observée chez quelques* R. rattus *sauvages et quelques* R. norvegicus *de laboratoire, mais pas chez* M. domesticus *sauvage,* M. musculus *de laboratoire, le lapin* Oryctolagus cuniculus *et le rat marsupial* Isoodon macrourus.

MOTS CLÉS : *Gallegostrongylus australis, Nématoda, Angiostrongylidae, Muridae, Gastropoda, cycle évolutif, pathologie.*

INTRODUCTION

Gallegostrongylus australis Spratt, Haycock & Walter, 2001 (Nematoda: Angiostrongylidae) was reported from subpleural nodules in the lungs of native bush rats, *Rattus fuscipes* (Waterhouse, 1839), swamp rats, *Rattus lutreolus* (Gray, 1841) and a single wild *Mus domesticus* Schwartz & Schwartz, 1943 (see Figueroa *et al.*, 1986) in southeastern Australia (Spratt, Haycock & Walter, 2001). Despite examination of 4,227 animals representing 28 species of native and three species of introduced murid rodents throughout Australia over a 20 year period, these authors reported a prevalence of *G. australis* infection of only 0.38 %. The parasite is known from only two coastal locations in far southeastern New South Wales near the Victorian border – Ludwigs Swamp, Nadgee State Forest and Sidling Swamp, Timbillica State Forest – where it occurred in *R. fuscipes* and *R. lutreolus*, and on one occasion in *M. domesticus* –, and from Mt. Barrow, Tasmania where it occurred in *R. lutreolus*. *R. fuscipes* does not occur in Tasmania.

Spratt *et al.* (2001) argued that a combination of four features justified erection of the new species, despite the low prevalence of infection and relatively minor morphological differences between *G. australis* and its two congeners, *G. andersoni* from gerbillids in West Africa and *G. ibicensis* from microtids and murids in the western Mediterranean. These were *i)* morphology, *ii)* geographic distribution, *iii)* host distribution and *iv)* life history traits. This paper addresses the fourth feature and describes comparative studies of the life cycle and pathogenesis of the parasite in six species of gastropod intermediate host, in two indigenous and three introduced murid hosts, in the rabbit, *Oryctolagus cuniculus*, (Linnaeus, 1758), and in the marsupial short-nosed bandicoot, *Isoodon macrourus* (Gould, 1842) (Peramelidae).

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MATERIAL AND METHODS

Life cycle studies were conducted between 1979 and 1981. The following species of snail, slug and semi-slug (nomenclature after Smith, 1992) were caught in the wild and subsequently bred and reared in the laboratory to provide lungworm-free intermediate hosts for life-cycle studies: *Helix aspersa* Müller, 1774, *Deroceras panormitanum* (Lessona & Pollonera, 1882), *Lehmannia nyctelia* (Bourguignat, 1861), *L. flava* (Linnaeus, 1758), *Limax maximus* Linnaeus, 1758 and *Milax gigates* (Draparnaud, 1801).

Initially, first-stage larvae (L_1) were collected, using a Baermann apparatus (Thienpont *et al.*, 1979), from nodules in the lungs of naturally-infected rats, transferred to lettuce by pipette and the lettuce fed to laboratory-reared gastropods. Subsequently, L_1 were collected in the same manner from experimentally infected murids and used to infect gastropods. After at least 40 days, third-stage larvae (L_3) were harvested from gastropods by digestion in 1.5 % pepsin hydrochloride at 37°C for two hours.

Collection of wild animals was conducted in accordance with the legislation of State and Territory fauna authorities and under permits issued by them for scientific research. *Rattus fuscipes* and *R. lutreolus* were trapped in the wild and subsequently bred and reared in the laboratory to provide lungworm-free definitive hosts for life-cycle studies. In addition, *R. fuscipes, R. rattus* and *Mus domesticus* were trapped in the wild, in areas in which the new lungworm species was known not to occur, acclimated to the laboratory and used for life cycle studies. Laboratory white rats (*R. norvegicus*) and BALB/c mice (*M. musculus*) were also used in experimental infection studies.

Rattus lutreolus, R. fuscipes, R. norvegicus, M. domesticus and *M. musculus* were anaesthetised lightly with ether and infective larvae were administered *per os* using a fine rubber tube attached to a syringe. Five *Rattus rattus* and eight *M. musculus* were fed chopped slugs containing third-stage larvae. Larvae were administered *per os* to laboratory white rabbits (*Oryctolagus cuniculus*) using a tooth gag and a tapered rubber tube attached to a syringe. Third-stage larvae in a drop of water were placed in minced meat using a pipette and the meat fed to wild-caught northern brown bandicoots (*Isoodon macrourus*).

Faecal samples of animals were examined daily using the Baermann technique (Thienpont *et al.*, 1979) from 25 days after infection (DAI) until the termination of the experiments. Data are presented as mean 10-day larval output (L_1/gm of faeces) at 10-day intervals for 30 days post patency.

Animals were observed daily for clinical signs of infection and experiments were terminated if animals showed signs of distress. Animals were killed by intraperitoneal injection of sodium pentobarbitone ("Euthatal") at different times after administration of infective larvae.

Lungs were examined for helminths by pressing pulmonary tissue between glass Petri dishes and examining under a stereomicroscope by means of transmitted light. Representative pieces of lung were fixed in 10 % neutral buffered formalin, blocked in paraffin, sectioned at 6 µm and stained with haematoxylin and eosin for histological examination. Worms and worm fragments were recovered, using jeweller's forceps, fixed in hot 10 % neutral buffered formalin, cleared in lactophenol and studied under a compound microscope.

Measurements are in micrometres unless otherwise stated, the mean of 7-8 larvae followed by the range in parentheses. Drawings were made using a drawing tube.

RESULTS

Development in gastropod intermediate hosts

Deroceras panormitanum was the most suitable experimental intermediate host of *G. australis*, larvae moulting L_{1-2} at 18-19 DAI, L_{2-3} at 28 DAI and infective to murid hosts at 35 DAI. Development was slightly slower in *Lehmannia nyctelia, L. flava, Limax maximus* and *Milax gigates* and L_3 were not infective to murids until 41-43 DAI. *Helix aspersa* was the least suitable experimental intermediate host larvae remaining as L_1 at 19 DAI, as L_1 or L_2 at 28 DAI with only a few developing to L_3 at 72 DAI, remaining in moulted cuticles of L_1 and L_2 and darkening thereafter.

Development in vertebrate definitive host

Summaries of the results of experimental infection of murid hosts with *G. australis* are presented in Tables I-III.

R. fuscipes: wild-caught (n = 4) and laboratory-bred (n = 4) *R. fuscipes* given 32 (19-64) L_3 *per os* were examined at 12 hr, 19 hr and at 3, 6, 9, 20 and 25 (n = 2) DAI. Third-stage larvae were found only in the peritoneal cavity and liver at 12 hr and only in the lungs at 19 hr. Third-stage larvae, larvae moulting L_{3-4} and L_4 were observed in the lungs at 3 DAI, L_4, moulting L_{4-5} and early L_5 at 6 DAI, early L_5 males and females at 9 DAI and adult males and fertilised females at 20 and 25 DAI. Maximum size was attained by 25 DAI (Table I). Mean time to patency in wild-caught animals given small numbers (19-64) of L_3 was 38.5 days and in those given large numbers (270-273) was 33.5 days (Table II).

	Rattus fuscipes				*R. norvegicus*	*R. rattus*	
	9 DAI [2]**	20 DAI [2]	25 DAI [4]	77-78 DAI [2]	17 DAI [2]	33 DAI [2]	58 DAI [1]
Male							
Length*	1.8 (1.0-2.5)	3.7 (3.4-3.9)	5.7 (4.8-6.5)	4.3 (3.8-4.7)	2.5 (1.9-2.9)	6.3 (6.0-6.6)	5.5
Max width	45 (41-48)	77 (52-102)	81 (73-92)	95 (67-122)	69 (65-73)	86 (79-92)	75
Nerve ring	76 (54-97)	92 (91-93)	135 (120-145)	120 (113-127)	99 (95-103)	104 (102-105)	
Excretory pore	77 (77-77)	103 (101-105)	178 (165-187)	154 (150-157)	105 (103-107)	176 (165-187)	
Oesophagus	218 (203-233)	218 (195-240)	278 (263-285)	289 (270-307)	263 (250-276)	268 (250-285)	285
Right spicule	141 (132-150)	151 (149-153)	157 (157-157)	143 (135-150)	155 (154-156)	133 (131-135)	150
Left spicule	141 (132-150)	151 (149-153)	157 (157-157)	143 (135-150)	155 (154-156)	133 (131-135)	150
Gubermaculum	48 (47-49)	49 (47-51)	59 (58-60)	49 (47-50)	48 (47-49)	45 (45-45)	42
Tail	10 (10-12)	7 (7-7)	9 (7-10)	11 (10-12)	11 (10-12)	14 (12-15)	10
	9 DAI [1]		25 DAI [2]	65-78 DAI [3]	17 DAI [2]	33 DAI [1]	58 DAI [1]
Female							
Length*	1.8		9.5 (8.0-11.0)	6.7 (5.0-7.8)	8.9 (8.4-9.3)	7.5	7.7
Max width	39		85 (85-85)	67 (60-73)	79 (77-81)	98	73
Nerve ring	90		117 (113-120)	82 (82-82)	94 (87-100)	66	130
Excretory pore	125		180 (165-195)	85 (85-85)	101 (97-104)	150	157
Oesophagus	203		330 (300-360)	246 (159-278)	260 (257-263)	360	278
Vulva	41		78 (78-78)	67 (63-69)	78 (75-81)	75	66
Anus	18		30 (29-30)	24 (22-25)	30 (30-30)	25	27

* Length in mm.
** Days after infection (number measured).

Table I. – Measurements* (µm) of *Gallegostrongylus australis* Spratt, Haycock & Walter, 2001 from experimentally infected rodent hosts.

	No infected/ No dosed	Sexes	Larval dose X (range) [N]*	Patency (days) X (range) [N]	Mean L1/gm at 10 days [N]	Mean L1/gm at 20 days [N]	Mean L1/gm at 30 days [N]	Duration of patency (days) X (range) [N]
Rattus fuscipes								
(W)	10/11	6M, 5F	29 (20-41) [11]	38.5 (34-46) [4]	537 (432-642) [2]	2,984 (1,178-4,789) [2]	5,932 (2,563-9,300) [2]	121,5 (84-159 [2]
(W)	3/3	3M	272 (270-273) [3]	33.5 (31-36) [2]	8,254 (251-16,526) [2]	2,492	4,972	48 (14-82) [2]
(WL)	4/6	3M, 3F	32 (19-64) [6]	***	nil	nil	nil	nil
Rattus lutreolus								
(WL)	18/18	4M, 2F	40 (28-66) [6]	29 (27-30) [6]	5382 (870-15,817) [5]	16,967 (1,272-36,146) [5]	5,811 [1]	47 (25-84) [3]**
		3M, 10F	217 (115-280) [13]	32 (28-39) [13]	57,672 (5,963-269,434) [13]	111,680 (14,571-383,842) [11]	124,134 (20,307-178,431) [6]	35.5 (11-45) [10]**
Rattus norvegicus								
(L)	2/6	4M, 2F	28 (14-50) [6]	56 (48-64) [2]	238 [1]	1046 [1]	2856 [1]	223.5 (7-392) [2]
	1/3	1M, 2F	169 (155-188) [3]	nil	nil	nil	nil	nil
Rattus rattus								
(W)	3/5	2M, 3F	fed inf. slugs	34.5 (33-36) [2]	605 [1]	94 [1]	103 [1]	49 [1]
Mus domesticus								
(W)	0/4	2M, 2F	18 (11-21) [4]	nil	nil	nil	nil	nil
Mus musculus								
(L)	0/4	4F	fed inf. slugs	nil	nil	nil	nil	nil
Mus musculus								
(L)	4/16	6M, 10F	24 (13-26) [16]	nil	nil	nil	nil	nil

* [N] = number of animals.
** = experiment terminated due to respiratory distress.
*** = four in life cycle studies pre-patency, two uninfected.
(W) = wild strain.
(WL) = wild strain, lab bred.
(L) = conventional lab strain.

Table II. – Results of experimental infection of murid hosts with *Gallegostrongylus australis* Spratt, Haycock & Walter, 2001.

	Rattus fuscipes	*R. lutreolus*	*R. norvegicus*	*R. rattus*	*Mus domesticus*	*M. musculus*
Clinical signs	nil	lethargy stary fur respiratory distress	nil	nil	nil	nil
Gross pathology	subpleural lesions	subpleural lesions	nil or minor subpleural lesions	nil or minor subpleural lesions	congestion nil or minor subpleural lesions	congestion nil or minor subpleural lesions
Histopathology (inflammation)						
3 DAI	nil					
6 DAI	nil					
9 DAI	nil					
20-25 DAI	+ acute				ne*	ne
33 DAI	++ acute				ne*	ne
42 DAI	+++ sub-acute				ne	ne
43 DAI		++ fibrosis			ne	ne
60 DAI		+++ fibrosis			ne	ne
77 DAI		+++ fibrosis			ne	ne
78 DAI	+++ fibrosis		++ fibrosis		ne	ne
91 DAI				++ fibrosis	ne	ne
114 DAI		++ fibrosis				
118 DAI		++ fibrosis				
128 DAI	++ fibrosis				ne	ne
159 DAI		++ fibrosis				
450 DAI	++ fibrosis				ne	ne

* ne = not examined; + = mild; ++ = moderate; +++ = severe.

Table III. – Summary of clinical signs, gross pathology and histopathology in murids experimentally infected with *Gallegostrongylus australis* Spratt, Haycock & Walter, 2001.

Moderate numbers of L_1 were passed in the faeces of *R. fuscipes*, the largest number in those receiving the higher numbers of L_3 (Table II). Duration of patency varied from 84-159 days in animals receiving low numbers of L_3 and from 14-82 days in animals receiving high numbers of L_3 (Table II). Patency occurred at 42 DAI in one *R. fuscipes* given 27 L_3 and L_1 were found in faeces for 26 days. Examination at 78 DAI revealed four grey, fibrotic, subpleural lesions approximately 10mm in diameter that contained some live and some dead adult *G. australis* surrounded by embryonated eggs and L_1. Patency occurred at 37 DAI in another *R. fuscipes* given 27 L_3 and L_1 were found in faeces for 75 days. Examination at 128 DAI revealed four, large, grey, fibrotic, subpleural lesions that contained degenerating adult nematodes. Females contained no fertilised eggs *in utero* and there were no live L_1 in the lungs. Patency was not detected in an additional *R. fuscipes* given 27 L_3 and on examination 77 DAI a single, grey, subpleural lesion that contained one male and two unfertilised females was observed. Finally, patency was not detected in an animal given 273 L_3. At examination 42 DAI, dead adult male and female *G. australis* were found in subpleural nodules which contained no embryonating eggs or hatched L_1. Signs of ill-health or respiratory distress were not observed in any of the animals (Table III).

R. lutreolus: patency occurred in laboratory-bred *R. lutreolus* given small numbers of L_3 (28-66) at 29 (27-30) DAI and at 32 (28-39) DAI in those given large numbers of L_3 (115-280) (Table II). Massive numbers of L_1 were passed in the faeces of *R. lutreolus* especially those given the larger numbers of L_3 (Table II). Duration of patency varied from 25-84 days in animals which were given low numbers and from 11-45 days in animals which were given high numbers of L_3 (Table II). However, in all instances, experiments were terminated due to respiratory distress in individual animals (Tables II, III). Four *R. lutreolus* were treated at the same time, in the same manner and using L_3 pooled from the same infected molluscs, as two *R. norvegicus* in which patent infections had failed to establish on two previous occasions. All four *R. lutreolus* reached patency 30.5 (28-32) DAI, produced enormous numbers 227,486 (109,390-383,842) of L_1/gm of faeces for 45 days before respiratory distress prompted termination of the experiment. Numerous lesions containing live adult male and female nematodes, embryonated eggs and L_1 were recovered from the lungs of each animal. One wild-caught and naturally infected animal was brought into captivity and continued to pass L_1 in the faeces for 132 days before developing signs of respiratory distress. At examination, two large, grey, subpleural lesions were present on the right lung and contained live adult male and female *G. australis*, embryonated eggs and L_1.

R. norvegicus (laboratory strain): Infection was established only in three of nine laboratory *R. norvegicus*

(Table II). One animal given 20 L_3 and examined 17 DAI contained three males and a female in the subpleural parenchyma although no gross lesions were apparent (Table I). These worms were extremely difficult to extract and were tightly bound in fibrous tissue. Two *R. norvegicus* were given 30 and 13 L_3 respectively but no L_1 were detected in the faeces after 48 and 81 days respectively. They were again given 25 and 158 L_3 but no L_1 were detected in the faeces after 168 and 98 days respectively. On a third occasion they were given L_3, at the same time, in the same manner and using L_3 pooled from the same infected molluscs as were given to four *R. lutreolus*. No L_1 were detected in the faeces of both *R. norvegicus* after 59 days and at examination on that day no lesions were observed. No nematodes were recovered from the lungs of one animal and a single, dead male *G. australis* was recovered from the lungs of the second animal. First-stage larvae were not observed in the faeces of one animal, given 16 L_3, up to 56 DAI but at 70 DAI there were 80 L_1/gm. Numbers continued to decrease and at 78 DAI only five larvae were observed, three of them dead. At examination that day a hard, mineralised lesion 10 mm × 8 mm was observed on the diaphragmatic surface of the left lung and a single, live male *G. australis* was recovered from the apex of the left lung. One *R. norvegicus* given 20 L_3, was patent 48 DAI and continued to pass L_1 in the faeces for 392 days. At examination 450 DAI there were small foci of necrotic tissue on each lung and a single, live male *G. australis* was recovered.

R. rattus: five wild-caught *R. rattus* were offered and observed to eat live *D. caruanae* and *L. nyctelia* infected for 49-91 days with *G. australis*. The lungs of one animal examined 33 DAI contained five subpleural lesions 2-4 mm in diameter. Each lesion contained live male and female worms (Table I), embryonated eggs and L_1. Although no L_1 were detected in faeces prior to examination, five were found in rectal faeces at this time. A second animal had no L_1 in the faeces and on examination 58 DAI, no gross lesions were observed in the lungs. A single male was recovered from one lung and a single, unfertilised female from the other lung (Table I). A third animal passed L_1 in the faeces 36 DAI and these persisted for 49 days. At examination 91 DAI, no L_1 were detected in rectal faeces but three subpleural lesions were observed on the lungs and two live male and two degenerating female *G. australis* were recovered from these. Two *R. rattus* were examined at 33 and 75 DAI; lung lesions, nematodes or nematode fragments were not observed (Table II).

M. domesticus (wild strain): mice (n = 4) were given 18 (11-21) L_3 and examined 40 DAI (Table II). The lungs of two animals were congested grossly and had small, focal, grey lesions 0.5-1 mm in diameter on or immediately under the pleura. These were easily detached from the pleura and contained numerous cells but no evidence of larval or adult nematodes or nematode fragments.

M. musculus (laboratory strain): mice (n = 16) were given 24 (13-26) L_3 and examined 3, 15, 22, 27, 28 (n = 2), 29, 35 (n = 5) and 42 (n = 4) DAI (Table II). Four L_3 were recovered from the lungs of one mouse 3 DAI, a single unfertilised female was recovered from the lungs of a mouse 15 DAI, a single dead female was recovered from the lungs of a mouse 27 DAI and a single live male was recovered from the lungs of a mouse 35 DAI. Larvae or adult nematodes were not recovered from eight of the 16 mice. The lungs of six animals examined at 22, 28, 29, 35 and 42 (n = 2) DAI were congested grossly and had small, focal, grey lesions 0.5-1 mm in diameter on or immediately under the pleura. These were easily detached from the pleura and contained numerous cells but no evidence of larval or adult nematodes or nematode fragments. Infections were not established in four laboratory *M. musculus* fed infected slugs and examined 42 DAI (Table II).

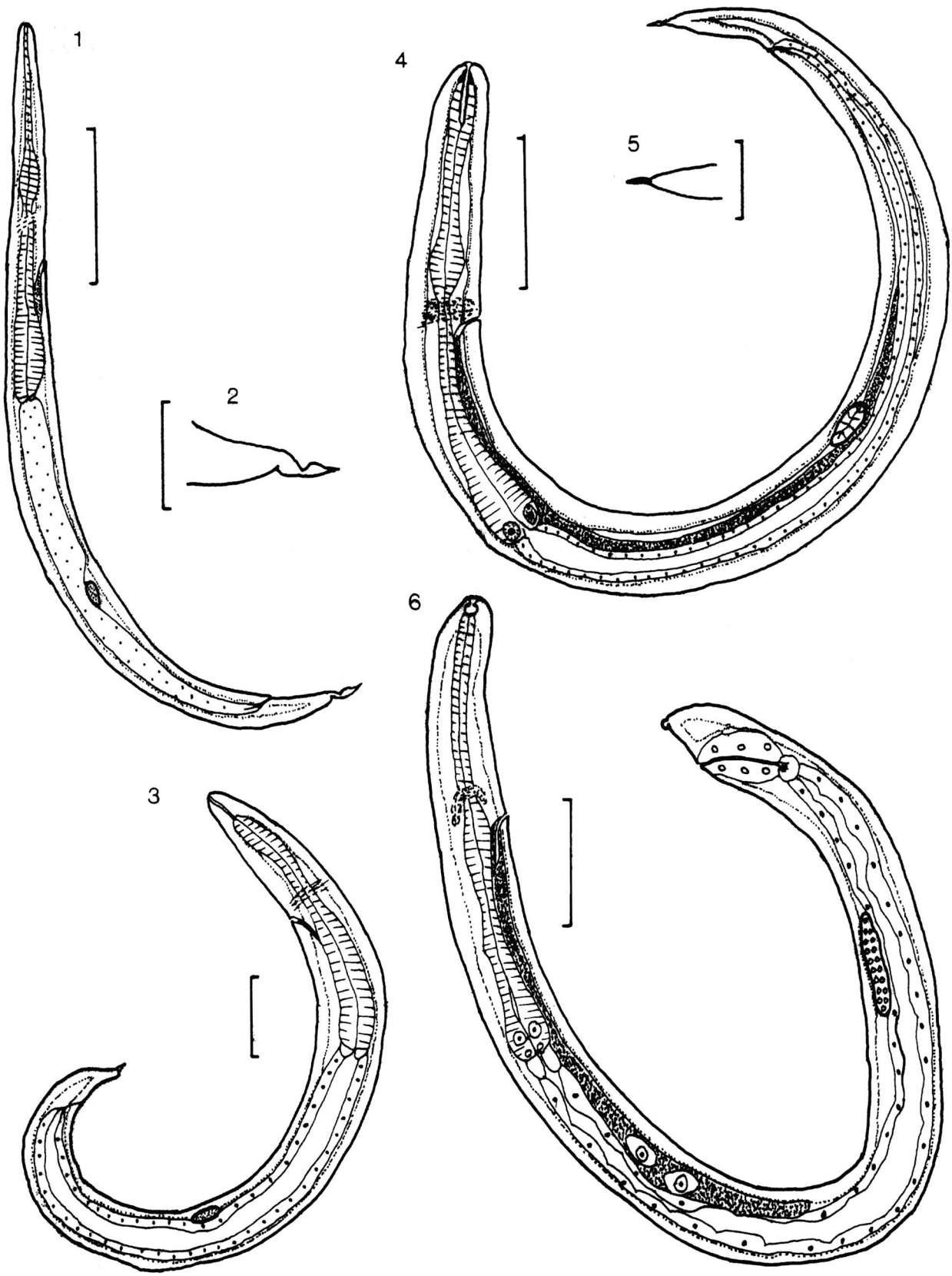
O. cuniculus (laboratory strain): rabbits were given 30 (n = 2) and 200 (n = 2) L_3 and examined 25, 26 and 27 (n = 2) DAI. The lungs of both rabbits given 30 larvae had three to six small, focal, grey lesions 1-2 mm in diameter on or immediately under the pleura. These were easily detached from the pleura and contained numerous cells but no evidence of larval or adult nematodes or nematode fragments. The lungs of both rabbits given 200 L_3 exhibited numerous such lesions on and under the pleura and throughout the pulmonary parenchyma. Although numerous infiltrating cells were apparent on squashes of lungs there was no evidence of larval or adult nematodes or nematode fragments.

I. macrourus: two wild-caught northern brown marsupial bandicoots (*I. macrourus*) were given 26 L_3 in mince and a third was fed infected slugs. The former animals were examined 71 and the latter 73 DAI. The lungs of all animals contained small, mineralised lesions but there was no evidence of larval or adult nematodes or nematode fragments.

MORPHOLOGY OF LARVAL STAGES

First-stage larva (Figs. 1-2)

Measurements of first-stage larvae (n = 8) from lungs of experimentally infected *Rattus fuscipes* 77 days after infection: L 293 (281-315), MW 13 (12-15), NR 84 (83-87), EP 100 (95-103), O 142 (125-150), claviform, GP 196 (185-207), kidney-shaped, AL 286 (273-307). Transverse cuticular annulations and lateral alae not detected. Tail tip with characteristic anterior dorsal and posterior ventral notches, terminating in point.



Figs 1-6. – *Gallegostrongylus australis*. 1. First-stage larva from lung of *Rattus fuscipes*, lateral view, bar = 50 µm. 2. Tail of first-stage larva, lateral view, bar = 10 µm. 3. Second-stage larva from *Deroceras panormitanum*, lateral view, bar = 40 µm. 4. Third-stage larva from *Lehmannia flava*, lateral view, bar = 50 µm. 5. Tail of third-stage larva, lateral view, bar = 10 µm. 6. Fourth-stage larva from lung of *Rattus fuscipes*, lateral view, bar = 50 µm.

Second-stage larva (Fig. 3)

Measurements of second-stage larvae (n = 8) digested from *Limax flavus* 20 DAI: L 415 (404-420), MW 34 (31-35), NR 69 (66-73), EP 73 (68-76), O 141 (135-145), GP 255 (247-260), AL 377 (366-382). Cuticle with fine transverse annulations. Fine lateral alae present commencing posterior to nerve ring and terminating anterior to anus. Tail tip sharply pointed.

Third-stage larva (Figs. 4-5)

Measurements of third-stage larvae (n = 8) digested from *Limax flavus* 28 DAI: L 495 (485-510), MW 25 (22-27), NR 79 (75-81), EP 84 (82-85), O 171 (155-175), GP 293 (290-295), AL 457 (445-472). Cuticle with transverse annulations. Conspicuous lateral alae present commencing approximately half way to excretory pore and terminating in region of anus. Tail tip sharply pointed. Measurements of third-stage larvae (n = 4) in the lungs of *R. fuscipes* 3 DAI: L 446 (429-454), MW 25 (24-26), NR 67 (61-74), EP 75 (60-83), O 154 (150-156), GP 273 (258-282), AL 420 (411-426). Cuticle with transverse annulations. Conspicuous lateral alae present commencing approximately half way to excretory pore and terminating in region of anus. Tail tip sharply pointed.

Fourth stage larva (Fig. 6)

Measurements of fourth-stage larvae (n = 7) in the lungs of *R. fuscipes* 3 DAI: L 537 (489-581), MW 26 (24-27), NR 71 (60-78), EP 81 (72-89), O 177 (156-198), GP 386 (330-436), AL 515 (470-543). Cuticle smooth, without transverse annulations or lateral alae. Buccal cavity conspicuous. Excretory system with two conspicuous large nuclei. Large cells present in anterior and mid-region of intestine. Genital enlage developing, elongate. Tail tip rounded, with knob-like appearance. Measurements of larva in *M. musculus* 3 DAI: L 548, MW 30, NR 82, EP 90, O 180, GP 353, AL 517.

Early fifth-stage larva

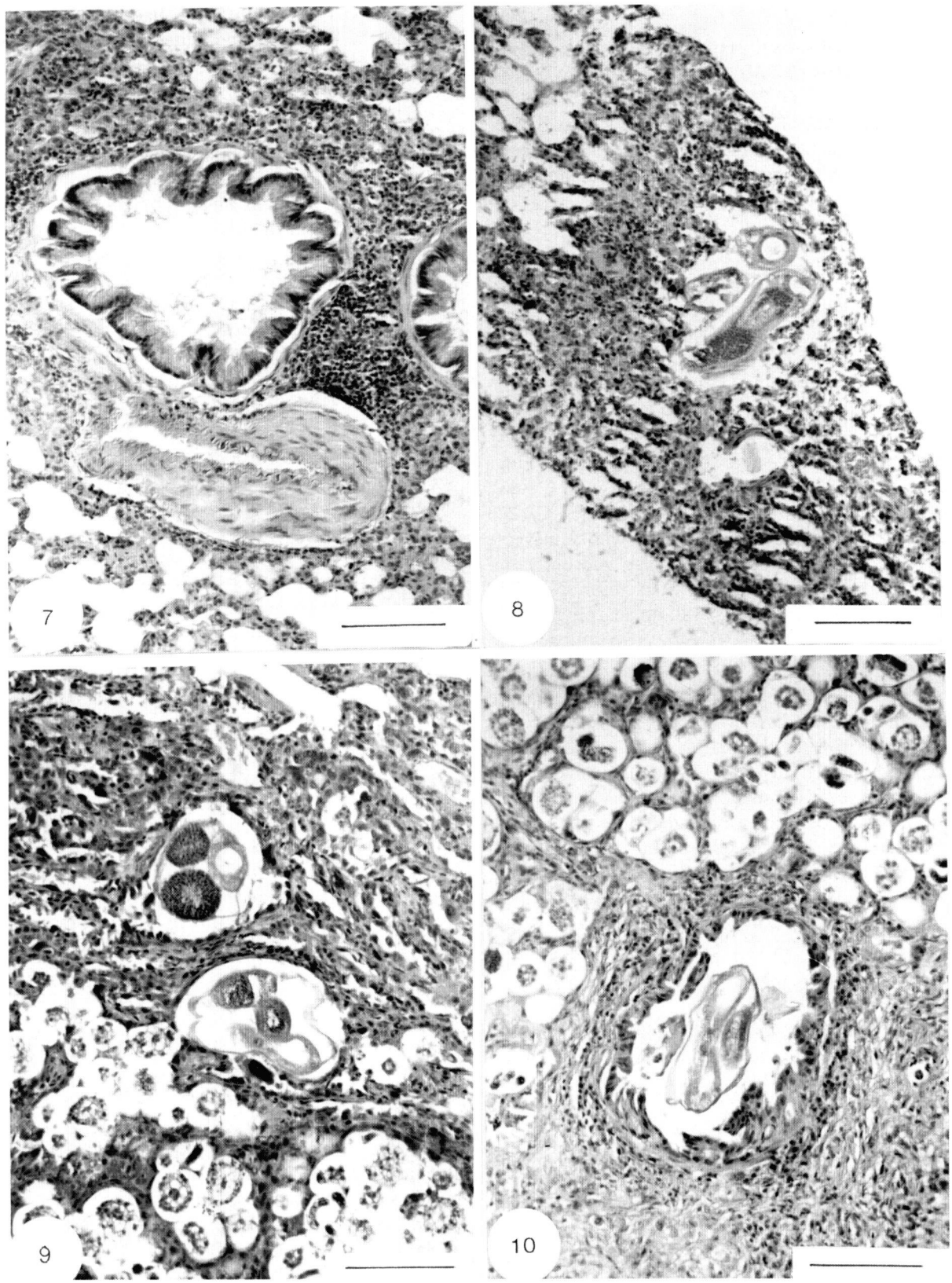
Measurements of an early fifth-stage female in the lungs of *R. fuscipes* 6 DAI: L 800, MW 35, NR 68, EP 95, O 187, V 45, A 20.

PATHOLOGY

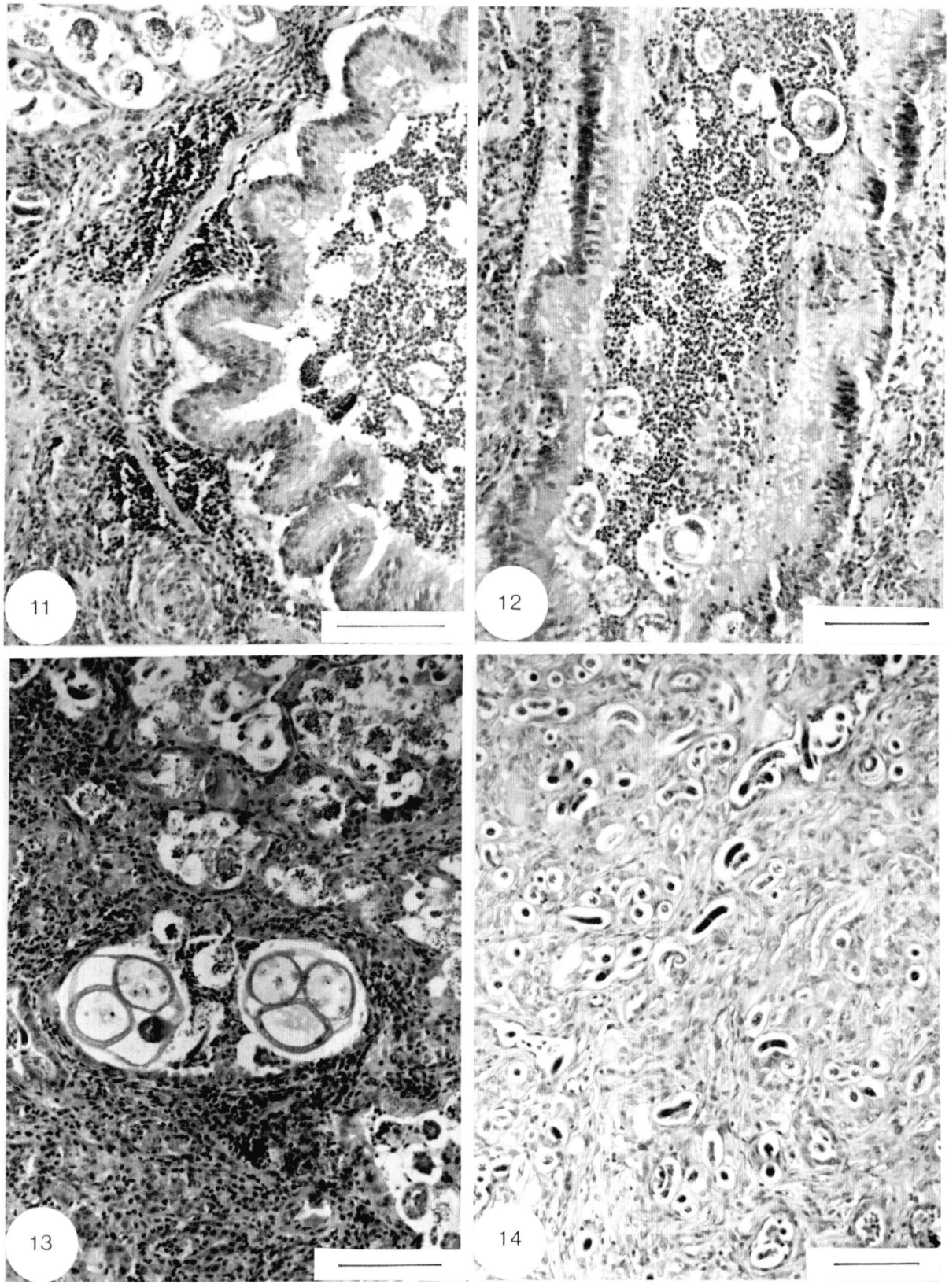
In most instances there was a marked contrast in the clinical signs between wild-caught *R. fuscipes* and lab-bred *R. lutreolus* infected experimentally with *G. australis*. In the former given 273 L_3, pulmonary weight (n = 4) represented 1.6 (0.6-3.3) % of the total body weight. Animals had glossy fur, appeared alert, were active and exhibited no signs of respiratory distress. In contrast, *R. lutreolus* given small and large numbers of L_3 had heavier lungs that represented 2.3 (1.6-2.8) % and 9.3 (3.3-12.2) % respectively of total body weight. Many animals became inactive, had stary fur, sat hunched in the corner of the cage and developed signs of severe respiratory distress, necessitating termination of experimental infections. Clinical signs were not observed in any of the other host species (Table III).

R. fuscipes: no gross lesions were observed at 3, 6 and 9 DAI by which time worms were early L_5 in the lungs. At 20 and 25 DAI, small subpleural greyish-white lesions 1-3 mm in diameter were apparent and there was interstitial thickening in the pulmonary parenchyma around worms. However, embryonated eggs and L_1 were not present in the parenchyma. Infiltrations of neutrophils occurred around bronchi and the pulmonary artery (Fig. 7). Acute inflammation developed around bronchioles containing subadult worms (Fig. 8). At 33 DAI, mature adult worms were surrounded by masses of embryonated eggs, neutrophils and foamy macrophages. Few fully developed L_1 were seen at this stage. Host response appeared more pronounced following patency (Table III). At 42 DAI, neutrophils, eosinophils, and foamy macrophages were present in areas containing large numbers of embryonated eggs and L_1 (Fig. 9). At 78 DAI, perivascular and especially peri-bronchial cuffing of neutrophils was conspicuous and bronchial walls were thickened by fibrosis (Fig. 10). One wild-caught *R. fuscipes* given 273 L_3 failed to develop a patent infection and on examination 42 DAI contained dead male and female *G. australis* in small nodules in the bronchial lumen of both lungs and surrounded by inflammation. No embryonated eggs or L_1 were observed in pulmonary parenchyma.

R. lutreolus: patency occurred 31 DAI in a laboratory-bred *R. lutreolus* given 279 L_3. The animal was passing 135,272 L_1/gm faeces 10 days after patency and two days before the experiment was terminated at 43 DAI. Examination revealed numerous subpleural lesions containing numerous male and female *G. australis,* and a mass of embryonated eggs and L_1 in each lung. The lungs represented 6.6 % of the total body weight of the animal. Mature adult worms in the subpleural parenchyma and bronchioles were surrounded by embryonated eggs and L_1, and large numbers of neutrophils and eosinophils. Substantial fibrosis had developed at the periphery of subpleural nodules (Table III). Perivascular and peribronchial cuffing was conspicuous and bronchioles were occluded with embryonated eggs, L_1 and infiltrating cells, especially neutrophils (Figs. 11, 12). At 60 DAI, this reaction was more pronounced (Table III) and some occluded bronchioles and alveoli were collapsed (Fig. 13). At 77 DAI, few adult worms were present, little functional parenchyma was apparent and embryonated eggs and L_1 were surrounded by infiltrating cells, including fibroblasts. At 114 DAI, no adult worms were present, perivascular



Figs 7-10. – Pathological changes in the lungs during experimental infection of *Gallegostrongylus australis* in *Rattus fuscipes*. 7. Infiltrations of neutrophils around bronchus and pulmonary artery 20 DAI, bar = 100 µm. 8. Bronchiolitis in respiratory bronchioles containing subadult nematodes and infiltrations of neutrophils in surrounding pulmonary parenchyma 20 DAI, bar = 100 µm. 9. Adult nematode and embryonated eggs surrounded by numerous neutrophils, eosinophils and foamy macrophages 42 DAI, bar = 100 µm. 10. Adult nematode in bronchiole, peri-bronchiolar cuffing of neutrophils and developing fibrosis in interstitium 78 DAI, bar = 100 µm.



Figs 11-14. – Pathological changes in the lungs during experimental infection of *Gallegostrongylus australis* in *Rattus lutreolus*. 11. Peribronchial cuffing of eosinophils and embryonating eggs (top left) 43 DAI, bar = 100 µm. 12. Hyperplasia of bronchiolar epithelium and occlusion of airway with embryonating eggs, L_1 and infiltrating leucocytes 43 DAI, bar = 100 µm. 13. Respiratory bronchiole occluded with adult nematode and numerous eosinophils, surrounding embryonated eggs and L_1 in alveolar sacs 60 DAI, bar = 100 µm. 14. Embryonated eggs and L_1 trapped in fibrous connective tissue 114 DAI, bar = 100 µm.

and peribronchial cuffing, primarily of eosinophils, was pronounced, and embryonated eggs and L_1 were trapped in fibrotic tissue (Fig. 14).

R. norvegicus: the hard, mineralised nodular lesion observed on the diaphragmatic surface of the left lung of an animal at 78 DAI with 16 L_3 consisted of epithelioid cells with fibrosis organised around disintegrated worms (Table III). Adults, eggs and L_1 were not observed. The histopathological changes resembled those seen in *R. lutreolus* at 114 DAI. In contrast, one *R. norvegicus* was given 20 L_3 and passed L_1 in the faeces for 392 days. At examination 450 DAI the small patches of necrotic tissue on each lung consisted of epitheliod cells organised around disintegrated worms. Nematodes, eggs and L_1 were not detected.

R. rattus: at 91 DAI, live adults were observed in bronchioles. Perivascular and peribronchial cuffing was conspicuous and alveolar septae were thickened by fibrosis (Table III). Embryonated eggs and L_1 were not observed.

DISCUSSION

The life cycle of *G. australis* is typical of the majority of the Metastrongyloidea with an obligatory period of development in a gastropod intermediate host prior to infection of the vertebrate definitive host (Anderson, 1992). Gastropods were infected experimentally by placing L_1 suspended in water on lettuce and feeding this to snails and slugs. However, it was not determined whether infection occurred through ingestion, through penetration of the foot or by both routes. Petter & Cassone (1975) demonstrated that experimental infection of the aquatic snails *Lymnaea stagnalis* and *Planorbarius corneus* by *G. andersoni* occurred by ingestion, not penetration.

G. australis developed relatively slowly in experimental intermediate hosts (which may not be those in nature) with relatively long periods between moults, the first moult occurring at 18-19 days, the second at 28 days and the larvae then requiring a maturation period before being infective to murid definitive hosts at 35 days. In contrast, Petter (1974) reported the first moult of *G. andersoni* at eight days and the second moult after 11 days in the aquatic snail, *Lymnaea stagnalis*. In addition to this snail, *Deroceras reticulatum* and *Planorbarius corneus* were suitable, *Arion hortensis* was partially suitable and *Physa acuta* was an unsuitable intermediate host for *G. andersoni* (Petter, *loc. cit.*). Although *Helix aspersa* supported development of *G. andersoni* it was an unsuitable intermediate host in our studies of *G. australis*. However, *Deroceras panormitanum, Lehmannia nyctelia, L. flava, Limax maximus* and *Milax gigates* were suitable intermediate hosts of *G. australis*.

Initial development of *G. australis* in murid definitive hosts was surprisingly rapid. As reported for *G. andersoni* (Petter, 1974), L_3 reached the peritoneal cavity and then the liver in 12-14 hours, and were present in the lungs within 19-24 hours. In *G. australis* the moult L_{3-4} occurred at 3-4 DAI and the moult L_{4-5} occurred at 6-7 DAI. Immature adult nematodes were present in the lungs at 9 DAI and mature males and fertilised females at 20 and 25 DAI (Table I). Worms reached adult size at 25 DAI (Table I) but no embryonated eggs or L_1 were present in the lungs at this time. *G. australis* appears to require a relatively long embryonation phase in the lungs because the first appearance of L_1 in the faeces, generally was around 35-36 days. However, time to patency was highly variable occurring 27 to 64 DAI, depending sometimes on the number of larvae given, sometimes on the species of murid host involved and sometimes on inexplicable causes (Table II). The shortest mean time to patency occurred in *R. lutreolus* (29 days) and the longest in *R. norvegicus* (56 days). In contrast, male and female *G. andersoni* attained adult size by 15 days, embryonated eggs were in alveoli of the lungs at this time and patency occurred 24 days after infection in *Tatera* cf. *nigrita* and > 29 days in *Meriones crassus* (Petter, 1974). Duration of patency (7-392 days) also was highly variable in murid hosts infected with *G. australis* . Variation occurred within and between definitive host species and at different numbers of L_3 given (Table II). In general, *G. australis* adults lived 3-4 months. Approximately 50 % of adult worms were dead at 78 DAI in *R. fuscipes* and approximately 85 % at this time in *R. lutreolus*. All adults were dead and degenerating at 114 DAI in *R. lutreolus* and at 128 DAI in *R. fuscipes*. However, in the wild caught infected *R. lutreolus* adults were alive 132 days after the animal was brought into captivity. And, in one *R. norvegicus* given only 20 L_3, L_1 were passed in the faeces for 14.5 months and a single live male worm was found at examination nearly 16.5 months after infection. Petter (1972) reported live *G. andersoni* in the lungs of experimentally infected *Tatera kempi* three and half months (approx 106 days) after infection but females contained neither eggs nor larvae. She reported grey lesions in the lungs of *T. kempi* but no nematodes at six months. Petter (1972, 1974) reported that *G. andersoni* infection was similar in four gerbillid species, *Taterillus* cf. *congicus, Tatera kempi, T.* cf. *nigrita* and *Meriones crassus* but considered the laboratory mouse (*M. musculus*) a less suitable host because egg development was incomplete in two of four animals and the laboratory rat (*R. norvegicus*) an unsuitable host because infection did not establish. A spectrum of traits may be used to assess the "success" of a parasite, virulence

or pathogenicity being but one of these (Combes, 1997). Nevertheless, the experimental life cycle and pathological studies reported here, in combination with the known host and geographical distribution of *G. australis* (Spratt *et al.*, 2001), suggest that the bush rat, *R. fuscipes*, and the swamp rat, *R. lutreolus*, are the most suitable definitive hosts of *G. australis*. The substantial host reaction of *R. lutreolus* to the parasite and the subsequent development of debilitating clinical signs in animals given small or large numbers of L_3, in contrast to the situation in *R. fuscipes*, suggests that the former host-parasite association may be the more recent one. Other traits suggest the opposite. Although nematode longevity was similar in these two host species experimental infection was established in 18 of 18 *R. lutreolus* but in only 17 of 20 *R. fuscipes* and in terms of parasite dispersal, far greater numbers of L_1/gm of faeces occurred in *R. lutreolus* than in *R. fuscipes*. Duration of patency cannot be used in this comparison because experiments with *R. lutreolus* were terminated due to respiratory distress in experimental host animals. Although natural infection has not been recorded in the recently introduced brown rat, *R. rattus* (Spratt *et al.*, 2001), the species was suitable as host of *G. australis*. Infection occurred in three of five animals, two of these reached patency in 33 and 36 days and one continued to produce L_1 in the faeces for 54 days. Infection was established in only three of nine individuals of a laboratory strain of *R. norvegicus* and one animal remained patent for 392 days however, infection has not been observed in this species in the wild (Spratt *et al.*, 2001). There was further evidence of the unsuitability of this host species relative to *R. lutreolus*. Infective larvae pooled from infected molluscs and given to *R. lutreolus* and *R. norvegicus* resulted in patent infection in all four *R. lutreolus* and no infection in one and non-patent infection in the other *R. norvegicus*, which had a single dead male in the pulmonary parenchyma at 58 DAI. The laboratory mouse, *M. musculus*, and the wild house mouse, *M. domesticus*, were unsuitable definitive hosts. Although L_3 passed from the gastrointestinal tract and reached the lungs, either developing larvae or adult worms were destroyed in the lungs prior to patency. Nevertheless, infection containing two immature and unfertilised females was reported by Spratt *et al.* (2001) on one occasion in wild *M. domesticus* in the same geographical location where natural infection occurred in *R. fuscipes* and *R. lutreolus*. Both the European rabbit, *O. cuniculus*, and the northern brown marsupial bandicoot, *I. macrourus*, were unsuitable hosts of the parasite, larvae apparently reaching the lungs but being destroyed therein possibly prior to patency.

The zoogeographic implications of the occurrence of this parasite in indigenous species of *Rattus* in Australia and the evolution of morphologically similar but biologically distinct species of *Gallegostrongylus* in rodent hosts in West Africa, the western Mediterranean and Australia as a consequence of the phenomenon of "hôte de capture" (Chabaud, 1965) were discussed previously (Spratt *et al.*, 2001).

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