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PATHOLOGY ASSOCIATED WITH PHYSALOPTERID LARVAE (NEMATODA: SPIRURIDA) IN THE GASTRIC TISSUES OF AUSTRALIAN REPTILES

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ABSTRACT: Cysts containing one or more physalopterid larvae were found commonly in the gastric tissues of snakes (Elapidae) and in all five families of lizards (Agamidae, Gekkonidae, Pygopodidae, Scincidae and Varanidae) in Western Australia. Snakes had been collected from many locations in tropical and arid Western Australia between 1912 and 1976, and lizards from the Great Victoria Desert between September 1978 and March 1979. Most cysts occurred in the submucosa; others were found within stomach muscle, and subserosally on the external stomach surface. Encysted and apparently viable larvae were associated with minimal host inflammatory response. Encysted dead and degenerating larvae occurred in cysts with inflammatory cell infiltrates, principally in snakes.

Key words: *Abbreviata* sp., physalopterid larvae, pathology, reptiles, Australia.

INTRODUCTION

Cysts containing physalopterid larvae first were described from Australian reptiles by Irwin-Smith (1922) in a skink (*Lygosoma taeniolatum*), and they are a common finding in the stomach tissues of many species of Australian reptiles. The cysts have been recorded from snakes (Elapidae), and from all five families of Australian lizards (Agamidae, Gekkonidae, Pygopodidae, Scincidae and Varanidae), often at high prevalence and intensity (Jones, 1978, 1995). Non-encysted migrating larvae also occur within the gastric tissues, especially in snakes (Jones, 1978). The presence of these migrating larvae, and the high prevalence of encysted larvae, prompt questions regarding their effects on the hosts, and the host response to these larvae in the tissues. My objective was to describe the larval cysts, the sites at which they occurred, and the pathological responses of the hosts.

MATERIALS AND METHODS

All material examined was taken from specimens preserved in the Department of Terrestrial Vertebrates, Western Australian Museum, Perth, Australia. Snakes had been collected from a variety of locations within the state (14°00′ to 34°00′S, 114°00E to 128°50′E), mostly from the tropical and arid regions, between 1912 and 1976. Lizards had been collected by Dr. E. R. Pianka from two sites in the Great Victoria Desert, (28°28′S, 122°50′E, and 28°13′S, 123°36′E), between September 1978 and March 1979. These habitats were described by Beard (1974) and by Pianka (1986). The reptiles had been fixed in 10% formalin, and then stored in 70% ethanol. Small segments of stomach which were seen macroscopically to contain larval cysts were excised from 24 snake and 51 lizard specimens, representing 25 species from six families, as follows: Elapidae: *Acanthophis antarcticus* (n = 3), *A. pyrrhus* (n = 5), *Pseudonaja modesta* (n = 6), *P. nuchalis* (n = 5), *Suta fasciata* (n = 4), *Echiopsis curta* (n = 1); Agamidae: *Ctenophorus isolepis* (n = 2), *Pogona minor* (n = 1); Gekkonidae: *Diplodactylus ciliaris* (n = 1), *D. conspicillatus* (n = 3), *Gehyra variegata* (n = 3), *Nephrurus levis* (n = 1), *N. laevissimus* (n = 4), *Rhynchoedura ornata* (n = 7); Pygopodidae: *Lialis burtonis* (n = 1), *Pygopus nigriceps* (n = 2); Scincidae: *Ctenotus calurus* (n = 1), *C. grandis* (n = 2), *C. helenae* (n = 5), *C. pantherinus* (n = 4), *Egernia striata* (n = 2), *Lerista muelleri* (n = 1); Varanidae: *Varanus caudolineatus* (n = 3), *V. eremius* (n = 7), *V. gouldii* (n = 1). These were dehydrated, embedded in paraffin, and serially sectioned at 5 μm. Tissue samples from snakes were stained with hematoxylin and eosin, and with Gabe's (1976) one-step trichrome, and those from lizards were stained with hematoxylin and eosin. The viability of larvae at the time the hosts were collected was determined on the basis of cuticular appearance and internal morphology. Larval death was determined by swollen cuticle, loss of cellular architecture, internal structures with necroses, and epithelioid cells from the cyst wall encroaching on the larvae.

To identify the larvae and their stage of de-

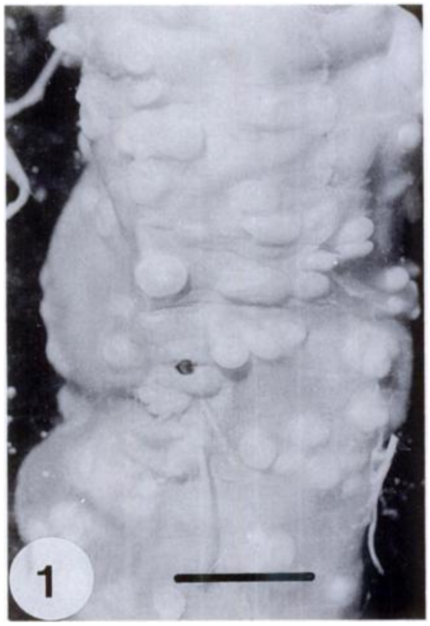

FIGURE 1. External view of stomach of *Varanus eremius*, showing many cysts containing physalopterid larvae. H&E. Bar = 5.0 mm.

velopment, 25 larvae were dissected out from cysts in the stomach walls of 24 snakes representing 12 species, and 296 larvae were evaluated from 100 lizards representing 20 species. These were examined by light microscopy after clearing in chlorolactophenol; lengths of nematode larvae (in mm) were measured with a map-measurer from drawings made with the aid of a drawing tube. Larval stages were determined on the basis of development and degree of differentiation of their sexual organs, as described in *Physaloptera maxillaris* (Cawthorn and Anderson, 1976).

A one factor factorial analysis of variance (Winer, 1971) was used to compare larval size between smaller and larger hosts.

RESULTS

All entire larvae examined, and those which were sectioned appropriately, possessed two lateral lips each bearing a submedial denticle and a single dorsal and ventral denticle, a cuticular collar, and a muscular esophagus divided into two portions. On these characteristics they were assigned to the nematode family Physalopteridae (Chabaud, 1975). There were no discernible differences between larvae from different sites within the stomach wall.

TABLE 1. Lengths of physalopterid larvae from cysts, in relation to number of larvae per lizard host.

Number of larval cysts per stomach	Number of larvae measured	Number of hosts	Mean length (mm)	SD
1	22	22	6.78	1.59
2	25	14	6.64	1.55
3	18	7	6.59	1.76
4 to 5	22	7	5.96	2.44
6 to 10	23	4	6.09	1.78
11 to 20	56	5	5.81	1.79
21 to 40	63	3	5.37	2.21
40+	67	2	6.51	2.42

In preserved material, cysts containing larvae appeared from the external surface as white raised spherical structures, 1 to 3 mm in diameter, beneath the serosal surface (Fig. 1), from which the larvae were readily extracted. When cysts were present in low numbers, their position appeared to be random, but when present in larger numbers they usually occurred in the distal portion of the stomach, especially around the pylorus. Their positions within the gastric tissues could not be ascertained in undissected material. Frequently, worms were noted to be leaving the serosal stomach surface and entering the peritoneal cavity. In some host species, particularly *Ctenotus calurus* (Scincidae) and *Gehyra variegata* (Gekkonidae) many empty cysts were present, and in some cases their walls were calcified. In *Ctenotus* spp. hosts, larvae usually were coiled in the subserosa in clusters of up to 30 worms, attached loosely and often forming a pedunculated sac.

Lengths of 296 larvae in cysts ranged from 3.0 mm to 12.4 mm ($\bar{x} \pm \mathrm{SD} = 6.15 \pm 2.06$ mm). Mean larval length was not affected by the number of cysts per stomach (Table 1). Larvae from hosts with a snout-vent length (SVL) >90 mm (principally *L. burtonis*, *P. nigriceps* and *E. striata*) were significantly ($P < 0.001$) lon-

TABLE 2. Lengths of encysted physalopterid larvae in relation to host snout-vent length (SVL).

Host snout-vent length (mm)	Number of worms measured	Mean length (mm)	SD
<40	6	6.06	2.00
40 to 49	19	7.20	1.65
50 to 59	73	5.27	1.69
60 to 69	51	5.71	1.22
70 to 79	33	6.92	2.86
80–89	89	5.83	2.00
>90	25	8.48	1.80

ger than those with an SVL <90 mm (Table 2). All larvae examined from lizards were third stage; three from snakes were fourth stage larvae.

Cysts were present in three host sites: in the submucosa, within the gastric musculature, or beneath the serosal tissues covering the external stomach surface (Tables 3 and 4). Cysts were located only in the submucosa in 13 of 19 lizard host species. In the lizards *C. grandis* (*n* = 2) and *G. variegata* (*n* = 3), cysts occurred exclusively within the gastric muscle, and in *C. helenae* (*n* = 5) and *L. muelleri* (*n* = 1) cysts were exclusively subserosal; in *C. pantherinus* cysts were subserosal in three hosts, and intramuscular in one host. In snakes most cysts also occurred in the submucosa, but most individuals also had some subserosal cysts, and a small number of intramuscular cysts.

On histopathological analysis, cyst walls from the submucosa were composed of collagen tissue, usually with little cellular infiltration. Most cysts were thin-walled (Fig. 2), but in larger lizards (*P. minor*, Fig. 3, and *V. eremius*) and in all snakes, a proportion of cyst walls was composed

TABLE 3. Sites of cysts containing physalopterid larvae in lizards, and number of lizard species showing evidence of resorption of larvae.

Host species	Number examined	Sites of larval cysts			Resorption
		Submucosal	Intramuscular	Subserosal	
Agamidae					
Ctenophorus isolepis	2	2	0	0	0
Pogona minor	1	1	0	0	0
Gekkonidae					
Diplodactylus ciliaris	1	1	0	0	0
D. conspicillatus	3	3	0	0	1
Gehyra variegata	3	0	3	0	0
Nephrurus levis	1	1	0	0	0
N. laevissimus	4	4	0	0	0
Rhynchoedura ornata	7	7	0	0	0
Pygopodidae					
Lialis burtonis	1	1	0	0	1
Pygopus nigriceps	2	2	0	0	1
Scincidae					
Ctenotus calurus	1	1	0	0	0
C. grandis	2	0	2	0	0
C. helenae	5	0	0	5	1
C. pantherinus	4	0	1	3	0
Egernia striata	2	2	0	0	2
Lerista muelleri	1	0	0	1	0
Varanidae					
Varanus caudolineatus	3	2	0	1	1
V. eremius	7	7	0	0	3
V. gouldii	1	1	0	0	0

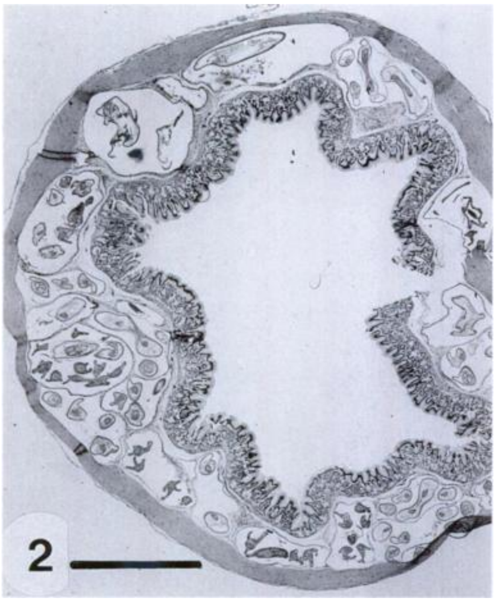

FIGURE 2. Section of stomach of *Nephrurus laevissimus*: numerous cysts containing physalopterid larvae occur in the submucosa throughout the circumference of stomach. H&E. Bar = 0.25 mm.

FIGURE 3. Section of stomach of *Pogona minor* with hyperplasia and lymphocytic infiltration surrounding larva in submucosa. H&E. Bar = 0.25 mm.

of many concentric layers of collagenous tissue. In these multilayered cysts, histiocytes or lymphocytes usually were confined to the outer layers. In heavy infections among *N. laevissimus* and many snakes, and in some lighter infections, cysts were multilocular with more than one worm in each cyst, and the submucosal space was considerably distended, with many contiguous cysts separating the muscularis mucosae from the outer muscle layers (Figs. 2 and 4). Cysts within the muscle layer usually were thin-walled, with little cellular infiltration (Fig. 5). In many specimens the cysts bulged out to the extent that the muscles over the external surface were thinned to a single layer of cells. The walls of cysts located subserosally in *Ctenotus* spp. skinks usually were multilayered, and often were pedunculated (Fig. 6). Cellular infiltration of these cyst walls by histiocytes occurred largely in the outer layers. Cyst walls from all three sites appeared to be derived from host tissues.

Cysts containing viable undamaged larvae had no significant cellular infiltrate.

TABLE 4. Sites of cysts containing physalopterid larvae in snakes (Elapidae), and the number of snake specimens showing evidence of resorption of larvae.

Host species	Number examined	Sites of larval cysts			Resorption
		Submucosal	Intramuscular	Subserosal	
Acanthophis antarctius	3	3	0	2	3
A. pyrrhus	5	5	2	3	5
Suta fasciata	4	4	1	2	2
Echiopsis curta	1	1	0	0	1
Pseudonaja modesta	6	6	0	2	4
P. nuchalis	5	5	1	3	5

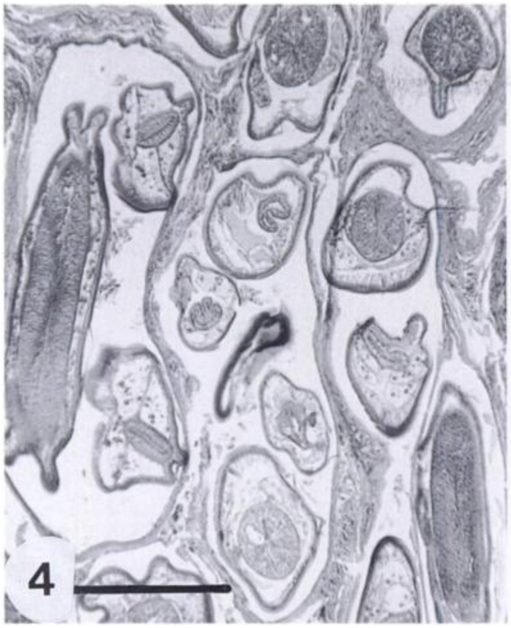

FIGURE 4. Section of stomach of snake *Pseudonaja nuchalis* with many contiguous thin-walled cysts containing larvae in submucosa. Gabe's trichrome. Bar = 0.20 mm.

FIGURE 5. Section of stomach of *Ctenotus grandis* with larval cyst within gastric muscle. H&E. Bar = 0.25 mm.

There was evidence of minor hemorrhage into the cyst wall in two lizards, and in three lizards there were congested blood vessels adjacent to the cysts. Several sections contained non-encysted free larvae within the submucosa or subserosa, and these were not associated with cellular reactions.

Three lizard specimens (*C. helenae*, *L. burtonis* and *V. caudolineatus*) each had a cyst in which the larva had been replaced by dense cellular tissue. Specimens from four more species of lizard (*D. conspicillatus*, *P. nigriceps*, *E. striata* and *V. eremius*) had sites in which the dense concentric layers of cyst wall were infiltrated with histiocytes, lymphocytes, and eosinophil leucocytes, and which were lined by epithelioid cells which appeared to be encroaching on the larvae undergoing resorption. In all snake species, and in most individual snakes examined, there were sites with progressive thickening of the collagen layers, containing histiocytes, lymphocytes and occasional eosinophil leucocytes, and in which the larvae were degenerating (Fig. 7) and being replaced by epithelioid cells.

DISCUSSION

In this study the size of larvae in cysts was unaffected by the number of cysts per stomach, though larvae in cysts from the largest hosts examined were significantly longer than those from smaller lizards. The number of larval cysts increases with host size both within and across species (Jones, 1995), and together these observations provide evidence for accumulation of larvae within the food chain. Larvae in cysts from the longest hosts (*P. nigriceps*, *L. burtonis*, and *E. striata*) were significantly longer than those from smaller lizards. *L. burtonis* feeds exclusively on vertebrates, and *E. striata* occasionally does so (Pianka, 1986), and this is evidence for the growth of larvae as they pass up the food chain.

Reports of physalopterid larval cysts in reptile tissues are scanty, and they are not

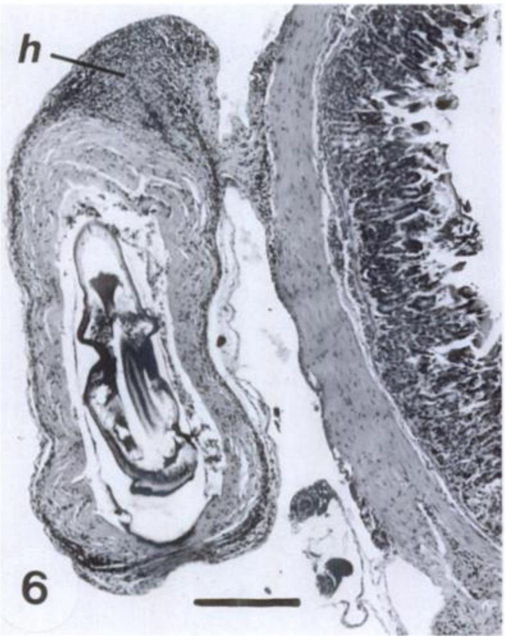

FIGURE 6. Section of stomach of *Ctenotus helenae* with larva in thickened pedunculate pocket of serosa; h = histiocytes. H&E. Bar = 0.25 mm.

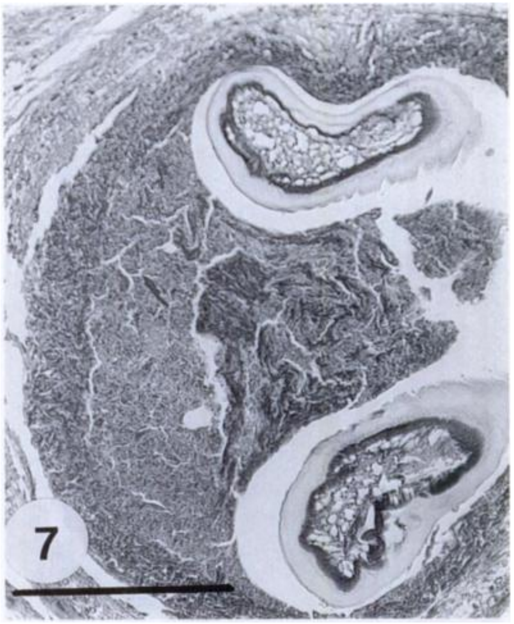

FIGURE 7. Section of stomach of snake *Acanthophis pyrrhus* with degenerating larvae surrounded by necrotic material in thick-walled submucosal cyst. Gabe's trichrome. Bar = 0.20 mm.

found in standard texts on reptile diseases (Reichenbach-Klinke, 1977; Frank, 1981). The cysts containing physalopterid larvae in *Lygosoma taeniolatum* described by Irwin-Smith (1922) were located on the outer surface of the stomach, beneath the mesentery, and were stated to have no connection with the host tissues. In the present study, cyst walls were identified as being composed of collagen on the basis of their structure and the bright green stain with trichrome, and therefore are believed to be derived from the host. Encysted third-stage physalopterid larvae occur in game birds (Boughton, 1937; Srivastava, 1971), and Srivastava (1971) reported considerable fibrous reaction around physalopterid larvae in striated muscle. Widmer (1970) found the prairie rattlesnake (*Crotalus viridis*) to be frequently infected with third-stage *Physaloptera* spp. larvae; these were attached to the stomach wall but not encysted, and he speculated that this reptile acts as a paratenic host. The high prevalence and intensity of physalopterid larvae in snakes and desert lizards in Australia, and their predominance in those hosts, especially smaller lizards, in which adult Physalopteridae do not occur, is evidence that these reptiles are paratenic hosts. Since nematodes in the genus *Abbreviata* are the predominant gastric helminths in larger Australian reptiles (Jones, 1978, 1988), and there are comparatively few carnivorous mammals in the desert areas, it is probable that the great majority of these larvae are *Abbreviata* spp. (Jones, 1995).

The most salient aspect of the present study was the relative lack of inflammatory response to the cysts, especially in the smaller lizards, despite heavy infections. Penetration of the gastric mucosa of *V. salvatori* by third-stage larvae of the gnathostomatid *Tanqua* sp., on the other hand, causes severe host reaction, resulting in the production of foreign body giant cells within a loose capsule, out of which the molting larvae eventually break to re-enter the stomach lumen (Pflugfelder, 1948). I found no evidence that larvae re-entered the stomach lumen.

There were few examples of resorption of larvae in lizards, but most sections of snake stomach contained from one to several larvae in various stages of resorption. Nonetheless, infections in most snakes were heavier than in lizards (except *N. laevissimus*), and the number of larvae undergoing resorption was a small proportion of the viable larvae in any one section.

One question is whether the larvae were killed by the host, or whether the host reaction set in after the death of the larvae. The fact that even in heavy infections, few cysts had evidence of host cellular response is evidence that there was no progressive process set in motion from the time of infection. Furthermore, there was no host response to non-encysted larvae, which commonly were found migrating within the tissues. These observations contrast with the considerable pathological reaction to non-encapsulated physalopterid larvae reported in a case of human infection (Nicolaides et al., 1977). There is little information on the longevity of encysted nematode larvae in reptiles; *Habronema* spp. (Spirurida) has been reported to survive for 154 days (Sharpilo, 1975). The lifespan of lizards is variable, but most can live for more than 5 yr, and some for 20 yr, with larger species generally being more long-lived than smaller species (Bustard, 1970; Greer, 1989). The presence of more dead and resorbing larvae in larger reptiles is evidence that these hosts would outlive the natural lifespan of third-stage physalopterid larvae. This is further evidence that the pathological reactions are in response to the death of the larvae, rather than their cause.

Another possibility that deserves study is the effect on encysted larvae of the great distension which occurs in snake stomachs when prey are ingested, and whether this results in injury or mortality.

The absence of any significant pathological reaction even when large numbers of encysted larvae are present is evidence for a long evolutionary association between these physalopterid species and their reptile hosts.

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