ATTEMPTS TO DIFFERENTIATE ELAPHOSTRONGYLINE

LARVAE (NEMATODA: PROTOSTRONGYLIDAE) USING GUINEA PIGS

(*Cavia porcellus*) AS ALTERNATE HOSTS

BY

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ABSTRACT

First- and third- stage larvae of *Parelaphostrongylus tenuis*, *P. andersoni* and *Elaphostrongylus cervi* could not be distinguished morphometrically. Third-stage larvae of *P. tenuis* did develop into recognizable adults in experimentally-infected guinea pigs, but *P. andersoni* and *E. cervi* did not. At present, elaphostrongyline larvae found in the feces of cervids in eastern North America are most likely to be either *P. tenuis* or *P. andersoni*; this study confirms that the experimental infection of guinea pigs will allow this distinction to be made. Because *E. cervi* is currently believed to be restricted to Newfoundland, its third-stage larvae are distinguished by geographic origin.

As many as 22 migrating *P. tenuis* larvae were recovered from outside the central nervous system (CNS) of each of seven guinea pigs necropsied between 1 and 27 days post-infection (DPI); all resembled third-stage larvae digested from snails and used for infections. From one to six developing *P. tenuis* larvae were recovered from the CNS of each of fourteen infected guinea pigs. These included third-stage larvae, longer than reported in published literature, which were recovered as early as 9 DPI and as late as 20 DPI in the CNS. Fourth-stage larvae were recovered between 18 and 47 DPI, and fifth-stage larvae between 20 and 61 DPI; these stages were only found in the CNS. The morphology of the buccal capsule and the tail distinguished the third-, fourth- and fifth- stage larvae. Descriptions and dimensions of third- and fourth-stage *P. tenuis* larvae recovered from the CNS are provided for the first time.

Lesions found in the stomach wall, mesentery and liver of guinea pigs infected with *P. tenuis* suggest that third-stage larvae migrate through the abdominal cavity towards the CNS. Similar lesions found in guinea pigs

infected with *P. andersoni* and *E. cervi* suggest that larvae of these species follow a similar route. However, *P. andersoni* apparently failed to reach the musculature of any of six experimentally-infected guinea pigs. Similarly, *E. cervi* was not recovered from the CNS or musculature of any of six experimentally-infected guinea pigs.

Administration of dexamethasone appears to have been ineffective in suppressing the immune response of guinea pigs to infection with these elaphostrongylines. The mean number of *P. tenuis* larvae recovered from the CNS of guinea pigs given dexamethasone was not significantly different from that recovered from guinea pigs given saline. Treatment with dexamethasone allowed neither *P. andersoni* nor *E. cervi* larvae to establish in guinea pigs.

ACKNOWLEDGEMENTS

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INTRODUCTION

North American cervids harbour at least four species of elaphostrongyline nematodes (Protostrongylidae: Elaphostrongylinae). Two of these, *Parelaphostrongylus tenuis* (Dougherty, 1945) Pryadko and Boev, 1971 and *Elaphostrongylus cervi* Cameron, 1931, can cause severe disease in some hosts. The other two species, *Parelaphostrongylus andersoni* Prestwood, 1972 and *P. odocoilei* (Hobmaier and Hobmaier, 1934) Boev and Schulz, 1950, are not usually pathogenic. All four species produce similar first-stage larvae which cannot be distinguished morphologically (Prestwood *et al.* 1974; Samuel and Holmes 1974; Lankester and Northcott 1979; Halvorsen and Wissler 1983; Ballantyne and Samuel 1984; Pybus and Shave 1984; Gray *et al.* 1985). Likewise, third-stage larvae, which develop in gastropods, do not differ significantly in dimensions (Gray *et al.* 1985), and there is some disagreement on whether their tail morphology is useful in distinguishing them to species (Prestwood 1972; Pybus and Samuel 1981; Ballantyne and Samuel 1984; Lankester and Hauta, in press). To date, adult male elaphostrongylines must be recovered from infected cervids for positive identification of these species.

There is obvious benefit in pursuing more practical and reliable methods of distinguishing members of this group of parasites. The increased interest in game ranching and the importation of exotic ungulates for this purpose, or for zoo specimens, has increased the need for more knowledge of the distribution of the elaphostrongylines and their pathogenicity in different hosts. Previous translocation of cervids has occasionally resulted in the introduction of one of these parasites into new areas (Watson and Gill 1985; Lankester and Fong, pers. comm.), or the introduction of a susceptible ungulate into areas in which

one of the pathogenic species is enzootic (Anderson 1971; Carpenter *et al.* 1973; Trainer 1973; Kistner *et al.* 1977; Brown *et al.* 1978; Nichols *et al.* 1986).

Parelaphostrongylus tenuis is a neurotropic parasite of white-tailed deer (*Odocoileus virginianus*) of southeastern Canada and the United States (see review by Anderson and Prestwood 1981; Kocan *et al.* 1982; Dew 1988). Adults are usually found in the subdural spaces and venous sinuses of the cranium of their hosts (Anderson 1963). Natural or experimental infections with P. tenuis have been reported in moose (*Alces*), caribou (*Rangifer tarandus caribou*), reindeer (*R. t. tarandus*), wapiti (*Cervus elaphus canadensis*), red deer (*C. e. elaphus*), mule deer (*Odocoileus hemionus*), black-tailed deer (*O. h. columbianus*), fallow deer (*Dama*), and various non-cervid hosts including sheep (*Ovis aries*), goats (*Capra* spp.), antelope (*Antilocapra americana*), llamas (*Lama guanicoe*), and guinea pigs (*Cavia porcellus*) (see review by Anderson and Prestwood 1981; Nichols *et al.* 1986). Infection in white-tailed deer is normally asymptomatic, whereas most other hosts develop neurologic signs of disease including lumbar weakness, ataxia, circling, fearlessness, paresis, and paraplegia; the end result of which is often death (Anderson and Prestwood 1981).

Parelaphostrongylus andersoni is found in the muscles of its hosts and appears to have a disjunctive distribution. It occurs in white-tailed deer of the southeastern United States (Prestwood *et al.* 1974), possibly in New Jersey (Pursglove 1977), and in south-eastern British Columbia (Pybus and Samuel 1981). It has recently been found in caribou (*R. t. caribou* or *R. t. groenlandicus*) in Labrador, in the central Northwest Territories, and on the Slate Islands, Ontario (Lankester and Hauta, in press). Adult parasites have been found throughout most skeletal muscles (Prestwood 1972; Pybus and

Samuel 1984a). Pybus and Samuel (1984a) also reported the recovery of adults from the epidural tissues and spaces of the central nervous system, and from fat associated with the abomasum, of experimentally-infected white-tailed deer. Experimental infections of mule deer have also been successful; adult parasites were recovered from all sites previously mentioned, and infections reached patency in this host (Pybus and Samuel 1984a). No clinical disease is known in naturally-infected white-tailed deer or caribou, but experimental infections of the former with large numbers of infective larvae affected their posture, gait and strength (Nettles and Prestwood 1976).

Adults of *P. odocoilei* were originally described from the dorsal skeletal musculature of naturally-infected Columbian black-tailed deer and mule deer of north central California (Hobmaier and Hobmaier 1934; Brunetti 1969). In Alberta, this species was identified experimentally by infecting mule deer with larvae recovered from naturally-infected mule deer (Platt and Samuel 1978a). Caribou of west-central Alberta also harbour natural infections of this muscleworm (Gray and Samuel 1986), and natural infection of mountain goats (*Oreamnos americanus*) has been reported (Pybus *et al.* 1984). Black-tailed deer, mule deer and moose have been infected experimentally with *P. odocoilei* (Platt and Samuel 1978b; Pybus and Samuel 1980, 1984a), but the apparent success of experimental infections in white-tailed deer needs confirmation (Platt and Samuel 1978b; Pybus and Samuel 1984a). Clinical signs of anorexia, listlessness and scouring have been reported in infected moose, mule deer and mountain goats (Platt and Samuel 1978b; Pybus and Samuel 1984b; Pybus *et al.* 1984).

Elaphostrongylus cervi was first reported in North America from caribou of Newfoundland (Lankester 1977; Lankester and Northcott 1979). It was

previously known only in cervids of Eurasia (see review by Lankester 1976) and New Zealand (Mason and McAllum 1976; Mason *et al.* 1976). Adult parasites are found in the central nervous system, beneath the skin and on deeper muscles of the fore and hind limbs (see review by Lankester 1976). It is a known cause of neurologic disease in cervids of Eurasia (see review by Kontrimavichus *et al.* 1976; Stuve and Skorping 1987) and causes lumbar weakness and posterior paralysis in wild caribou of Newfoundland (Lankester and Northcott 1979). Similar disease signs are observed in moose experimentally infected with this nematode (Lankester 1977; Stuve and Skorping 1987).

In order to confirm the identity of elaphostrongyline larvae, it remains necessary to experimentally infect cervids and recover adult males. The time and cost associated with this procedure might be reduced by using hosts other than cervids. Small laboratory animals such as guinea pigs, which are easier to handle and more economical to raise than cervids, have been used previously in studies with the elaphostrongylines, but with limited success. Some third-stage larvae of *P. tenuis* successfully migrated to the central nervous system (CNS) of guinea pigs and developed to recognizable fifth-stage larvae (immature adults) (Anderson and Strelive 1966; Spratt 1967). Similarly, some third-stage larvae of *E. cervi* migrated to the CNS of guinea pigs and developed to recognizable adults (Watson and Gill 1985). On the other hand, *P. andersoni* and *P. odocoilei* apparently do not develop in guinea pigs; infective third-stage larvae of these two species appear to be overcome by the host's immune response (Pybus and Samuel 1984c).

In this study, the use of guinea pigs as alternate hosts for *P. tenuis*, *P. andersoni* and *E. cervi* was re-examined. Some guinea pigs were given an anti-

inflammatory drug, dexamethasone, in an effort to suppress their immune responses and thereby increase the likelihood of all three species developing to recognizable adults. In addition, first- and third-stage larvae of each species were compared in an attempt to find morphological differences.

METHODS AND MATERIALS

Collection and Examination of Fecal Material

First-stage larvae of *Parelaphostrongylus tenuis* were isolated from white-tailed deer feces collected near Grand Marais, Minnesota. Recovery of adult nematodes in the crania of white-tailed deer from this area confirmed infections with *P. tenuis*. Examination of deer musculature has not revealed the presence of any other elaphostrongyline nematodes (Lankester, unpubl. data).

First-stage larvae of *P. andersoni* were isolated from the feces of woodland caribou on the Slate Islands, Ontario (48°43'N, 86°37'W), where this muscleworm is believed to be the only elaphostrongyline present (Lankester and Hauta, in press).

First-stage larvae of *Elaphostrongylus cervi* were isolated from woodland caribou feces collected off range in central Newfoundland (48°00'N, 55°00'W). Caribou from this area are known to harbour *E. cervi* (Lankester and Northcott, 1979). Efforts to find other muscleworms in these herds have so far been unsuccessful (Lankester and Fong, unpubl. data).

First-stage protostrongylid larvae were recovered from feces using the Baermann filter technique. Pellets were placed on tissue paper ("Kimwipes", Kimberly-Clark #57-360) and suspended in distilled water in glass funnels (14 cm in diameter). After 24 hours, samples of 10 to 15 ml of water were drained off and examined for larvae. Larvae were concentrated in distilled water in settling flasks and refrigerated one to seven days before being used to infect snails.

Exposure of Snails to Larvae

Terrestrial snails, *Mesodon thyroidus* and *Triodopsis albolabris*, reared from eggs in the laboratory, were exposed to first-stage larvae on filter paper lining Petri dishes 6 cm in diameter. Five juvenile snails were placed on the filter paper in covered dishes. Snails were left five to ten days to ensure successful, heavy infections. They were checked periodically and those that had crawled off the filter paper were returned. After the infection period, snails were kept in glass finger bowls with moist paper towels in an environmental chamber at 20°C. Lettuce and chalk were provided weekly.

Examination of Snails and Larvae

After thirty to fifty days, third-stage larvae were digested from snail tissues using artificial pepsin solution (3 g pepsin, 4 ml concentrated hydrochloric acid and 500 ml distilled water). Snail tissue was suspended in solution over small pieces of wire screening, and digests were incubated at 37°C for up to 12 hours. Examinations were conducted hourly for third-stage larvae.

For morphometric studies, first- and third- stage larvae of each species were heat-relaxed in a drop of water on a slide over a hot-plate, and then drawn and measured using a Wild drawing tube and stage micrometer. All larvae were stored in 10% glycerin in 70% alcohol. Drawings and measurements of stored larvae were made in glycerin after larvae were cleared by allowing the alcohol to evaporate.

Infection and Maintenance of Guinea Pigs

Juvenile guinea pigs, six to ten weeks of age, were infected with 100 to 150 third-stage larvae administered orally in approximately 2 ml of water, using a plastic pipette. Animals were trained to accept water in this manner for at least one week prior to infections. Following the infections, guinea pigs were isolated and observed for fifteen to twenty minutes for signs of regurgitation. Guinea pigs were infected in small groups on different days.

Guinea pigs infected with *P. tenuis* or *E. cervi* were given sub-cutaneous injections of either dexamethasone (0.4 mg in 0.2 ml of solution) or saline (0.2 ml). All guinea pigs infected with *P. andersoni* were given injections of dexamethasone, at dosages of either 0.2 mg or 0.4 mg per animal (as previous infections of guinea pigs by Pybus and Samuel (1984) were unsuccessful). Injections were given on alternate sides of the abdomen, every other day (EOD), commencing one week prior to infections and continuing for the duration of the experiment.

Guinea pigs, in groups of two or three, were housed in metal cages either with solid or wire-mesh flooring. In the former, wood shavings were added to a depth of 2 cm as bedding material. Animals were fed fresh guinea pig or rabbit pellets, supplemented with oranges and hay. They were observed daily and weighed every six to ten days.

At pre-determined intervals, or after the onset of neurologic signs, animals were killed with an over-dose of sodium pentobarbitol (240 mg/kg), injected into the peritoneal cavity. A fecal sample was obtained from the rectum and placed in water or saline for subsequent examination for first-stage larvae.

Necropsy of Guinea Pigs.

The skin was removed and the external muscles were examined using a magnifying glass (10X) and a stereo microscope (25X). The abdominal cavity was opened, rinsed with saline, and the abdominal viscera were removed into saline. The thoracic cavity was opened and rinsed, and the lungs and heart placed in saline. All viscera were stored in saline for six to ten hours before being examined by pressing between two thick glass plates. The pressed tissues and saline rinses were examined for nematodes using a stereo microscope at 25X.

The animal was decapitated and the brain removed with the dura mater intact. The brain was examined for gross lesions and divided into right and left cerebral hemispheres, cerebellum, and brain stem (medulla, pons and associated tissues). Each portion of the brain was teased apart in saline and pressed in search of larvae.

Dorsal portions of the vertebral column were removed to expose the spinal cord. Lateral nerves were cut close to the vertebrae and the cord was freed from the column with the dura mater intact. The cord was divided into cervical, thoracic and lumbar-sacral regions. Each region was examined following the same procedure used to examine the brain.

The skeletal musculature was examined grossly before being removed, teased apart in saline, and pressed. The abdominal wall and muscles of the rib cage of animals euthanized before 18 days post-infection (DPI) were also examined by digestion in pepsin at 37°C.

Larvae recovered from guinea pigs were heat-relaxed, either in hot saline or hot glycerin-alcohol, and stored in glycerin-alcohol. After clearing to glycerin, larvae were drawn in glycerin, or in lactophenol which facilitated

manipulating larvae on slides.

Statistical tests followed Steele and Torrie (1980). Analysis of variance and Scheffe's tests were used to compare mean dimensions of larvae. The standard T-test was used when comparing two means. The minimum level for statistical significance was $P \leq 0.05$ for all tests. Mean values given in the text are followed by standard deviation (S.D.).

RESULTS

Larval Morphometrics

Mean dimensions (± S.D.) of the first- (L1) and third- (L3) stage larvae of all three species studied are given in Table 1. Scheffe's analysis revealed that the mean lengths of first-stage larvae of *P. tenuis* and *P. andersoni* were not significantly different, but those of *E. cervi* were longer. However, the ranges in length of first-stage larvae of each species overlapped. Individual larvae therefore could not be distinguished by dimensions or by morphology. The mean lengths of third-stage larvae of these species were all significantly different, but there was also considerable overlap in the ranges in length and other dimensions of each species.

Tail shapes of third-stage larvae were highly variable (Fig. 1), and could not be used to distinguish the three species. Larvae of *P. tenuis* frequently had pointed tails with a large dorsal hump, just anterior to the tail tip, giving the tip a digitiform appearance. However, some *P. tenuis* larvae had more gently rounded tails with a less-pronounced dorsal hump, or with no hump at all. Similar variation was seen in tails of *P. andersoni* larvae. The tail tips were less digitiform than in *P. tenuis*. A distinct dorsal hump was usually present, but in some larvae it was barely discernible. Third-stage *E. cervi* larvae had tail shapes that included the variations seen in *P. tenuis* and *P. andersoni*.

TABLE 1. Mean dimensions ($\pm$ S.D.), in microns (μm), of first- (L1) and third- (L3) stage elaphostrongyline larvae, recovered January to August 1988.

	P. tenuis		P. andersoni		E. cervi	
	L1's	L3's	L1's	L3's	L1's	L3's
Length*	370 ± 16 (21)[a]	1162 ± 54 (83)[a]	363 ± 18 (25)[a]	989 ± 39 (21)[b]	413 ± 25 (14)[b]	1051 ± 36 (25)
	(332 - 399)	(1036 - 1310)	(330 - 399)	(900 - 1062)	(370 - 450)	(956 - 1121)
Width	18 ± 1 (17)	51 ± 6 (83)	21 ± 5 (15)	46 ± 5 (21)	20 ± 2 (12)	43 ± 5 (25)
	(17 - 20)	(35 - 67)	(16 - 33)	(40 - 53)	(17 - 24)	(30 - 51)
Nerve Ring	96 ± 7 (3)	142 ± 8 (65)	97 ± 3 (8)	122 ± 5 (19)	103 ± 8 (6)	127 ± 10 (17)
	(91 - 104)	(126 - 170)	(93 - 102)	(107 - 130)	(97 - 117)	(107 - 150)
Excretory Pore	102 ± 5 (17)	167 ± 9 (82)	98 ± 4 (16)	126 ± 7 (15)	107 ± 5 (11)	136 ± 16 (19)
	(91 - 113)	(138 - 185)	(93 - 107)	(114 - 144)	(100 - 115)	(120 - 198)
Esophagus	177 ± 7 (19)	443 ± 26 (83)	171 ± 8 (16)	361 ± 20 (21)	184 ± 8 (13)	375 ± 28 (25)
	(166 - 191)	(376 - 498)	(160 - 192)	(300 - 390)	(168 - 196)	(307 - 467)
Genital Primordium	239 ± 11 (14)	730 ± 39 (58)	238 ± 7 (3)	670 ± 22 (18)	241 ± 17 (6)	689 ± 57 (14)
	(224 - 260)	(640 - 860)	(233 - 246)	(612 - 711)	(220 - 263)	(547 - 770)
Tail	38 ± 3 (20)	51 ± 5 (82)	34 ± 3 (19)	39 ± 5 (21)	42 ± 4 (14)	35 ± 5 (25)
	(33 - 43)	(37 - 61)	(29 - 40)	(31 - 48)	(37 - 48)	(28 - 49)

* All dimensions are of larvae heat-relaxed and measured in water; ranges are provided in brackets.
[a] Different superscripts, within the same larval stage (L1 vs. L3), denote statistically significant differences in mean length.

Fig. 1. Tail profiles (100X) of third-stage elaphostrongyline larvae, heat-relaxed and drawn in water. (A) *Parelaphostrongylus tenuis*, originating from white-tailed deer, Grand Marais, Minnesota.

(B) *Parelaphostrongylus andersoni*, from woodland caribou, Slate Islands, Ontario. (C) *Elaphostrongylus cervi*, from woodland caribou, Newfoundland.

A

B

C

20 μm
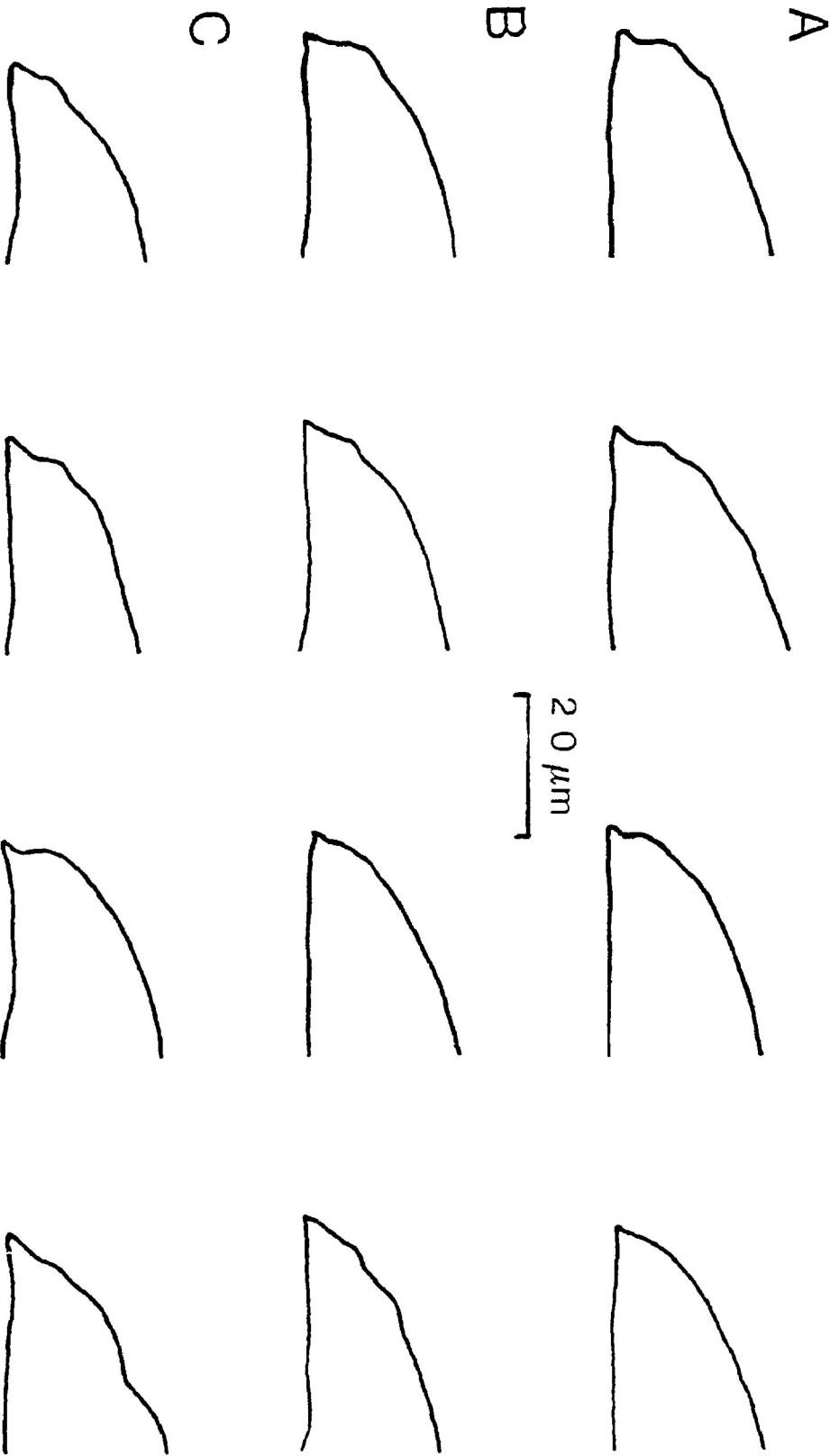
Necropsies and Recovery of Larvae

A total of 144 larvae was recovered from the 16 guinea pigs experimentally infected with *P. tenuis* (mean intensity: 9.0 ± 7.8 larvae per animal)(Table 2). Fifty-three of these larvae were recovered from the central nervous system (CNS) of 14 animals (3.8 ± 1.7 larvae per animal). Migrating larvae (MIG), defined here as larvae recovered from sites outside the CNS, were recovered from most animals necropsied up to 27 DPI, and were indistinguishable from infective third-stage larvae.

Of the larvae recovered from the CNS of guinea pigs, twenty-four (mean=3.4 ± 1.7) were recovered from animals given dexamethasone, and twenty-nine (mean=4.1 ± 1.8) from animals given saline. The difference between these means was not statistically significant (t_{12}=0.765, P>0.4). The numbers of migrating larvae recovered from dexamethasone versus saline treated guinea pigs were not compared as only one saline-treated animal (T-11), necropsied up to 27 DPI, was examined for migrating larvae. Clinical signs of disease, which included lameness and disorientation, were observed in six animals (Table 2).

No larvae were recovered from the musculature of any of the eight guinea pigs infected with *P. andersoni* (Table 3), under either treatment with dexamethasone (0.2 mg or 0.4 mg per animal). Clinical signs of disease were not observed in any of these animals. Migrating (MIG) third-stage larvae (n=7), indistinguishable from infective larvae, were recovered from one animal (A-2); this animal was given 42 additional third-stage larvae 24 hours prior to necropsy.

No larvae were recovered from the CNS or musculature of any of the eight guinea pigs infected with *E. cervi* (Table 4); clinical signs of disease were not observed. One migrating third-stage larva, indistinguishable from

Table 2. Summary of treatments and infections of guinea pigs given third-stage
Parelaphostrongylus tenuis larvae.

Guinea pig	Sex	Larvae given	Treatment[1]	Necropsy (DPI[3])	Clinical signs	Larvae recovered[2] CNS (%)	MIG	Tot (%)
T-1	M	114	Dex	1	no	0	12	12 (10.5)
T-2	M	119	Dex	3	no	0	18	18 (15.1)
T-3	F	116	Dex	9	no	3 (2.6)	22	25 (21.6)
T-4	F	116	Dex	12	no	4 (3.4)	20	24 (20.7)
T-5	M	83	Dex	15	no	2 (2.4)	3	5 (6.0)
T-6	F	79	Dex	18	no	1 (1.3)	2	3 (3.8)
T-7	F	155	Sal	18	no	4 (2.6)	*	4 (2.5)
T-8	M	143	Sal	20	no	6 (4.2)	*	6 (4.2)
T-9	M	144	Sal	21	no	3 (2.1)	*	3 (2.1)
T-10	M	147	Sal	22	no	5 (3.4)	*	5 (3.4)
T-11	F	151	Sal	27	yes	4 (2.6)	14	18 (11.9)
T-12	M	152	Dex	35	yes	6 (3.9)	0	6 (3.9)
T-13	F	154	Dex	38	yes	3 (1.9)	0	3 (1.9)
T-14	F	149	Dex	41	yes	5 (3.4)	0	5 (3.4)
T-15	M	154	Sal	47	yes	6 (3.9)	0	6 (3.9)
T-16	M	-	Dex	53	-	-	-	-
T-17	F	151	Sal	61	yes	1 (0.7)	0	1 (0.6)
T-18	F	-	Sal	71	-	-	-	-

[1] Guinea pigs were given 0.2 ml injections, EOD, of either saline (Sal) or dexamethasone
(Dex; 2 mg/ml).
[2] Larvae were recovered from the central nervous system (CNS) or were considered migrating
to the CNS (MIG), if found anywhere outside the CNS.
[3] DPI = days post-infection
* Only the CNS was examined in these animals

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Table 3. Summary of treatments and infections of guinea pigs given third-stage
Parelaphostrongylus andersoni larvae.

Guinea pig	Sex	Larvae given	Treatment[1]	Necropsy (DPI[3])	Clinical signs	Larvae[2] recovered
A-1	M	98	Dex2	32	no	0
A-2*	F	105	Dex1	38	no	7*
A-3	F	98	Dex2	67	no	0
A-4	M	97	Dex1	70	no	0
A-5	F	98	Dex1	84	no	0
A-6	M	-	Dex1	86	-	-
A-7	F	100	Dex2	87	no	0
A-8	M	-	Dex2	90	-	-

[1] All animals were given dexamethasone injections (2 mg/ml), EOD; only the
amount given varied: 0.1 ml (Dex1) or 0.2 ml (Dex2).
[2] This column reflects efforts to find larvae in any location.
[3] DPI = days post-infection
* This guinea pig was given 42 more larvae 24 hours prior to necropsy.

Table. 4. Summary of treatments and infections of guinea pigs given third-stage *Elaphostrongylus* *cervi* larvae.

Guinea pig	Sex	Larvae given	Treatment[1]	Necropsy (DPI[3])	Clinical signs	Larvae[2] recovered
C-1	F	105	Dex	31	no	0
C-2	M	109	Sal	42	no	0
C-3	F	103	Sal	70	no	0
C-4	M	105	Dex	74	no	0
C-5	M	105	Sal	88	no	0
C-6	F	-	Sal	91	-	-
C-7*	F	108	Dex	93	no	1*
C-8	M	-	Dex	95	-	-

[1] Guinea pigs were given 0.2 ml injections, EOD, of either saline (Sal) or dexamethasone (Dex; 2 mg/ml).

[2] This column reflects efforts to find larvae in any location.

[3] DPI = days post-infection

* This guinea pig was given 83 more larvae 24 hours prior to necropsy.

infective larvae, was recovered from one animal (C-7); this animal was given 83 additional third-stage larvae 24 hours prior to necropsy.

The following sections give a chronological account of the necropsy findings for each guinea pig, grouped by species of nematode. Descriptions and dimensions of *P. tenuis* larvae recovered are given in a separate section.

Larvae generally were easy to locate in tissue presses, being straw-coloured in comparison to tissues. Grossly visible lesions occurred in the CNS of only three animals; all were infected with *P. tenuis*. No gross lesions associated with elaphostrongyline infections were apparent in the musculature of any animal. First-stage larvae were not found in the feces of any guinea pig at the time of necropsy.

Necropsy of Guinea Pigs Infected with P. tenuis

<u>1 DPI</u>

Guinea Pig: T-1 (M)
Clinical Signs: none
Necropsy:

Twelve third-stage larvae (L3) were recovered from saline rinses of the abdominal cavity and viscera. Larvae recovered from viscera and saline rinses of this and all other animals were usually alive and active.

A small (3-4 mm diameter) hemorrhage and two small greyish areas were present in the stomach wall. The latter lesions appeared grey under the stereo microscope on pressing and were likely accumulations of inflammatory cells (an assumption made throughout the text). Similar areas of diffuse hemorrhage and probable inflammation were also observed in the wall of the duodenum,

near the stomach, and in the wall of the colon, near the caecum. No other lesions or larvae were observed.

3 DPI

Guinea Pig: T-2 (M)
Clinical Signs: none
Necropsy:

Three larvae (L3) were recovered from rinses of the abdominal cavity and viscera. Much of the surface of the pyloric region of the stomach was covered with diffuse hemorrhage, and a small greyish area (described previously) was evident upon pressing. The mesentery in this region was hemorrhagic and contained many small yellowish capsules (< 2 mm dia.). One larva (L3) was found on pressing the mesentery and 13 more were recovered from digests of the mesentery and pancreas. All larvae recovered in tissue digests at this necropsy and subsequent ones were dead. One small hemorrhage was evident on the pancreas. Two greyish areas (3-4 mm dia.) were present on the duodenum. One larva (L3) was recovered on pressing the liver.

The capsules, seen in this animal and in subsequent necropsies, were golden brown in colour, relatively transparent, usually contained dark granular material, and consisted of a tough, gelatinous outer wall. Although some capsules in subsequent necropsies contained larvae, in this animal the contents were indistinct.

9 DPI

Guinea Pig: T-3 (F)
Clinical Signs: none
Necropsy:

A total of three larvae (L3) was recovered within the tissues of the CNS. No lesions were apparent on the surface of the brain or spinal cord. All three larvae were found in the saline that contained the thoracic region of the spinal cord, after it had been teased apart.

Three larvae (L3) were found in saline rinses of the abdominal cavity and viscera. The pyloric region of the stomach was reddened and the wall felt thickened. Tissue presses revealed one small grey area (3-4 mm) near the pyloric sphincter. The pancreas and mesentery adjacent to this region of the stomach were hemorrhagic. Fourteen larvae (L3) were recovered from digests of the mesentery. Some of the lymph nodes in the mesentery were hemorrhagic as well, but no larvae were found on pressing them.

Four larvae (L3), and six capsules with indistinct contents, were discovered by pressing lesions visible on the liver. These lesions were whitish in colour and were present on the diaphragmatic surface of the left lateral and medial lobes. Most were simple lines that resembled thin tracks (hereafter referred to as "track-like") approximately 0.5 mm wide, 2 mm deep, and varying from 1 to 2 cm in length. Some of these lesions were wider (2-3 mm) and more extensive.

One small grey area was revealed on pressing the diaphragm. One larva (L3) was found in the rinse of the thoracic cavity. No other lesions or larvae were found.

12 DPI

Guinea Pig: T-4 (F)
Clinical Signs: none
Necropsy:

A total of four larvae (L3) was recovered from the CNS. One of these was recovered on pressing the lumbar-sacral cord, and two others were in the saline that contained the thoracic cord after it was teased apart. The fourth was found in the saline that contained the right cerebral hemisphere after it was teased apart. All of these larvae were recovered on the second day; initial examination of the surface of the CNS and teasing the tissues did not reveal them.

Five larvae (L3) were recovered from saline rinses of the abdominal cavity and viscera. Diffuse hemorrhage was apparent in the pyloric region of the stomach and the associated mesentery. Eleven more larvae (L3) were recovered in digests of the mesentery and pancreas.

Three larvae (L3) were found in presses of liver tissue: one was found in apparently normal tissue, while the other two were found associated with white track-like lesions observed on the diaphragmatic surface. These lesions were present on all lobes of the liver and were similar in appearance to those described previously. Approximately thirteen capsules, with indistinct granular contents, were also found associated with lesions on the liver. The ventral half of the medial lobe of the liver was replaced by a solid yellow fibrous lesion, but no larvae were found associated with this area.

One larva (L3) was found in the saline that had contained the thoracic viscera. The lungs appeared normal except for one or two small, hard nodules (2-3 mm dia.) on the surface; presses and digests were negative for larvae.

15 DPI

Guinea Pig: T-5 (M)
Clinical Sign: none
Necropsy:

Two larvae (L3) were recovered on pressing regions of the CNS: one of these was found in the cervical cord and the other in the brain stem.

Three larvae (L3) were recovered from the saline that contained the mesentery and pancreas, the tissues of which were hemorrhagic. Track-like lesions were present on the diaphragmatic and visceral surfaces of the right medial lobe of the liver. A yellowish lesion (3 mm dia.) was also present on the visceral side of the left lateral lobe. A small yellow nodule (1 mm dia.) was found on surface of the lung. No other larvae or lesions were found.

18 DPI-a

Guinea Pig: T-6 (F)
Clinical Signs: none
Necropsy:

One larva (L3) was found on pressing the cerebellum. Two larvae (L3) were found in saline rinses of the abdominal viscera. The mesentery was hemorrhagic and thickened. Many capsules were found on pressing the mesentery, but their contents were indistinct. Each lobe of the liver had at least one track-like lesion present on either the visceral or diaphragmatic surface; some of these tracks were associated with bright red hemorrhages. A small yellow nodule was found on the surface of the lung. No larvae were recovered on pressing any tissue.

18 DPI-b

Guinea Pig: T-7 (F)
Clinical Signs: none
Necropsy:

A total of four larvae was recovered from the CNS. One (L3) was recovered from the saline after teasing apart the thoracic cord. A female fourth-stage larva (L4) was found on pressing the brain stem. A second female (L4) was found in the saline immediately following the removal of the right cerebral hemisphere from the rest of the brain. A male (L4) was found on pressing the right cerebral hemisphere, but was badly damaged during its examination and could not be measured.

Only the CNS of this and the following three animals was examined due to time constraints.

20 DPI

Guinea Pig: T-8 (M)
Clinical Signs: none
Necropsy:

A total of six larvae was recovered from the CNS. Two (L3) were found on pressing the spinal cord; one was in the thoracic region and the other in the cervical region. The latter was badly damaged and could not be measured. A female fifth-stage larva (L5), still within its fourth-stage cuticle, was found on pressing the medulla oblongata. A male (L4) was found in the saline in which the medulla oblongata was teased apart. On pressing the remainder of the brain stem, a larva that appeared to be a fourth-stage male was recovered, but it was badly damaged prior to its examination and could not be positively identified or measured. A second female (L5) was found protruding from neural tissue on the dorsal side of the cerebellum.

21 DPI

Guinea Pig: T-9
Clinical Signs: none
Necropsy:

A total of three larvae was recovered from the CNS; all were within the tissues of the spinal cord. On pressing the thoracic region, two larvae were recovered: one male (L5), still within its fourth-stage cuticle, and one (L4) that could not be identified to sex (based on characteristics discussed in a following section). The third larva, a male (L5) also still within its fourth-stage cuticle, was found on pressing the cervical region.

22 DPI

Guinea Pig: T-10
Clinical Sign: none
Necropsy:

A total of five larvae was found in the CNS. Two, a male (L5) and a female (L4), were recovered on pressing the lumbar-sacral region of the spinal cord. Another female (L4) was found in the saline in which the thoracic cord was teased apart. A second male (L5) was freed on teasing apart the medulla oblongata. The fifth larva, a female (L4) still within its third-stage cuticle, was found on pressing the right cerebral hemisphere.

27 DPI

Guinea Pig: T-11 (F)
Clinical Signs:

Signs were first observed 7 DPI, when this animal had difficulty maintaining its balance. It circled towards the right, and often fell over onto its right side. These signs worsened over the next week, at which time a white liquid discharge was discovered in the right ear. Treatment with

Gentocin Otic (Schering Canada, Pointe Claire, Quebec) was initiated immediately but the condition did not improve and the animal was found dead 27 DPI.

Necropsy:

Four larvae were recovered from the CNS; none were evident upon examination of the surface of the intact brain and spinal cord. A male (L5) was found free in saline containing the brain stem, after it was separated from the rest of the brain. The right cerebrum was gently teased apart in saline and another male (L5) was found within the tissue; a female (L5) was recovered from the saline that contained this part of the brain, after it was teased apart. Another female (L4) was discovered in the saline that contained the left cerebrum, 24 hours after it had been teased apart and examined. The tissues within this part of the brain were yellowish and more fragile, compared to the rest of the brain, and a large hemorrhage (<4 mm dia.) was evident in this tissue.

Four larvae (L3) were found in saline rinses of the abdominal cavity and viscera. Five larvae (L3) were found by pressing the mesentery and pancreas, and an additional five (L3) were recovered on digesting these tissues. A white nodule (<2 mm dia.) and several track-like lesions were found on the visceral surface of the right lateral lobe of the liver. A similar track-like area was present on the diaphragmatic surface of the left lateral lobe.

The left lateral lobe of the lung was hemorrhagic and adhered to the pericardium and the thoracic wall. Hemorrhages (4-5 mm dia.) were present on the right and left sides of the ribs below the latissimus dorsi muscles and behind the scapula. Presses and digests of these and other tissues were negative for larvae.

35 DPI

Guinea Pig: T-12 (M)
Clinical Signs:

Clinical signs were first observed in this animal 27 DPI. The guinea pig held its head slightly tilted to the left and appeared to have difficultly walking. Ambulatory difficulty increased over the next few days. The guinea pig was found lying on its back 35 DPI, and if returned to an upright position it immediately fell sideways onto its back again; it was euthanized and examined.

Necropsy:

A total of six larvae (all L5) was recovered from the CNS. A male was located in the sub-dural space of the lumbar-sacral cord. Two more males were projecting from nerve tissue of the anterior end of the thoracic region of the cord, where it had been severed from the cervical region. A fourth male was found in the saline that contained the shredded brain stem. Two larvae (one male, one female) were found by teasing apart the cerebellum.

A small nodule (1-2 mm dia.) was found in the stomach wall upon pressing; dark granular material, with no distinct form, was evident within this nodule. A few areas of the liver also had small white nodules embedded in the tissue, but presses were negative for larvae. No lesions or larvae were found in any other tissues.

38 DPI

Guinea Pig: T-13 (F)
Clinical Sign:

This animal developed diarrhea 16 DPI, which persisted on and off for the next two weeks. Difficulty walking was observed 25 DPI; occasionally its left

hind leg would splay outward while it was walking. A gradual weakening of both hind limbs and the left front limb was noticed over the next ten days. Thereafter, mobility decreased rapidly and the animal was euthanized.

Necropsy:

A red hemorrhage was evident on the surface of the cervical region of the spinal cord. Two larvae, a male (L5) and a female (L4), were teased out from the tissues of this region. Another male (L5) was recovered from the tissues of the cerebellum. All tissues of the CNS were digested following teasing and pressing: a larva-like structure was found in the digest of the cervical cord, but it could not be positively identified because of its degenerate state.

The pancreas was hemorrhagic and capsules with indistinct contents were observed on pressing. A few white nodules were present on the diaphragmatic surface of the liver. No larvae were recovered from presses or digests of any tissue.

41 DPI

Guinea Pig: T-14 (F)
Clinical Signs:

At 37 DPI this animal could not remain upright, falling over to the right side; both the right fore- and right hind- limbs were immobile, but the animal was still able to manoeuver itself about the cage. It was euthanized four days later.

Necropsy:

A total of five larvae (all L5) was recovered from the CNS. One larva, in poor condition and therefore not measured, was recovered by digesting the thoracic cord. A large anterior portion of another was recovered from the

rinse in which the spinal cord was separated from the head, just behind the atlas. Two males were recovered from the tissues of the medulla oblongata: one near the surface, very close to the cerebellum, and the other deeper in the tissues. The fifth larva, a female that was damaged during its removal, was recovered from the brain stem, anterior to the medulla oblongata.

The mesentery associated with the stomach was hemorrhagic. White nodules were present on every lobe of the liver, and three nodules were found on the diaphragm. No larvae were recovered from presses of any tissues.

47 DPI

Guinea Pig: T-15 (M)
Clinical Signs:

A slight weakening of the right hind leg of this animal was first observed 31 DPI. When walking, both legs, viewed from behind, appeared to curl inward when extended back. Three days later, the left front leg was weak; the animal occasionally fell to the left, and was often found lying on its left shoulder. A tendency to tilt the head to the left and to circle to the left became evident. Over the next two weeks, signs did not worsen but instead often appeared to dissipate. Three days prior to necropsy, the left hind limb was weak and the animal was limping; both hind feet curled back so that the upper surfaces contacted the ground when walking.

Necropsy:

Six larvae were recovered from the CNS. Five of these, or segments of them, were found associated with the thoracic cord, which was damaged during removal from the vertebral column. A male (L4) was found in the saline containing this part of the cord prior to teasing; it may have escaped from the damaged tissue. Two females (L5) were recovered on pressing. A male (L5)

was also found deep in the tissue. A portion of a fifth larva (L5) (not measured) was found attached to a piece of the dura mater, which still adhered to the vertebral column. The sixth larva, a female (L5) still within its fourth-stage cuticle, was found in the tissues of the cerebrum.

The mesentery associated with the greater curvature of the stomach was hemorrhagic, and the liver had several white track-like lesions on the diaphragmatic surface; larvae were not recovered from presses of any tissues.

53 DPI

Guinea Pig: T-16 (M); CONTROL
Clinical Signs: none
Necropsy:

No lesions associated with a nematode infection were found in this animal, and all tissue presses were negative.

61 DPI

Guinea Pig: T-17 (F)
Clinical Sign:

Neurologic signs were first observed in this animal 31 DPI, with a weakening of the right hind leg becoming more apparent over the next few days. At 53 DPI, the guinea pig was less active than usual and three days later it was limping noticeably. These signs did not worsen before necropsy at 61 DPI.

Necropsy:

A large hemorrhage (7-8 mm dia.) was evident on the cerebellum, within the meninges. A single male larva (L5) was found in the subdural space associated with this hemorrhage. The surface of the cerebellum was yellow and thickened.

A slight hemorrhage was observed in the subcutaneous tissues over each knee joint. No gross damage was observed on any organ and presses of tissues were negative.

71 DPI

Guinea Pig: T-18 (F); CONTROL
Clinical Sign: none
Necropsy:

No lesions or larvae were found on examination of tissues.

Necropsy of Guinea Pigs Infected with P. andersoni

32 DPI

Guinea Pig: A-1 (M)
Clinical Signs: none
Necropsy:

No lesions associated with a nematode infection were found on examination of this animal.

38 DPI

Guinea Pig: A-2 (F)
Clinical Signs: none
Necropsy:

This animal was originally infected 38 days prior to necropsy (Table 3); a second infection of 42 third-stage larvae was administered 24 hours prior to necropsy.

The ventral and dorsal surfaces of the stomach were reddened and a discrete area (5-6 mm dia.) of dark brown hemorrhage was present on the ventral surface of the pyloric region. Presses of the mesentery and pancreas

revealed approximately 28 capsules. Most of these capsules appeared to contain a larva in various stages of decomposition. One capsule was successfully opened and a live larva (L3) was freed. Another larva (L3) was recovered from the saline in which the mesentery was rinsed, and 2 dead larvae were recovered from digests of this tissue.

Six capsules were located on pressing the liver. Three were successfully opened: two live and one dead larvae (L3) were recovered. No other larvae were recovered on pressing any other tissue.

67 DPI

Guinea Pig: A-3 (F)
Clinical Signs: none
Necropsy:

Several petechial hemorrhages were observed on the surface of fascia over the longissimus dorsi muscles, especially near the vertebral column. Careful examination of fascia and muscles did not reveal any larvae. Small (1-2 mm dia.) hemorrhages were present on both triceps muscles, but no larvae were found.

A white lesion, 3 mm in diameter by 7 mm deep, was present on the diaphragmatic surface of the left medial lobe of the liver. Four shallow (1 mm deep) white lesions, surrounded by reddened tissue, were also present on the left lateral lobe of the liver. Pressing this area revealed one capsule that contained dark granular material with no distinct shape. Three more capsules were located by pressing the mesentery, but their contents were also indistinct.

70 DPI

Guinea Pig: A-4 (M)
Clinical Signs: none
Necropsy:

No gross lesions were apparent. Four capsules were found on pressing the liver and eight more on pressing the mesentery. All contained dark granular material; the contents of five had larval shapes, but attempts to recover larvae failed as they were degenerate.

84 DPI

Guinea Pig: A-5 (F)
Clinical Signs: none
Necropsy:

An area of diffuse hemorrhage was present on the left dorsal lobe of the liver, approximately 1 mm deep. One superficial white area was present on the diaphragmatic surface of the left ventral lobe. Neither of these areas nor any other part of the liver was found to contain larvae. Ten capsules were found on pressing the mesentery. The granular contents of most were indistinct, but a few appeared larva-like in shape; attempts to open capsules and release larvae were not successful as the contents were degenerate.

86 DPI

Guinea Pig: A-6 (M); CONTROL
Clinical Signs: none
Necropsy:

No lesions were found upon examination of any tissues.

87 DPI

Guinea Pig: A-7 (F)
Clinical Signs: none
Necropsy:

Distinct petechial hemorrhages were present on the right adrenal gland. The portion of the pancreas nearest the pyloric region of the stomach was hemorrhagic. Pressing and digesting of tissues produced no larvae.

90 DPI

Guinea Pig: A-8 (M); CONTROL
Clinical Signs: none
Necropsy:

No lesions or abnormalities were found on examining this animal.

Necropsy of Guinea Pigs Infected with E. cervi

31 DPI

Guinea Pig: C-1 (F)
Clinical signs: none
Necropsy:

Hemorrhaging (4-5 mm dia.) was apparent in the stomach wall. Several capsule-like structures associated with the mesentery appeared to contain dark granular material that resembled degenerating larvae. A few small white areas (<2 mm dia.) were found on the liver. No larvae were found in presses of any tissues.

A pale green area (2-3 mm dia.) in the intercostal muscles, at the ventral or distal end of the 12th rib, appeared to be an aggregation of pigmented cells. Examination of this area, and of petechial hemorrhages on the right longissimus dorsi muscle beneath the lumbar-dorsal fascia, revealed no larvae,

and no lesions were found in any other tissue.

42 DPI

Guinea Pig: C-2 (M)
Clinical Signs: none
Necropsy:

Large superficial white lesions covered most of the diaphragmatic surfaces of the left lateral and medial lobes of the liver, as well as much of the visceral surface of these and the caudate lobe. The left kidney was white in colour over much of its surface directly facing the stomach, and the greater curvature of the stomach was reddened. Diffuse hemorrhage was evident in the pancreatic tissue and small capsules containing indistinguishable granular material were observed. No larvae were recovered on pressing any tissue.

70 DPI

Guinea Pig: C-3 (F)
Clinical Signs: none
Necropsy:

Two capsules were located on pressing the mesentery; the contents were larva-like in shape, but were degenerate. Superficial lines (<0.5 mm wide), pale pink in color, were present on the liver but larvae were not found on pressing the tissue. No lesions or larvae were found on examining any other tissue.

74 DPI

Guinea Pig: C-4 (M)
Clinical Signs: none
Necropsy:

The surfaces of the mesentery and pancreas most closely associated with the stomach were hemorrhagic. The ventral surface of the pyloric region of the stomach was reddened as well. Two white lesions (5 mm dia.) were found on the diaphragmatic surface of the medial lobe of the liver. On pressing the liver, two larva-like structures were found, but these were degenerate and impossible to identify. All other presses and digests of tissues were negative.

No larvae were found associated with small hemorrhages (2-3 mm dia.) present on the internal abdominal wall (internal oblique and transverse muscles) and the right longissimus dorsi muscle.

88 DPI

Guinea Pig: C-5 (M)
Clinical Signs: none
Necropsy:

A superficial white lesion (2 cm by 1 cm) was present on the diaphragmatic surface of the medial lobe of the liver; presses of liver tissue were negative. A single capsule-like structure was found on pressing the mesentery; the contents of this structure were granular and larva-like in shape, but could not be released from the capsule or positively identified.

91 DPI

Guinea Pig: C-6 (F); CONTROL
Clinical Signs: none
Necropsy:

No lesions were found upon examination of this animal.

93 DPI

Guinea Pig: C-7 (F)
Clinical Signs: none
Necropsy:

This animal was initially infected 93 days prior to necropsy; it was given 83 more third-stage larvae 24 hours prior to necropsy.

Two small hemorrhagic areas were present on the cardiac portion of the stomach, near the lesser curvature and in direct contact with the liver. No larvae were found associated with these areas on pressing, but three grey areas (3-4 mm dia.) were located. The mesentery was hemorrhagic; capsules were found within this tissue that contained indistinct shapes of granular material. Some capsules contained recognizable larvae that appeared degenerate. A superficial white area on the diaphragmatic surface of the medial lobe of the liver was pressed and four capsules were discovered (contents indistinct). A single larva (L3) was found on pressing the remainder of the liver.

95 DPI

Guinea Pig: C-8 (M); CONTROL
Clinical signs: none
Necropsy:

No lesions were found upon examination of this animal.

Changes in Body Weights of Infected Guinea Pigs

Five guinea pigs infected with *P. tenuis* lost weight prior to being necropsied (Fig. 2). These animals all showed typical signs of neurologic disease and began to lose weight following the onset of signs (Table 1). Another guinea pig (T-15, male(M)) also showed clinical signs but continued to

Fig. 2.   Changes in body weights of guinea pigs infected with *Parelaphostrongylus tenuis*, and treated with saline or 0.4 mg dexamethasone. Onset of clinical signs varied among animals ($\bigtriangledown$). T - 18 and T - 16 were the uninfected control animals.

Saline Treated

Dexamethasone Treated
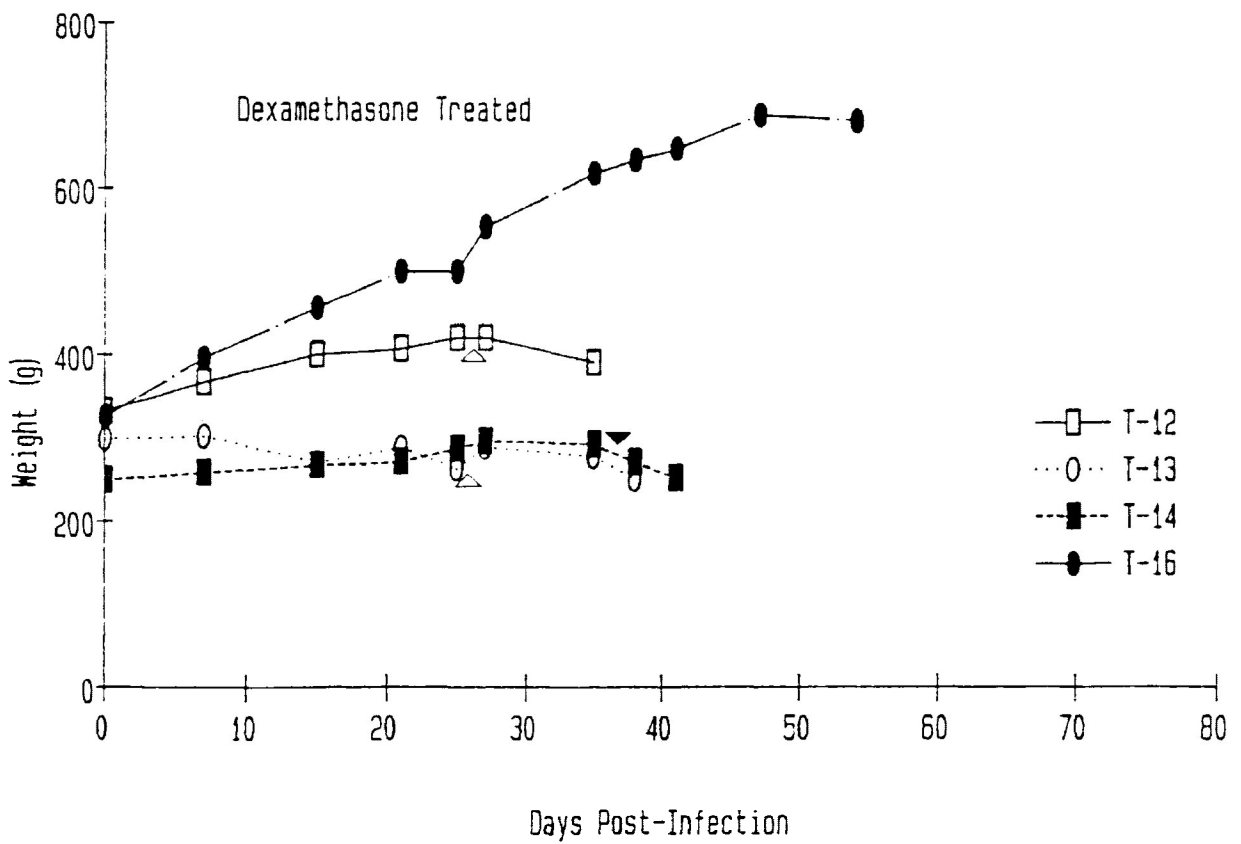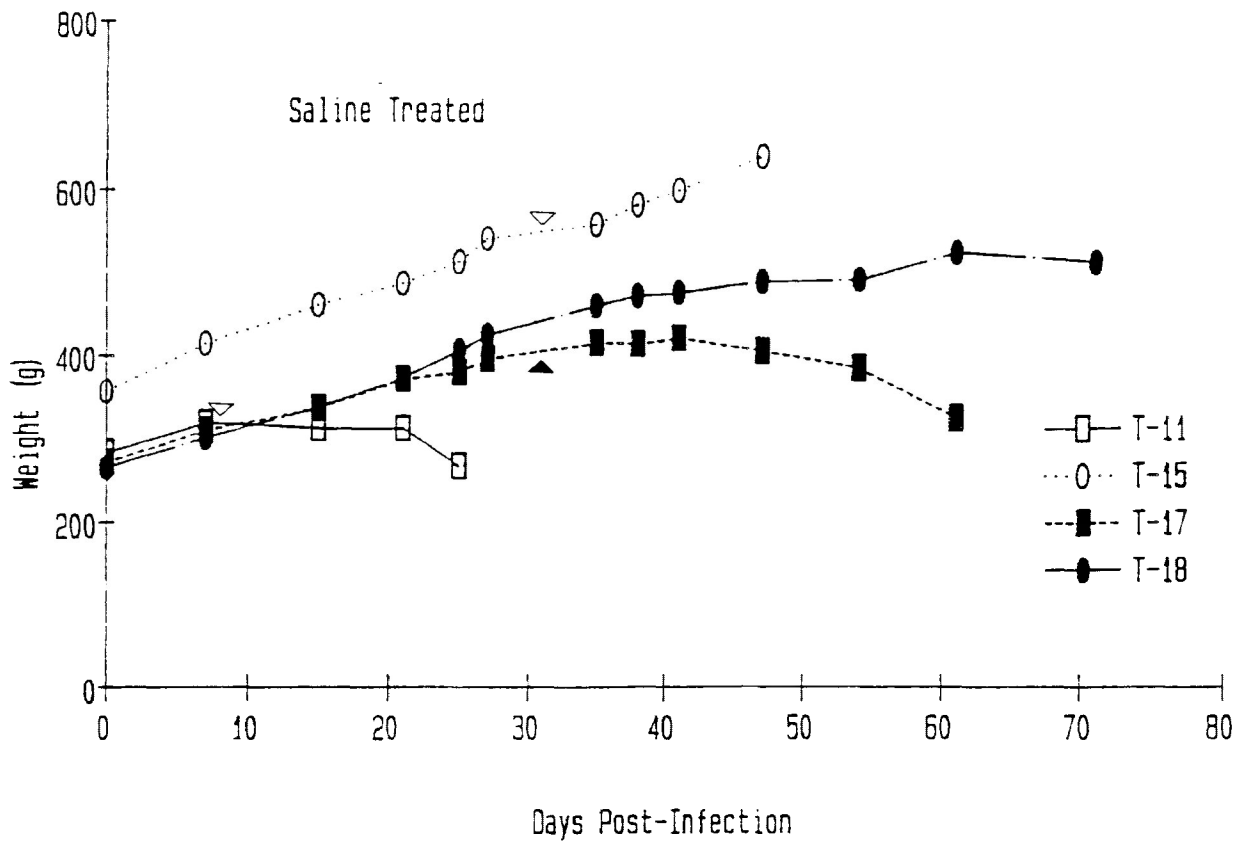
gain weight until it was necropsied. The two control animals (T-18, female (F), and T-16, M) and all infected guinea pigs with no disease signs, showed a steady increase in weight. Weights of only the control guinea pigs and animals with neurologic disease are shown in Fig. 2. Males usually gained more weight than females, whether infected or not, and whether given dexamethasone or saline.

Guinea pigs infected with *P. andersoni* gained weight steadily throughout this study (Fig. 3). The two control animals (A-6, M; A-8, M) gained more weight than any of the infected animals. Both groups of infected animals consisted of two females and one male; infected males were slightly heavier than infected females, but both sexes gained weight at approximately the same rate in each treatment group.

Guinea pigs infected with *E. cervi* gained weight steadily during this study (Fig. 4). Infected males gained more weight than infected females, whether they had been treated with dexamethasone or saline. The male control (C-8) also gained more weight than the female control (C-6).

Development of P. tenuis in Guinea Pigs

The mean dimensions of developing *P. tenuis* larvae recovered from guinea pigs are given in Table 5. Some were damaged and could not be measured (as noted previously), but the total number recovered is recorded in the table. Larvae were identified as third-, fourth-, or fifth-stage larvae based on length, the structure of the buccal capsule, and tail morphology. Migrating third-stage larvae were recovered from the viscera up to 27 DPI (Fig. 5). They could not be distinguished from infective larvae initially given to guinea pigs (0 DPI), except that they were inactive in pepsin (mean length =

Fig. 3. Changes in body weights of guinea pigs infected with *Parelaphostrongylus andersoni*, and treated with 0.2 mg dexamethasone or 0.4 mg dexamethasone. A -6 and A - 8 were the uninfected control animals.

Dexamethasone Treated (0.2 mg)

Weight (g)

Days Post-Infection

A-2
A-4
A-5
A-6

Dexamethasone Treated (0.4 mg)

Weight (g)

Days Post-Infection

A-1
A-3
A-7
A-8
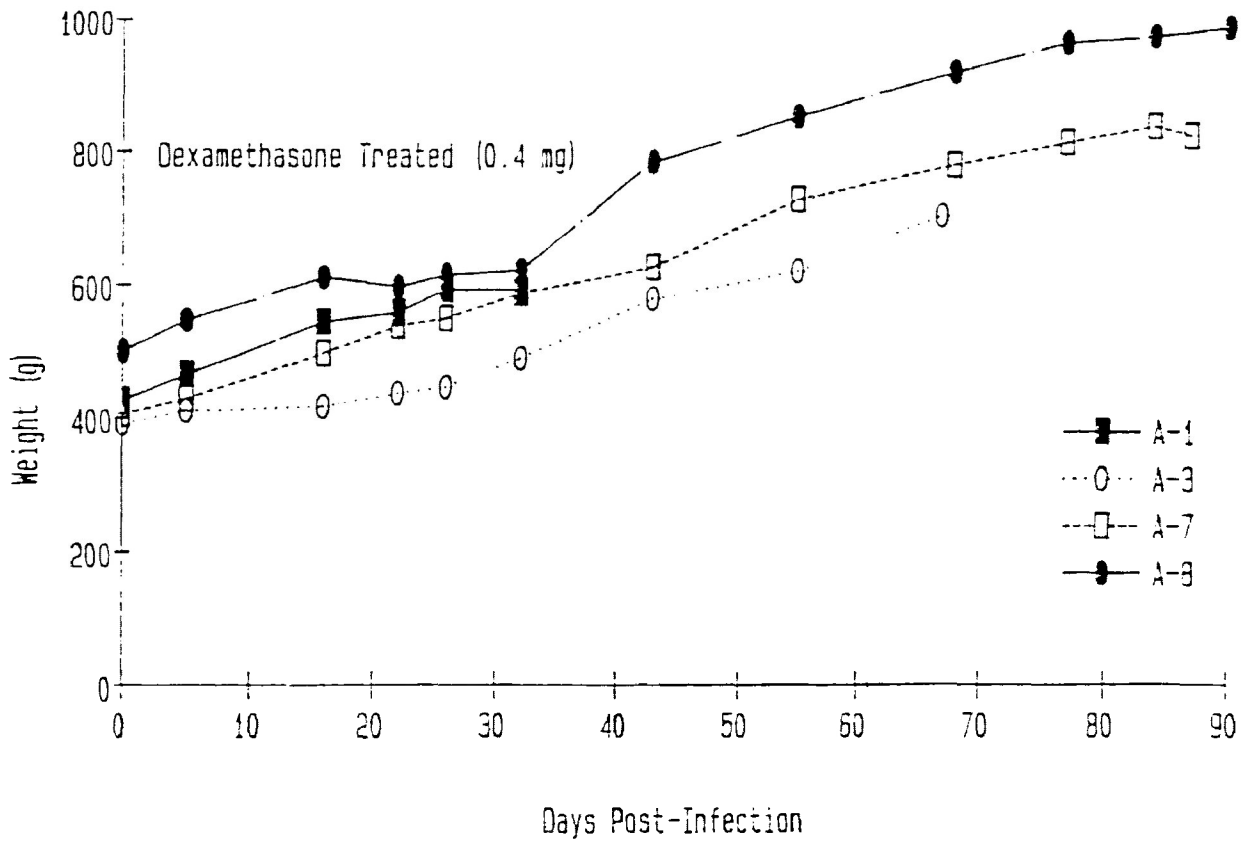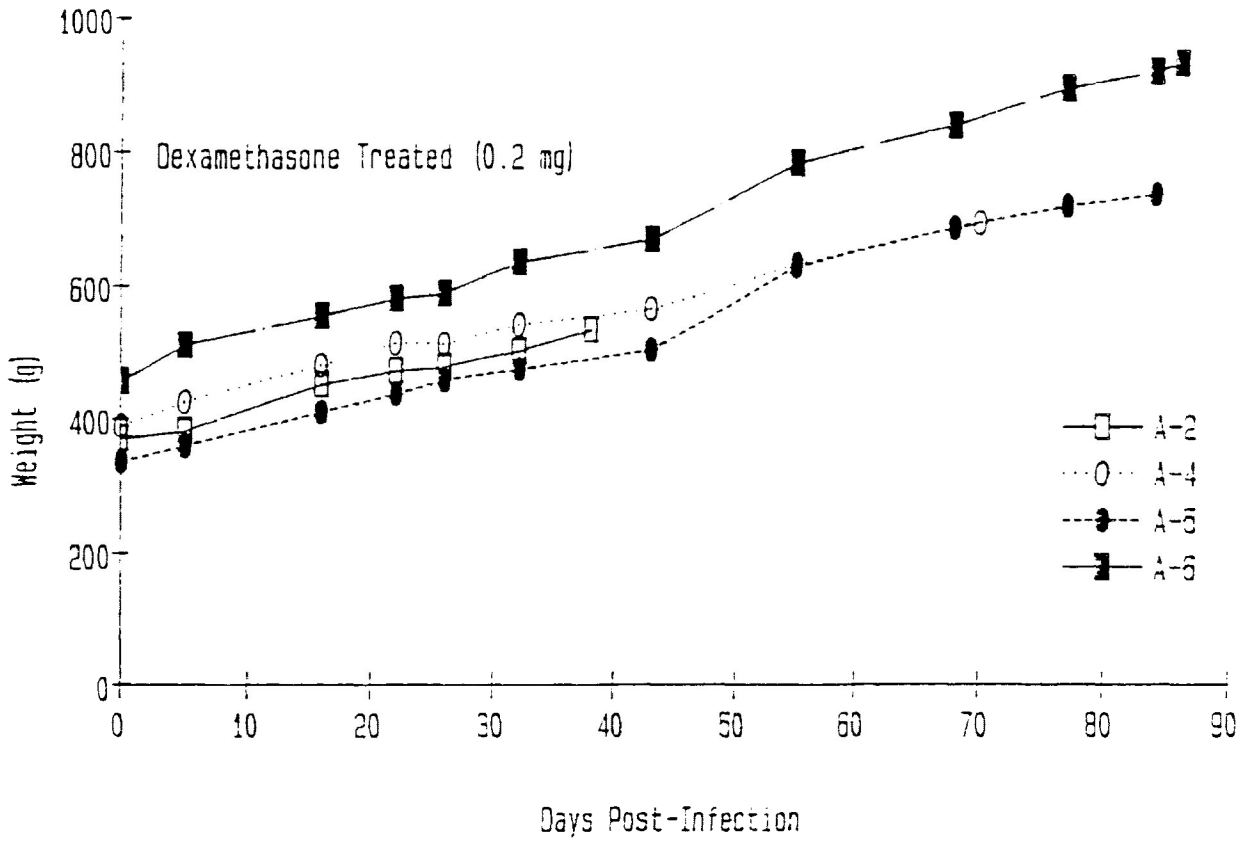
Fig. 4.   Changes in body weights of guinea pigs infected with
Elaphostrongylus cervi, and treated with saline or 0.4 mg
dexamethasone. C -6 and C- 8 were the uninfected control animals.

Saline Treated

Weight (g)

Days Post-Infection

C-2
C-3
C-5
C-6

Dexamethasone Treated

Weight (g)

Days Post-Infection

C-1
C-4
C-7
C-8
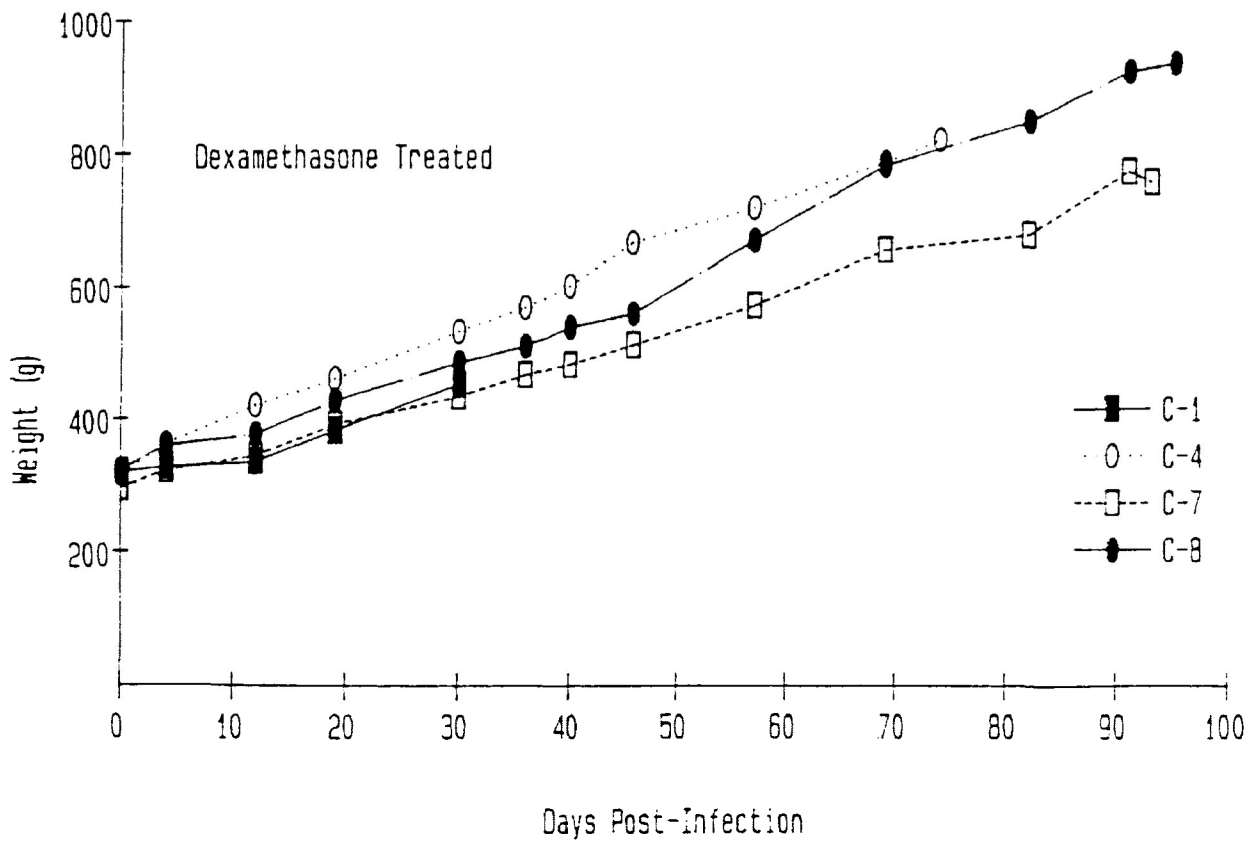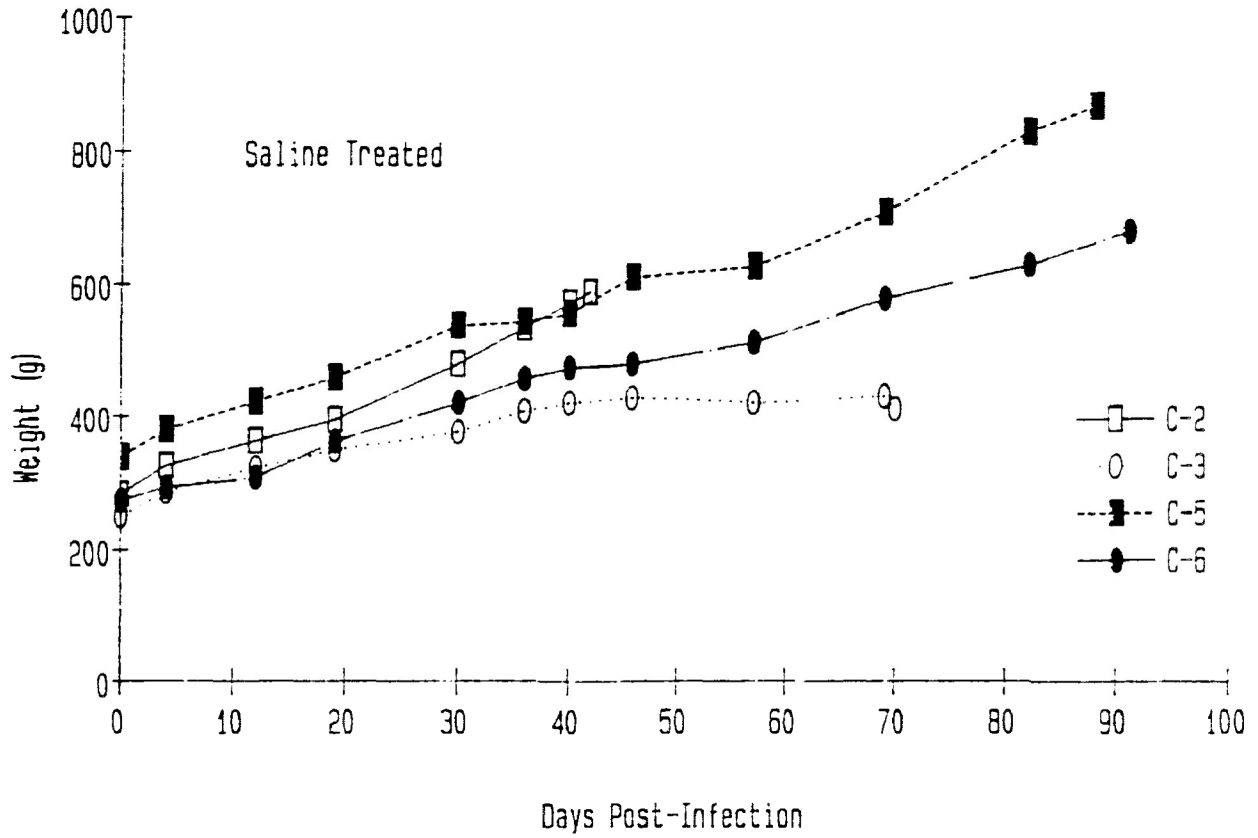
TABLE 5. Mean dimensions of third- (L3), fourth- (L4), and fifth- (L5) stage Parelaphostrongylus tenuis larvae recovered from the central nervous system (CNS), or outside the CNS (MIG), of guinea pigs between 1 and 61 days post-infection (DPI).

	0 DPI[a]	1 DPI	3 DPI	9 DPI	
		MIG	MIG	MIG	CNS
	L3	L3	L3	L3	L3
N (n)[b]	25	7 (12)	12 (18)	16 (22)	3
Length[c]	1011 ± 34 (943 - 1118)	1006 ± 39 (940 - 1047)	970 ± 37 (912 - 1038)	938 ± 45 (848 - 1000)	970 ± 65 (894 - 1008)
Width	46 ± 2 (42 - 49)	44 ± 3 (42 - 48)	44 ± 2 (41 - 49)	48 ± 5 (42 - 56)	44 ± 0
Nerve Ring[+]	123 ± 6 (112 - 136)	98 ± 16 (76 - 123)	112 ± 13 (92 - 128)	109 ± 13 (82 - 128)	81 ± 7 (77 - 90)
Excretory[+] Pore	144 ± 5 (136 - 155)	142 ± 7 (132 - 151)	138 ± 6 (127 - 147)	133 ± 9 (105 - 150)	127 ± 11 (117 - 139)
Esophagus[+]	383 ± 11 (360 - 401)	364 ± 26 (321 - 390)	352 ± 25 (294 - 390)	358 ± 41 (284 - 479)	401 ± 9 (390 - 408)
Genital Primordium[+]	624 ± 25 (582 - 682)	641 ± 66 (594 - 687)	611 ± 33 (552 - 657)	587 ± 36 (541 - 670)	608 ± 42 (578 - 638)
Anus[++]	44 ± 3 (38 - 50)	41 ± 4 (36 - 45)	40 ± 5 (33 - 50)	39 ± 4 (31 - 46)	33 ± 5 (28 - 37)

41

TABLE 5. Continued

	12 DPI		15 DPI		18 DPI[d]
	MIG	CNS	MIG	CNS	MIG
	L3	L3	L3	L3	L3
N (n)[b]	18 (20)	4	3	2	2
Length[c]	896 ± 43 (837 - 976)	1161 ± 68 (1066 - 1222)	895 ± 33 (858 - 921)	1378 ± 10 (1371 - 1385)	917 ± 78 (862 - 973)
Width	43 ± 3 (39 - 53)	48 ± 2 (46 - 50)	45 ± 2 (43 - 47)	53 ± 0	51 ± 3 (49 - 53)
Nerve Ring[+]	110 ± 6 (99 - 119)	97 ± 23 (72 - 117)	109 ± 3 (106 - 111)	128 ± 4 (125 - 131)	111 ± 6 (106 - 115)
Excretory[+] Pore	127 ± 6 (120 - 137)	113 ± 32 (78 - 140)	127 ± 9 (119 - 136)	139 ± 6 (134 - 143)	129 ± 1 (128 - 129)
Esophagus[+]	343 ± 23 (300 - 390)	417 ± 45 (381 - 467)	353 ± 6 (346 - 357)	410 ± 34 (386 - 434)	367 ± 28 (347 - 386)
Genital Primordium[+]	572 ± 44 (541 - 674)	752 ± 0	571 ± 15 (558 - 587)	-*	-
Anus[++]	38 ± 4 (30 - 47)	39 ± 4 (35 - 44)	35 ± 3 (33 - 38)	41 ± 2 (40 - 43)	37 ± 3 (35 - 39)

TABLE 5. Continued.

	18 DPI[d]		20 DPI		
	CNS	CNS	CNS	CNS	CNS
	L3	L4[e]	L3	L4	L5
		F		M	F
N (n)[b]	2	2	1 (2)	1 (2)	2
Length[c]	1113 ± 81 (1055 - 1170)	3937 ± 661 (3470 - 4405)	1339	3683	5171 ± 18 (5158 - 5184)
Width	57 ± 2 (55 - 58)	73 ± 4 (70 - 75)	69	75	108 ± 4 (105 - 111)
Nerve Ring[+]	111 ± 15 (101 - 155)	127 ± 43 (97 - 158)	119	-*	149 ± 9 (142 - 155)
Excretory[+] Pore	128 ± 38 (101 - 105)	157 ± 37 (131 - 183)	148		-
Esophagus[+]	438 ± 42 (408 - 468)	505 ± 26 (486 - 523)	368	489	587 ± 34 (563 - 611)
Genital Primordium[+]	724 ± 0 (541 - 674)	NA[f]	960	NA	NA
Anus[++]	41 ± 4 (38 - 44)	46 ± 2 (43 - 48)	35	22	69 ± 28 (49 - 89
Vulva[++]	NA	NA	NA	NA	156 ± 62 (112 - 200)
Gubernaculum	NA	NA	NA	NA	NA
Spicules	NA	NA	NA	NA	NA

TABLE 5. Continued.

	21 DPI		22 DPI		27 DPI
	CNS	CNS	CHS	CNS	MIG
	L4[e,g]	L5	L4	L5	L3
		M	F	M	
N (n)[b]	1	2	3	2	9 (14)
Length[c]	2069	4007 ± 665 (3537 - 4477)	2186 ± 839 (1305 - 2976)	5741 ± 336 (5503 - 5978)	934 ± 103 (839 - 1175)
Width	62	96 ± 6 (92 - 100)	68 ± 7 (62 - 76)	89 ± 6 (85 - 94)	47 ± 6 (36 - 56)
Nerve Ring[+]	73	120 ± 28 (100 - 140)	111 ± 3 (108 - 113)	120 ± 0	111 ± 20 (90 - 147)
Excretory[+] Pore	137	-*	127 ± 3 (125 - 131)	140 ± 7 (135 - 145)	134 ± 12 (125 - 152)
Esophagus[+]	495	547 ± 45 (515 - 578)	465 ± 55 (411 - 520)	527 ± 90 (521 - 534)	360 ± 46 (297 - 427)
Genital Primordium[+]	NA[f]	NA	NA	NA	563 ± 83 (428 - 684)
Anus[++]	32	NA	42 ± 6 (35 - 47)	NA	39 ± 5 (33 - 50)
Vulva[++]	NA	NA	NA	NA	NA
Gubernaculum	NA	58 ± 0	NA	77 ± 3 (75 - 80)	NA
Spicules	NA	137	NA	158 ± 13 (149 - 167)	NA

44

TABLE 5. Continued.

	27 DPI			35 DPI	
	CNS	CNS	CNS	CNS	MIG
	L4[e]	L5	L5	L5	L5
	F	F	M	F	M
N (n)[b]	1	1	2	1	5
Length[c]	3705	3580	6.94 $\pm$ 2.20** (5.38 - 8.49)	16.80**	17.31 $\pm$ 2.19** (13.64 - 19.03)
Width	-*	94	91 $\pm$ 2 (89 - 92)	85	93 $\pm$ 7 (84 - 102)
Nerve Ring[+]	84	103	122 $\pm$ 4 (119 - 125)	100	121 $\pm$ 6 (111 - 126)
Excretory[+] Pore		155	-		137 $\pm$ 8 (130 - 150)
Esophagus[+]	418	467	558 $\pm$ 8 (500 -561)	537	590 $\pm$ 12 (572 - 600)
Anus[++]	32	33	NA[f]	34	NA
Vulva[++]	NA	89	NA	100	NA
Gubernaculum	NA	NA	90 $\pm$ 0	NA	90 $\pm$ 11 (80 - 107)
Spicules	NA	NA	183 $\pm$ 3 (180 - 183)	NA	194 $\pm$ 12 (180 - 210)

TABLE 5. Continued.

	38 DPI		41 DP1		47 DPI
	CNS	CNS	CNS	CNS	CNS
	L4[e]	L5	L5	L5	L4
	F	M	F	M	M
N (n)[b]	1	2	1	2	1
Length[c]	3650	17.9 ± 1.33** (16.96 - 18.84)	25.91**	15.67 ± 1.87** (14.34 - 16.99)	3520
Width	64	97 ± 0	112	93 ± 17 (81 - 105)	64
Nerve Ring[+]	109	105 ± 8 (99 - 111)	115	115 ± 15 (105 - 126)	115
Excretory[+] Pore	-*	117 ± 8 (111 - 122)	130	135	142
Esophagus[+]	455	609 ± 18 (597 - 622)	568	583 ± 47 (549 - 616)	475
Anus[++]	38	NA[f]	39	NA	29
Vulva[++]	NA	NA	118	NA	NA
Gubernaculum	NA	89 ± 8 (82 - 96)	NA	77 ± 7 (72 - 82)	NA
Spicules	NA	199 ± 7 (192 - 205)	NA	191 ± 5 (188 - 195)	NA

TABLE 5. Continued.

	47 DPI		61 DPI
	CNS	CNS	CNS
	L5[e]	L5	L5
	F	M	M
N (n)[b]	3	1	1
Length[c]	13.88 ± 7.80** (4.9 - 19.27)	17.61**	39.90**
Width	110	135	130
Nerve Ring[+]	123	100	135
Excretory[+] Pore	123	100	167
Esophagus[+]	471	609	860
Anus[++]	40 ± 5 (35 - 45)	NA[f]	NA
Vulva[++]	109 ± 4 (105 - 112)	NA	NA
Gubernaculum	NA	75	112
Spicules	NA	178	190

[a] Dimensions of larvae recovered from snails, prior to infecting guinea pigs
[b] Number of larvae measured (number recovered, if different from N)
[c] All larvae were heat-relaxed, stored in glycerin-alcohol and cleared to glycerin before
       measurements were taken; all dimensions in microns (μm) unless stated otherwise

Fig. 5. Time intervals in which third-, fourth-, and fifth- stage *Parelaphostrongylus tenuis* larvae were recovered from the central nervous system (CNS), or outside the CNS (MIG), of guinea pigs necropsied 1 to 61 days post-infection (DPI).

Larval Stages

MIG L3

CNS L3

CNS L4

CNS L5

0   10   20   30   40   50   60   70

Days Post-infection
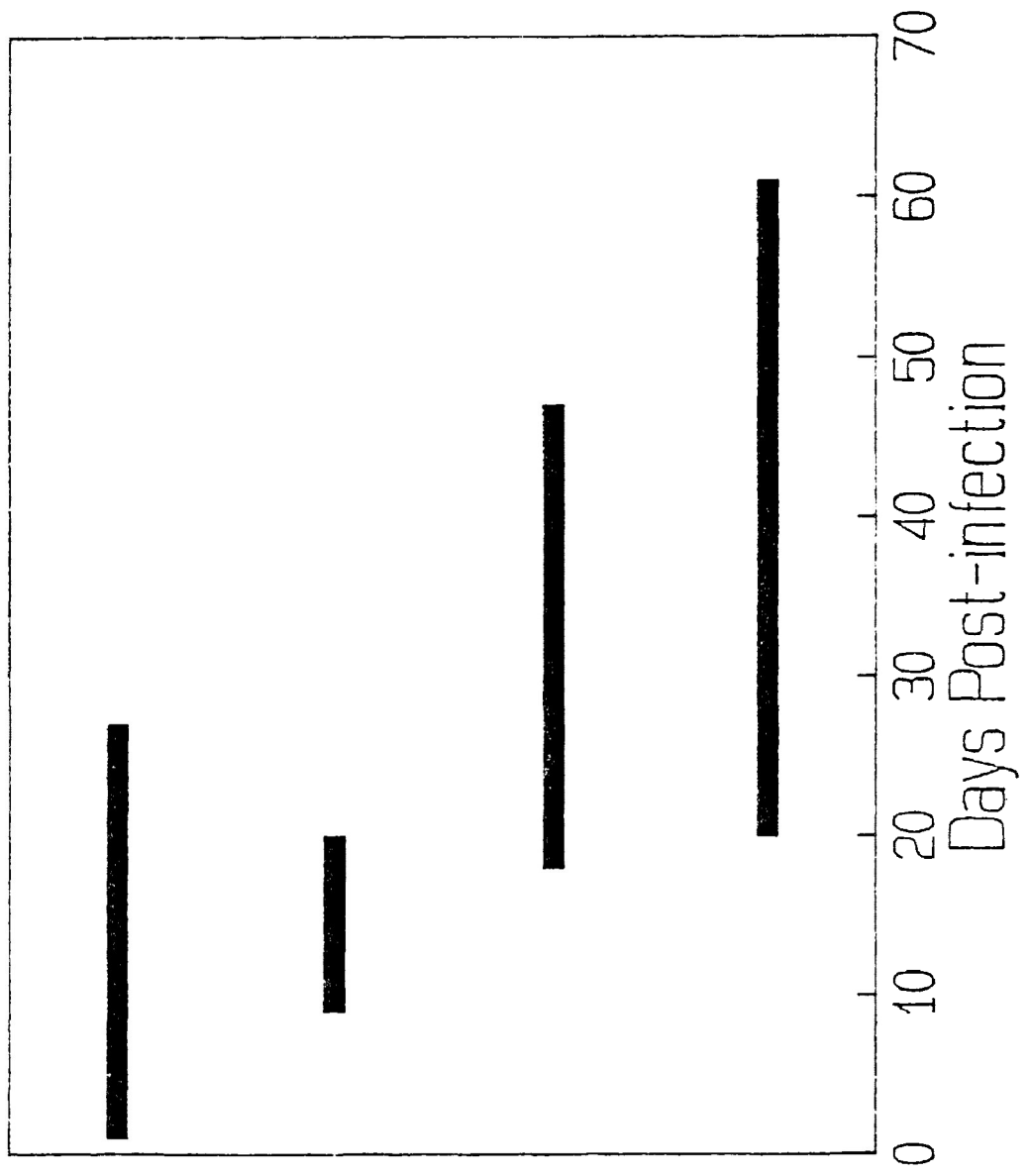
936 μm; range = 837 - 1175 μm). Third-stage larvae recovered from the CNS between 9 and 20 DPI were somewhat longer (mean length = 1156 μm; range = 894 - 1385 μm), but otherwise were similar morphologically to migrating third-stage larvae. Fourth-stage larvae were found in the CNS between 18 and 47 DPI, and fifth-stage larvae between 20 and 61 DPI.

The greatest number of larvae recovered from the CNS were in the thoracic region of the spinal cord (Table 6). In total, 28 larvae were recovered from the spinal cord and 25 from the brain and brain stem. More than half of the total number of larvae recovered from the CNS were fifth-stage larvae. On re-organizing the data in Figure 5 and Table 6, it is apparent that early in the infection (9 - 18 DPI) the majority of larvae were at the third-stage (Table 7). During the second time interval (20 - 35 DPI), numbers of third-stagelarvae had decreased markedly and fourth-stage larvae were more numerous, but fifth-stage larvae were the most prevalent. Late in the infection (36 - 61 DPI), no third-stage larvae were recovered, and the majority of larvae found were fifth-stage. There was no change in the location of larvae recovered with increased time after infection; some larvae were found in almost every region of the CNS during each time interval (Table 7). The thoracic region of the cord and the brain stem yielded the most developing larvae.

Third-stage larvae, recovered from snails or from guinea pigs, had a characteristic buccal capsule. In lateral view, the heavily cuticularized region of the buccal capsule appeared as a thick rod turned back upon itself at the anterior end (Fig. 6a). This part of the buccal capsule was 15 μm long and 4 μm wide, and the posterior region of the buccal capsule extended into the esophagus. The tail morphology of third-stage larvae also differentiated this

Table 6. Total numbers of Parelaphostrongylus tenuis third-
(L3), fourth- (L4) and fifth- (L5) stage larvae recovered
from each region of the central nervous system of infected
guinea pigs.

Location	L3	L4	L5	Total
Spinal Cord				
Lumbar-sacral	1	1	2	4
Thoracic	7	3	8	18
Cervical	2	1	3	6
Brain				
Brain Stem	1	3	7	11
Cerebellum	1	0	5	6
Cerebrum	1	4	3	8
Total	13	12	28	53

Table 7. Total numbers of <u>Parelaphostrongylus tenuis</u> larvae recovered from the central nervous system of infected guinea pigs during three time intervals.

| Location | DPI[a] | | | |
	9-18	20-35	38-61	Total
<u>Spinal cord</u>				
Lumbar-sacral	1,0,0[b]	0,1,2	0,0,0	1,1,2
Thoracic	6,0,0	1,2,3	0,1,5	7,3,8
Cervical	1,0,0	1,0,1	0,1,2	2,1,3
<u>Brain</u>				
Brain stem	1,1,0	0,2,4	0,0,3	1,3,7
Cerebellum	1,0,0	0,0,3	0,0,2	1,0,5
Cerebrum	1,2,0	0,2,2	0,0,1	1,4,3
<u>Total</u>	11,3,0	2,7,15	0,2,13	13,12,28

[a] The first two time intervals include necropsies of five guinea pigs; the last interval includes only four animals.
[b] Each series represents the number of each larval stage recovered (third, fourth, fifth).

Fig. 6. Anterior regions of *Parelaphostrongylus tenuis* larvae recovered from the central nervous system of infected guinea pigs: (A) third-stage larvae; (B) fourth-stage larvae; (C) fifth-stage larvae.

C

B

A
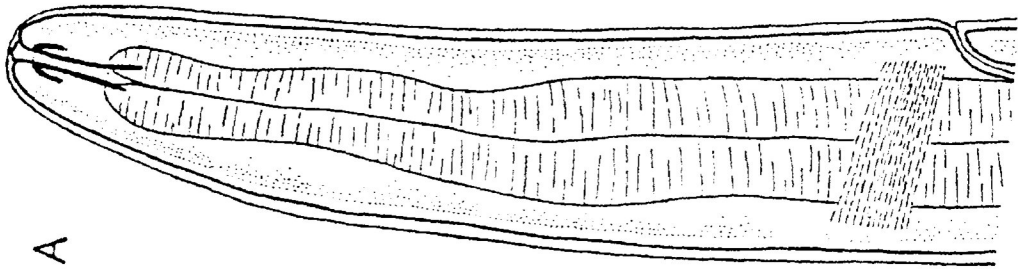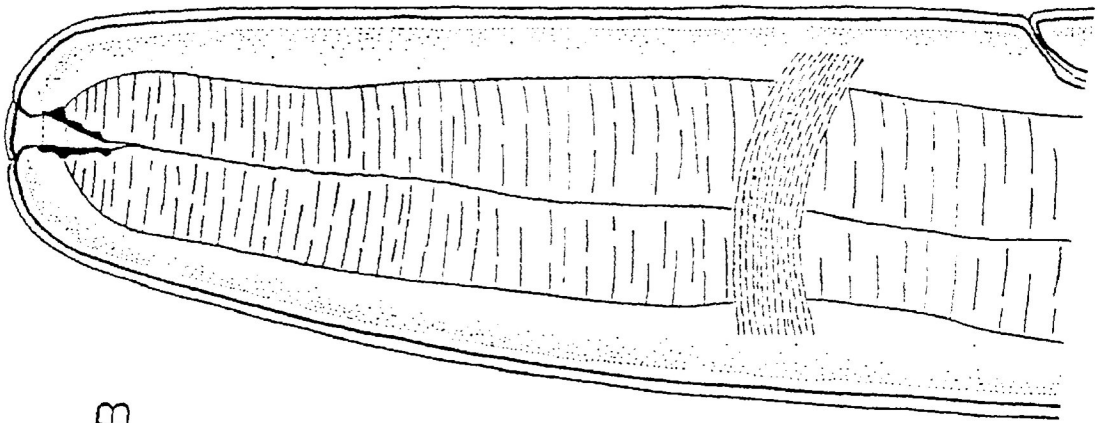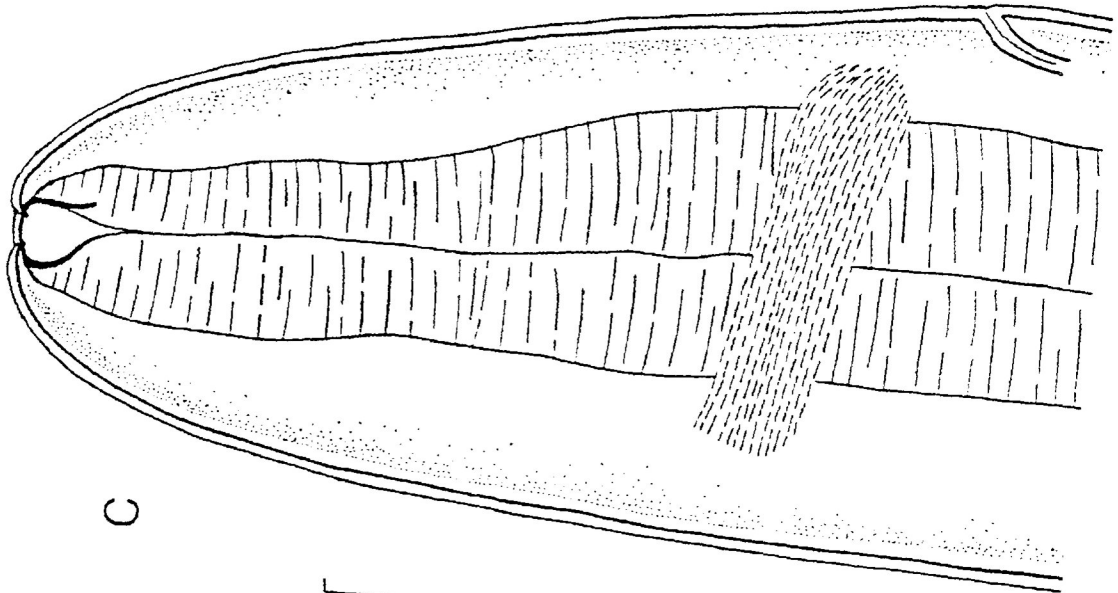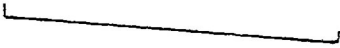
50 μm

stage from the others, and was described previously (Fig. 1).

In fourth-stage larvae, the walls of the distinctive buccal capsule were heavily cuticularized (Figs. 6b & 7), but lacked the hooked anterior region of the third-stage larvae. One or two lateral projections appeared to be present on the internal walls of the capsule; the outer walls, lining the cavity, were smooth. The length of the thickened portion of the capsule was approximately 9 μm, and the width at the level of the esophagus was approximately 4 μm.

Fourth-stage larvae were usually distinguishable as males or females by the shape of the tail (Figs. 8 & 9). The tail of female larvae was more pointed, and a vagina was usually evident under the cuticle; paired uteri were present, but a vulva had not yet formed (Fig. 8). The tail of male larvae was blunt and areas of organizing tissue that would give rise to the bursal rays were evident, but spicules were not (Fig. 9).

One fourth-stage larva, still within its loosened third-stage cuticle, was recovered 22 DPI (Figs. 7 & 8); it was recognizable as a female larva, and was within the range in length of third-stage larvae. Another larva, that was possibly a developing male, was recovered 21 DPI, but could not be identified reliably. It had a buccal capsule similar to that in other fourth-stage larvae (Fig. 6b). The developing genital primordium was distinct; there was no evidence of a developing vagina as seen in female fourth-stage larvae, and the tail region was not as blunt as in males.

Fifth-stage larvae, or immature adults, were also distinguishable as males and females, and generally were greater in length than fourth-stage larvae. The buccal capsule (Figs. 6c & 10) was not heavily cuticularized, was V-shaped and extended very close to the oral opening. Female larvae had pointed tails with a prominent vulva located anterior to the anus (Fig. 12). Males had a

Figs. 7 - 9. Fourth-stage *Parelaphostrongylus tenuis* larvae recovered from the central nervous system of infected guinea pigs. 7. Anterior region of female, still within third-stage cuticle, recovered 22 DPI. 8. Posterior region of female, still within third-stage cuticle, recovered 22 DPI. 9. Posterior region of male, recovered 47 DPI.

7

8

9

50 μm
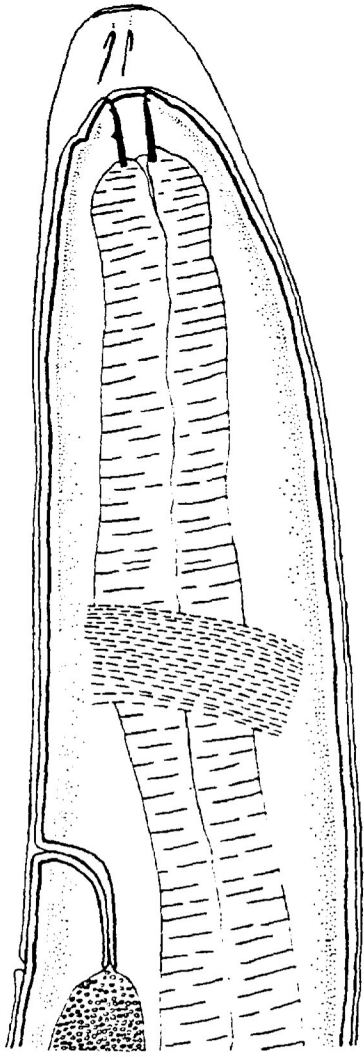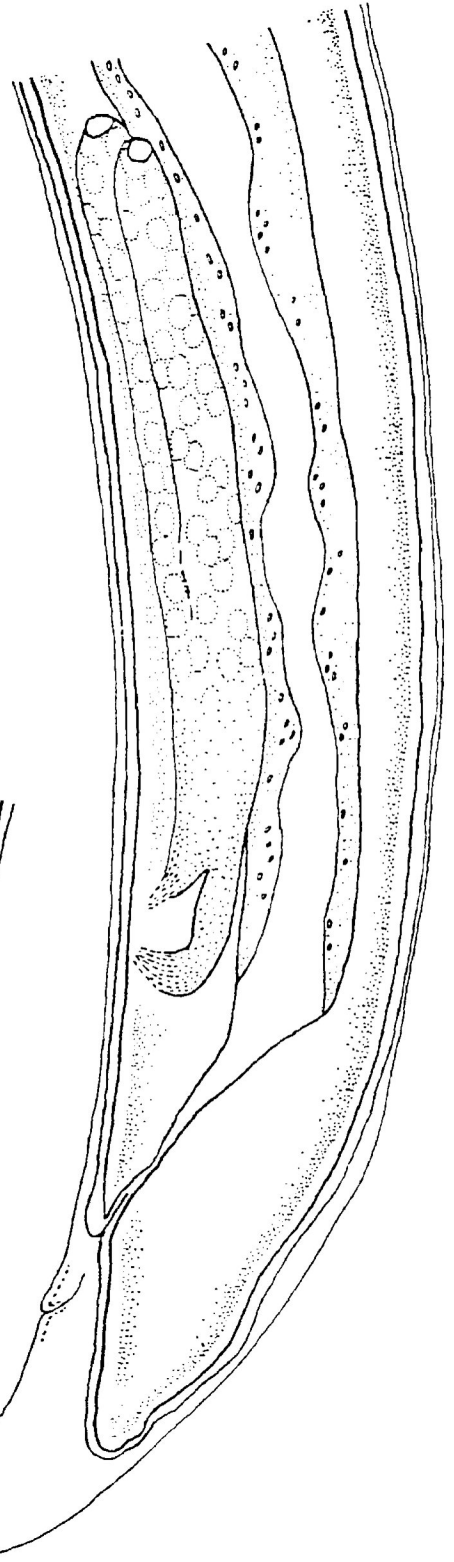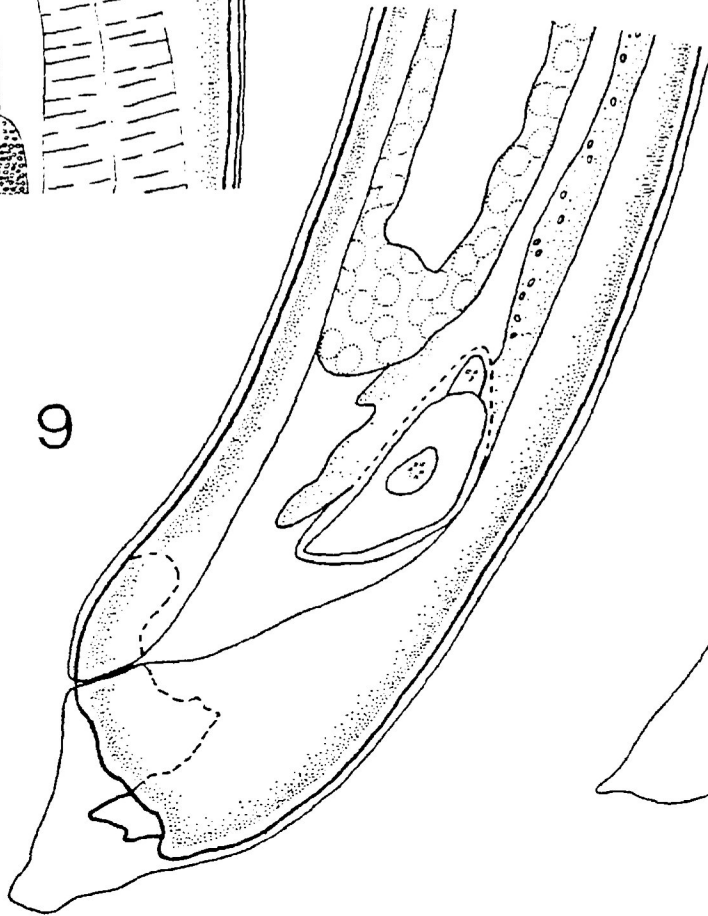
Figs. 10 - 13.   Fifth-stage *Parelaphostrongylus tenuis* larvae recovered from the central nervous system of infected guinea pigs.

10. Anterior region of male, still within fourth-stage cuticle, recovered 21 DPI.   11. Posterior region of male, still within fourth-stage cuticle, recovered 21 DPI (bursal rays omitted).   12. Posterior region of female, still within fourth-stage cuticle, recovered 47 DPI.

13. Gubernaculum, with crura, of male recovered 61 DPI.

10

11
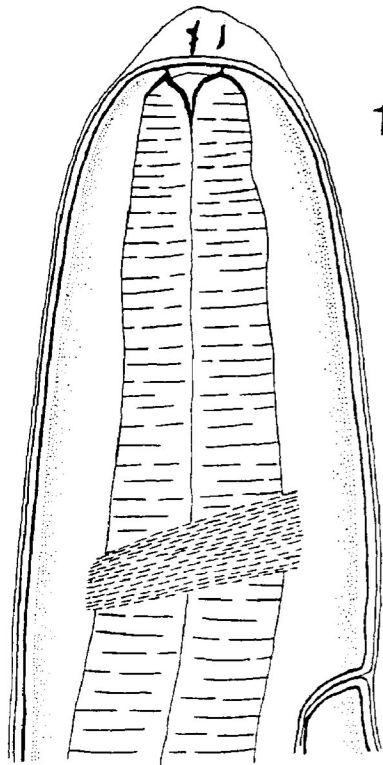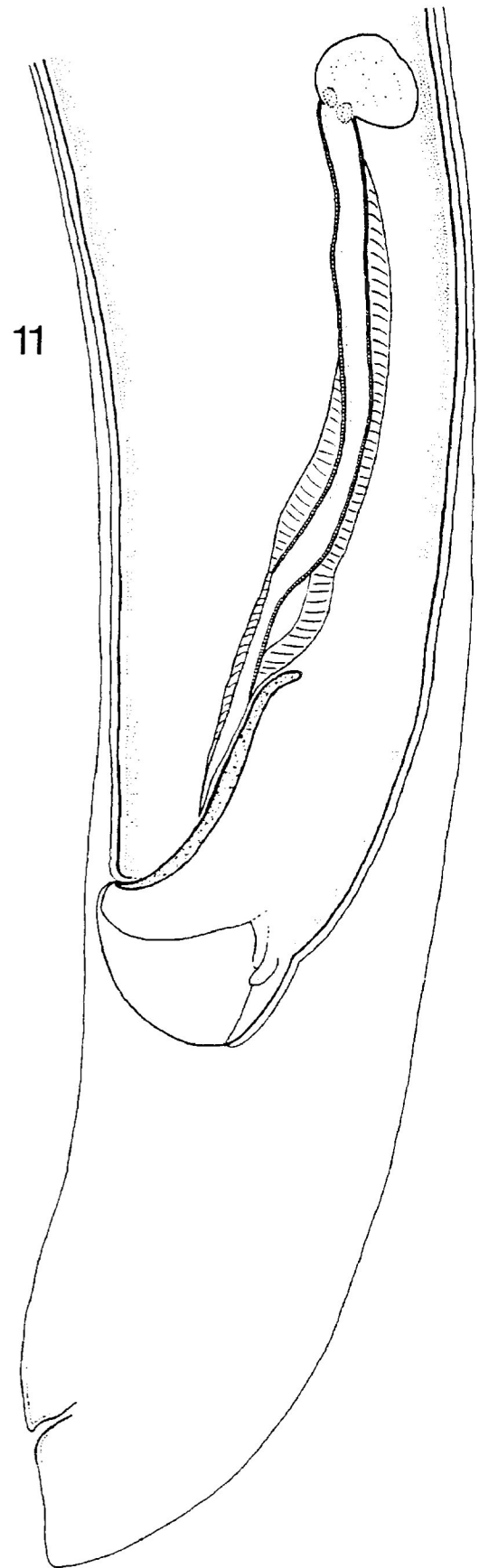
50 µm
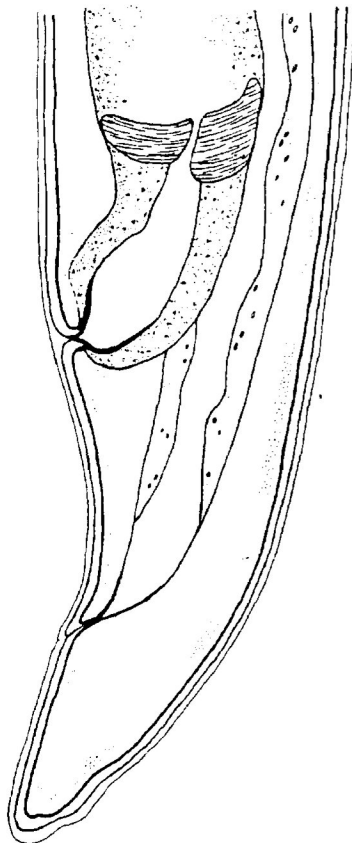
12
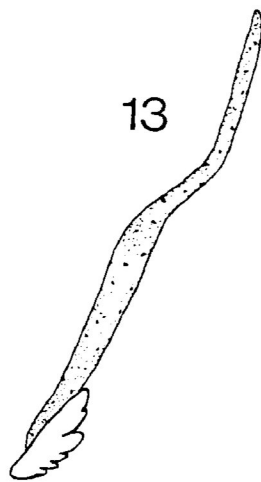
13

well-developed bursa, and the spicules and gubernaculum were evident (Fig. 11). The uteri of females and the testes of males were well defined at this stage.

Two female fifth-stage larvae, still within their fourth-stage cuticles, were recovered (Figs. 10 & 12); one was found 20 DPI and the other at 47 DPI. Both larvae possessed a distinct vulva and the fourth-stage cuticle over them was loosened and had no vulvar opening.

Two male fifth-stage larvae, still within the fourth-stage cuticle (Fig. 11), were recovered 21 DPI. The bursa of these larvae was well-developed. Spicules were evident, but were weakly sclerotized and difficult to accurately discern. The gubernaculum was also weakly sclerotized and distinct crura were not evident.

The sclerotized structures of male fifth-stage larvae were studied todetermine if the degree of sclerotization increased with time. At 27 DPI, the spicules of male fifth-stage larvae were more visible than in the larvae recovered before this. A crura was evident, although light in colour, in one of the two males recovered at 27 DPI, but was not apparent in any of the males recovered before 27 DPI. All males recovered after 27 DPI possessed distinct crura and were recognizable as *P. tenuis* (Fig. 13). Slight differences in the degree of sclerotization of male reproductive structures seemed evident between 20 and 27 DPI; an increase in sclerotization of the spicules and gubernaculum, based on the colour of these structures becoming darker with time, seemed apparent. However, it was difficult to discern appreciable differences in these structures after 27 DPI. Only the total length of larvae increased with time (Table 5).

DISCUSSION

First- and third- stage larvae of *P. tenuis*, *P. andersoni* and *E. cervi*, examined in this study, could not be distinguished morphologically. The mean length of first-stage *E. cervi* larvae was significantly greater than that of *P. tenuis* and *P. andersoni*, but the ranges in length for all three overlapped. The mean lengths of third-stage larvae of all three species also were significantly different, but again, the ranges overlapped and precluded the practical use of this characteristic in distinguishing these elaphostrongylines. All dimensions of first- and third- stage larvae studied here were similar to those published previously for *P. tenuis* (Anderson 1963; Ballantyne and Samuel 1984), *P. andersoni* (Prestwood 1972; Ballantyne and Samuel 1984), and *E. cervi* (Lankester and Northcott 1979).

The tail morphology of third-stage elaphostrongyline larvae was highly variable and also could not be used reliably to distinguish the three species studied. Although previous studies suggested that these larvae were distinguishable by tail shape (Prestwood 1972; Ballantyne and Samuel 1984), the present study gave incongruous results. Ballantyne and Samuel (1984) described *P. tenuis* as having a large, dorsal hump on its tail, while *P. andersoni* had a smaller and more anterior-dorsal hump. In the present study, specific tail shapes were not apparent for any species; similar variations in tail shapes were observed in all three species. Lankester and Hauta (in press) described third-stage *P. andersoni* larvae with variable tail-shapes including all three forms described by Ballantyne and Samuel (1984). Further study of the tail morphology of these larvae is recommended to determine the degree of variation and the practicality of this feature as a distinguishing characteristic.

This study confirms that guinea pigs can be used to rear identifiable adult *P. tenuis* from unidentified larvae collected off feces of wild cervids. Despite the failure of *P. andersoni* and *E. cervi* to develop in this laboratory host, the technique still provides a useful diagnostic tool considering the known distribution of the elaphostrongyline nematodes in North America. *E. cervi* is so far known only from Newfoundland (Lankester and Northcott 1979; Lankester and Hauta, in press), and *P. odocoilei* is currently known only in western Canada and the United States (Hobmaier and Hobmaier 1934; Brunetti 1969; Platt and Samuel 1978a). Therefore, only the larvae of *P. tenuis* and *P. andersoni* need to be distinguished in eastern North America, and the experimental infection of guinea pigs achieves this. However, possible concurrent infections of these two species would not be revealed.

Recovery of the different developmental stages of *P. tenuis* larvae from guinea pigs in this study indicates that they are also a useful laboratory host for studying the development of this particular species. Third-stage larvae, indistinguishable from infective larvae, were found in various tissues outside the CNS up to 27 DPI; these larvae are herein referred to as migrating larvae. Third-stage larvae were also found in the CNS of guinea pigs as early as 9 DPI, and up to 20 DPI. Fourth-stage larvae were found only in the CNS, between 18 and 47 DPI. Fifth-stage larvae also were found only in the CNS, but between 20 and 61 DPI. Since all larvae found outside the CNS as late as 27 DPI were still third-stage, yet fourth- and fifth- stage larvae were found in the CNS as early as 20 DPI, it is apparent that *P. tenuis* will only develop in the CNS of its host. This confirms conclusions reached by previous authors (Anderson 1963, 1965a; Anderson and Strelive 1967; Spratt 1967).

The length of time required for third-stage *P. tenuis* larvae to reach the

CNS of its host indicates that they migrate through the tissues, as has been suggested previously (Anderson and Strelive 1967), rather than being carried to the CNS in the blood. As mentioned, larvae were not recovered from the CNS of guinea pigs in this study before 9 DPI. Previously, *P. tenuis* larvae were first recovered in the CNS, either intact or in histological sections, at 10 DPI in both infected guinea pigs (Spratt 1967) and white-tailed deer (Anderson 1965a). Spratt (1967) also examined guinea pigs at 3, 5 and 8 DPI, but failed to recover larvae from the CNS. Likewise, Anderson and Strelive (1967) examined the CNS of white-tailed deer at 65, 90 and 144 hours after infection with *P. tenuis*, but failed to recover any larvae. Conversely, *Angiostrongylus cantonensis*, a neurotropic nematode that is known to be blood-borne, can be found in the brain of rats after only 17 hours (Mackerras and Sandars 1955).

Third-stage *P. tenuis* larvae apparently penetrate the stomach of their host and travel over or through the abdominal viscera, and possibly follow nerves in the body wall, when migrating to the CNS (Anderson and Strelive 1967; Spratt 1967). In this study, larvae were found in the abdominal cavity of guinea pigs as early as 1 DPI. Hemorrhages were apparent on the stomach wall in some animals up to 12 DPI, and also in the mesentery associated with the stomach in most up to 61 DPI. The majority of larvae recovered were found on pressing the mesentery. White track-like lesions on the liver, and capsule-like structures in visceral tissues, were seen in many of the animals; larvae, or remnants of larvae, were often found associated with these lesions. However, some larvae were free of the visceral tissues and could be flushed out of the abdominal and thoracic cavities with saline.

Spratt (1967) reported that third-stage *P. tenuis* larvae reached the abdominal cavity of guinea pigs by 2 DPI. Histologically, he found heavy

infiltrations of eosinophils around larvae in the stomach wall and suggested that larvae spending two or more days in this location were overcome by host defense mechanisms. In his study, migrating larvae were found in the mesentery,liver and lungs up to 20 DPI. Similar lesions were noted in these tissues, and histological examinations revealed larvae surrounded by eosinophils.

Comparable evidence of visceral migration by third-stage *P. tenuis* larvae is available from studies of infected white-tailed deer (Anderson and Strelive 1967). Intact third-stage larvae were recovered from the abdominal and thoracic viscera of deer fawns examined 65, 90 and 144 hours after infection (Anderson and Strelive 1967). Histologically, Anderson and Strelive (1967) found numerous larvae and marked eosinophilia in the submucosa, smooth muscle layer and serosa of the abomasum of fawns. These authors also found a pronounced eosinophilia in and around nerves below the vertebral column of the lumbar-sacral region of the fawn necropsied 6 DPI (144 hours), providing evidence that some larvae from the abdominal cavity may migrate to the CNS along nerves in this region.

The development of *P. tenuis* in the CNS of guinea pigs is obvious from the results of the present study, but specific details are difficult to establish. The larval stages were readily distinguished, and a few recently-molted fourth- and fifth- stage larvae were recovered. However, the precise length of time spent by each stage prior to molting to the next was impossible to ascertain. Third-stage larvae were recovered in the CNS as early as 9 DPI, yet fourth-stage larvae were not recovered until 18 DPI. This suggests that third-stage larvae require approximately 9 days in the CNS prior to molting to the fourth stage. However, it cannot be assumed that all third-stage larvae arrived in the CNS at 9 DPI. The recovery of fifth-stage larvae as early as 20 DPI

suggests that at least some fourth-stage larvae exist for only a short time before molting. Interestingly, third-, fourth- and fifth-stage larvae were all recovered from the CNS of one guinea pig at 20 DPI. Together, these results indicate that individual larvae may arrive in the CNS at different times post-infection and likely develop at different rates within the CNS.

Further research into this apparent migration route and the development of *P. tenuis* is warranted as it may be possible to relate the molting of larvae in the CNS to the onset of clinical signs of neurologic disease in susceptible host species. Except for one guinea pig that showed disease signs at 7 DPI, possibly due to an ear infection, each of the six animals studied here that eventually showed signs began to exhibit them some time between 16 and 37 DPI. The earliest onset of disease coincided approximately with the first evidence of the third molt, when the first fourth-stage larva was recovered (18 DPI). The onset of disease signs in all other animals occurred during the period in which larvae would most likely be molting from the fourth to the fifth stage (after 20 DPI). However, no definite conclusions can be reached based on these results since only a few infected animals showed clinical signs and the onset of these signs was variable.

This study provides the first detailed description of third-and fourth-stage *P. tenuis* larvae recovered from the CNS of any host. Third-stage larvae in the CNS, as well as those found outside the CNS, were similar to those described from snails by Anderson (1963). However, the sclerotized wall of the buccal capsule of third-stage larvae studied here was turned back upon itself, and appeared hook-shaped dorsally and ventrally. Anderson (1963) illustrated the dorsal and ventral walls of the buccal capsule of third-stage larvae as straight, thick, solid lines. Some of the third-stage larvae recovered here in

the CNS were also slightly longer than those described by previous authors (Anderson 1963; Ballantyne and Samuel 1984).

Fourth-stage larvae were longer, on average (mean=3124 µm), than third-stage larvae (mean= 1156 µm), but the ranges overlapped. Unlike the previous stage, fourth-stage larvae could be differentiated as males and females by the shape of the tail and the development of reproductive structures. Previous studies had reported the recovery of fourth-stage larvae from white-tailed deer (Anderson 1963) and reindeer (Nichols *et al.* 1986), but detailed descriptions of larvae were not provided. Fifth-stage larvae were generally much longer than fourth-stage larvae, although the ranges in lengths of these two stages also overlapped. Male and female fifth-stage larvae, or adults, were distinct; the vulva and vagina of adult females were well developed, as were the gubernaculum, spicules and bursa of adult males.

A few recently-molted fourth- and fifth- stage larvae, still within the loosened cuticle of the previous stage, were recovered from some guinea pigs and provide valuable information on the development of *P. tenuis*. Larvae may remain within the loosened cuticle for some time following the molt, but very few larvae like this were found; therefore, it is likely that the loosened cuticle is lost soon after molting. Recently-molted larvae were some of the shortest for that stage, which supports the view that those recovered had molted fairly recently. For example, the recently-molted fourth-stage female larva, recovered 22 DPI, was the shortest fourth-stage larva recovered and was within the range of lengths of third-stage larvae, yet it was clearly distinguishable from the third stage. Four recently-molted fifth-stage worms were recovered; one was found at 20, two at 21 and one at 47 DPI. These recently-molted fifth-stage worms were also some of the shortest adults

recovered, and were within the length range of the previous stage, yet were distinct from fourth-stage larvae. The one recovered 47 DPI may have reached the CNS later than other third-stage larvae, or it may have been inhibited from developing as rapidly as other larvae found in the same guinea pig. Again, it is difficult to reach conclusions due to the small sample size, but the recovery of these recently-molted larvae indicates approximate time intervals during which the molts can be expected to occur.

In nematodes, it has been suggested that molting may be more closely related to the differentiation of new structures, or to changes in habitat, than to the growth of worms (Bird 1971). The similarity in range in length of third-stage larvae recovered in the CNS of guinea pigs in this study (837-1385 μm) to ranges reported previously for third-stage larvae recovered from snails (900 - 1085 μm) (Anderson 1963; Ballantyne and Samuel 1984) indicates that third-stage larvae apparently do not increase greatly in length prior to undergoing the third molt. Fourth-stage larvae from the CNS of guinea pigs had a greater range in length (1305 - 4405 μm), which suggests that some growth occurs prior to the fourth molt. However, the greatest growth increase was seen after the final (fourth) molt to the adult or fifth stage (range = 3.5 to 39.9 mm).

The distinct buccal capsules of each larval stage recovered in this study and the sclerotized structures of adults, which were not present in previous stages, support the hypothesis that molting functions mainly in the differentiation of new structures. Replacement of the cuticular linings of the esophagus (pharynx), vulva and posterior intestine (proctodaem) of nematodes has been described previously (Croll and Matthews 1977). Reproductive structures found only in adult *P. tenuis* in this study, including the vulva of

females and the spicules and gubernaculum of males, indicates that molting is required for the differentiation of these structures. These sclerotized structures were evident in all fifth-stage larvae recovered as early as 20 DPI, yet were absent in fourth-stage larvae recovered as late as 38 to 47 DPI. Further detailed studies of the development of *P. tenuis* could be useful in studying molting in nematodes; the use of guinea pigs would facilitate such studies since the small early stages are easier to find in this laboratory host than in larger cervid hosts.

In the CNS, the development of *P. tenuis* is thought to occur in the neural parenchyma of the spinal cord for approximately one month, after which adult worms enter the spinal subdural space and migrate anteriorly to the cranium (see review by Anderson and Prestwood 1981). In previous studies with white-tailed deer, most larvae were found within neural tissue in animals examined up to approximately 30 DPI (Anderson 1963, 1965a). Some adults left the tissues as early as 25 DPI, and most were in the subdural space of infected deer by 40 DPI (Anderson 1965a). However, most developing larvae recovered from guinea pigs in this study were still within the tissues of the CNS, even after 47 DPI. Only two adult worms were found outside the tissues; one was in the subdural space of the lumbar-sacral region of the spinal cord at 35 DPI, and the other in the subdural space of the cerebellum at 61 DPI. Anderson and Strelive (1966) recovered adult *P. tenuis* from the neural parenchyma of seven guinea pigs examined 30 - 37 DPI, and from the cranial dura mater of one animal examined 127 DPI. Spratt (1967) recovered some adult worms from the subdural spaces of guinea pigs after 40 DPI, but many of the larvae were still within the tissues of the CNS up to 53 DPI. In moose, an unexpectedly large number of worms also fail to leave the neural

parenchyma before 40 DPI (Anderson 1964); this delay has been reported in wapiti as well (Anderson *et al.* 1966). The failure of adult worms to leave the neural parenchyma after one month is considered to be responsible for neurologic disease in these hosts (Anderson 1964; Anderson *et al.* 1966; Anderson and Strelive 1968; Tyler et al. 1980).

An anterior migration of *P. tenuis* larvae in the CNS, towards the cranium, also was not evident from this study. Larvae of all stages were found in all regions of the CNS on various days post-infection. Third-stage larvae were found in the thoracic cord at 9 DPI and in the cerebrum as early as 12 DPI, but the last third-stage larvae recovered at 20 DPI were found in the spinal cord. Fourth-stage larvae were recovered in the medulla and cerebrum at 18 DPI, in the cerebrum and lumbar-sacral region of the cord at 22 DPI, and in the thoracic cord at 47 DPI. Similarly, adult worms were found in the cerebellum at 20 DPI, yet were still found within the thoracic cord at 47 DPI. These results suggest either that third-stage larvae enter the CNS at different locations or, once inside the neural tissues, they may migrate at different rates or even in different directions (i.e. larvae are not necessarily moving from posterior regions to anterior regions). The cranial movement of *P. tenuis* in cervids is thought to occur only after adult worms enter the subdural space (Anderson and Prestwood 1981), and this is rarely achieved in guinea pigs (Anderson and Strelive 1966; Spratt 1967; this study).

Clinical disease seen here in some guinea pigs was similar to that seen by other authors in this host (Anderson and Strelive 1966; Spratt 1967), and was fundamentally like that seen in susceptible cervids (Anderson 1964, 1965b, 1965c, 1971; Anderson *et al.* 1966; Anderson and Strelive 1968; Carpenter *et al.* 1973; Trainer 1973; Lankester 1974; Kistner *et al.* 1977; Nettles *et al.* 1977a,

1977b; Woolf *et al.* 1977; Tyler *et al.* 1980). In this study, neurologic disease was observed in six of the sixteen infected guinea pigs. The onset of signs varied from 7 to 35 DPI, and included lameness, diarrhea and disorientation. Signs became progressively more severe in most guinea pigs, leading to paralysis of one or more limbs; weight loss usually followed the onset of clinical signs. In a variety of cervids, signs of infection with *P. tenuis* include lumbar weakness, ataxia, circling, blindness, paresis, and paraplegia (see review by Anderson and Prestwood 1981).

Previous authors (Anderson and Strelive 1966; Spratt 1967) have concluded that guinea pigs are poor alternate hosts of *P. tenuis* since infections do not become patent. Nonetheless, their value as hosts in which to study the development and morphology of this neurotropic nematode should not be overlooked. In white-tailed deer, the pre-patent period is approximately 82-91 days (Anderson 1963, 1965a), but guinea pigs infected up to 142 days still did not pass first-stage larvae (Spratt 1967). Although gravid female worms have not been recovered from infected guinea pigs, males do mature sufficiently to allow specific identification. Also, the recovery of small, developing worms in the CNS of guinea pigs is likely to be more successful than in large cervids. It is also noteworthy that the intensity of infection in the CNS achieved in this study (mean=3.8 larvae per guinea pig) is similar to that seen in naturally-infected white-tailed deer (1.8 to 9 worms per deer) (Alibasoglu *et al.* 1961; Anderson 1963, 1965a; Smith *et al.* 1964; Karns 1967; Gilbert 1973; Thurston and Strout 1978).

Results from the present study and published literature indicate that *P. tenuis* adults grow at similar rates in both guinea pigs (Spratt 1967; present study) and cervids (Anderson 1963, 1964; Anderson *et al.* 1966). No difference

was evident in the maximum length reached by male worms, but female worms recovered from cervids after approximately 60 DPI were considerably longer than those from guinea pigs (Fig. 14). This limitation on growth of female worms in guinea pigs may prevent *P. tenuis* from becoming patent in this host. Nevertheless, similar growth rates of *P. tenuis* among the hosts compared (Fig 14) support the conclusion that guinea pigs are suitable alternate hosts for some developmental studies involving this nematode.

Male adult elaphostrongylines, recovered from the CNS of guinea pigs in this study, were recognizable as *P. tenuis* as early as 27 DPI. The spicules of males at this time were large enough to distinguish them from *P. odocoilei* (see Platt and Samuel 1978a) and *P. andersoni* (see Lankester and Hauta, in press), and the presence of a crura distinguished them from *E. cervi* (see Anderson 1978). The distinct crura of *P. tenuis* (see Dougherty 1945) were not evident in male worms recovered from guinea pigs before 27 DPI, and the spicules were also shorter than previously described by Anderson (1956, 1963) and Spratt (1967). Male worms recovered before 27 DPI could therefore be confused with *P. odocoilei*, but not with *P. andersoni*, whose spicules are much shorter (Platt and Samuel 1978a). Evidently, some growth in spicule length must occur after molting to the adult is complete, although it is possible that the spicules were simply too lightly sclerotized before 27 DPI to accurately determine their maximum dimensions. The crura is the last structure to become sufficiently sclerotized to be visible with the light microscope. Although the morphology of adult male *P. tenuis* recovered before 27 DPI may be confused with other elaphostrongylines, the presence of the developing worms in the CNS, and the geographic locality from which larvae originated, would ensure a positive identification.

Fig. 14. Comparison of the range in length of *Parelaphostrongylus tenuis* adults recovered from the central nervous system of guinea pigs (Anderson and Strelive 1966; Spratt 1967; this study), white-tailed deer (Anderson 1963) and other cervids (moose, wapiti and mule deer) (Anderson 1964; Anderson *et al.* 1966). Single points are plotted where ranges not available.
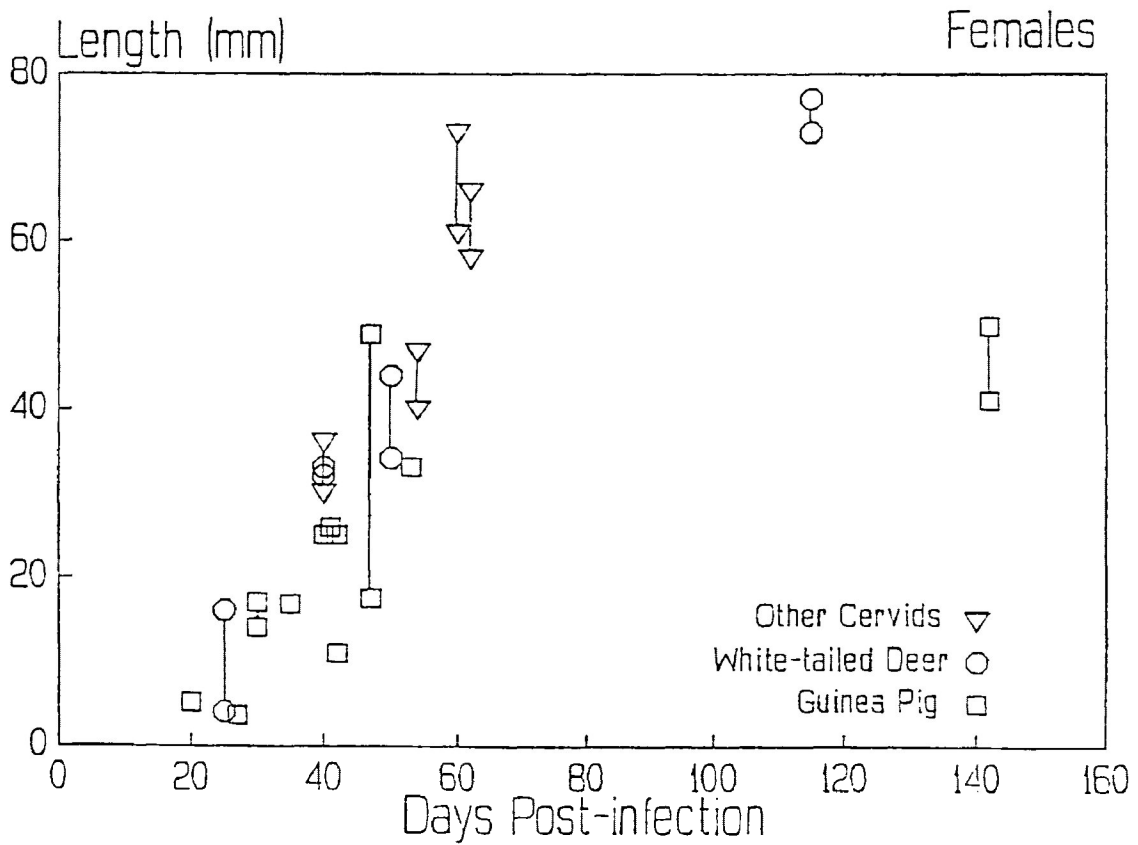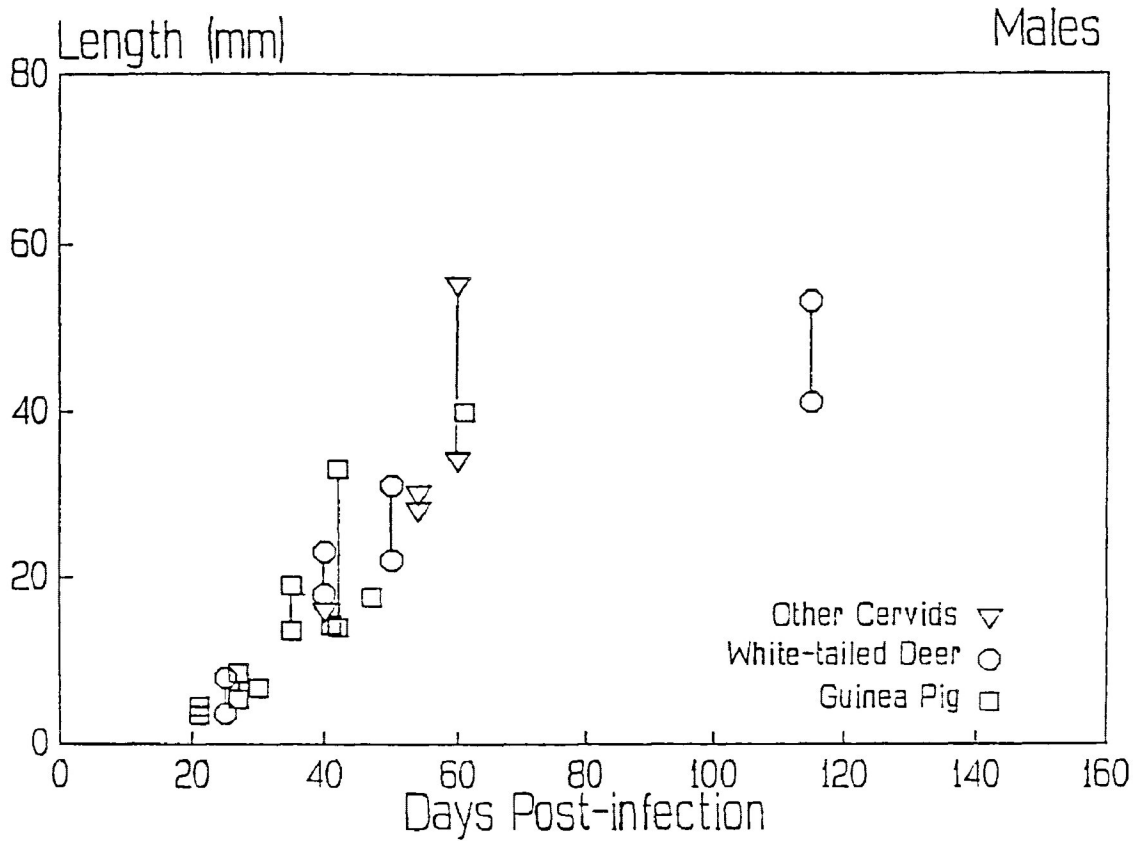
The failure to infect guinea pigs here with *P. andersoni* confirms earlier results by Pybus and Samuel (1984c). No larvae were recovered from the musculature of any of the guinea pigs infected in this study, and none of the muscle lesions reported in cervids infected with *P. andersoni* (Prestwood 1972; Nettles and Prestwood 1976; Pybus and Samuel 1981; Pybus 1983) were observed. Pybus and Samuel (1984c) also failed to recover adult muscleworms from infected guinea pigs, but recovered a few third-stage larvae from the viscera of two animals. Seven third-stage larvae were recovered from the viscera of only one animal (A-2) in this study, that had been given infective third-stage larvae a second time, 24 hours prior to necropsy. Apparently some larvae are able to migrate into the abdominal cavity and survive for a short time.

Tissue lesions found in guinea pigs infected with *P. andersoni* (Pybus and Samuel 1984c; present study) suggest that the initial migration route of this species is similar to that of *P. tenuis*. Lesions found in the stomach wall and liver of guinea pigs in this study were similar to those reported in previous infections of guinea pigs with *P. andersoni* (Pybus and Samuel 1984c); these lesions were also similar to those described for guinea pigs infected with *P. tenuis* (Spratt 1967). Capsules, containing larva-like structures or amorphous granular material, were found in the liver and mesentery of most guinea pigs in this study, up to 84 DPI. Pybus and Samuel (1984c) examined similar capsules histologically, and determined that they resulted from the "granulomatous encapsulation of larvae"; these authors concluded that larvae were overcome by a local host immune response. Pybus and Samuel (1984c) also concluded that some *P. andersoni* larvae moved through the mesentery to reach the liver, while others appeared to reach it via the blood. Other

evidence from their study indicated that some larvae reach the thoracic cavity, possibly by penetrating the diaphragm.

No studies have yet been conducted with cervids to determine the migratory path taken by *P. andersoni* larvae to the muscles of their hosts. The suggestion that the initial migration route followed by *P. andersoni* might be similar to that for *P. tenuis* is supported by Pybus and Samuel (1984a); these authors recovered adult *P. andersoni* from the epidural spaces and tissues of experimentally-infected deer. However, in the present study using guinea pigs, *P. andersoni* did not survive long enough nor migrate sufficiently to provide any further evidence of the route followed by this species. At the present time, studies on the migration of *P. andersoni* to the muscles of its host are limited to its normal cervid hosts; discovery of a suitable, small, laboratory host could facilitate such studies.

Contrary to a previous study by Watson and Gill (1985), *E. cervi* failed to develop here in guinea pigs. No larvae were recovered and no lesions were observed in the CNS. Lesions found on muscles of two animals were not associated with larvae. Watson and Gill (1985) infected eight guinea pigs and recovered one to two adult *E. cervi* from the CNS of each of five animals, after at least 74 DPI. Only one male worm was recovered, and infections did not become patent. All of the specimens recovered in their study were at the fifth-stage and were found beneath the cranial dura mater at the base of the brain (Watson and Gill 1985). Clinical signs of neurologic disease observed in these five animals were similar to those reported in various cervids (Roneus and Nordkvist 1962; Lankester 1977; Lankester and Northcott 1979; Bye and Halvorsen 1984; Stuve and Skorping 1987), and the histopathologic lesions

described in the CNS were similar to lesions in guinea pigs infected with *P. tenuis* (Anderson and Strelive 1966).

Reasons for the failure of *E. cervi* to develop in guinea pigs in this study are not obvious. The ages of guinea pigs used were similar in both studies (< 6 months.) and the housing and maintenance of animals was also very similar. Although unlikely, differences in the method of infection may be responsible. In this study, animals were given infective third-stage *E. cervi* larvae orally using a plastic pipette, whereas Watson and Gill (1985) administered larvae *per os* via a stomach tube. Guinea pigs were never observed to vomit after being given larvae. The success of *P. tenuis* infections in this study indicates that the oral infection method was successful at least in delivering larvae to the gut. The recovery of a single third-stage *E. cervi* larva from one guinea pig, and the presence of lesions and capsules in this and other infected animals, also support the success of the oral infection method. Possible strain differences between hosts, particularly relating to the effectiveness of immunological responses, could not be compared, and this factor cannot be ignored as a possible reason for the different results obtained.

The infectivity of third-stage *E. cervi* larvae may vary depending upon the host or the geographic location from which they originate; this may have been a factor resulting in the lack of development of this species in the present study. It is also possible that the apparent difference in infectivity of *E. cervi* may reflect more than just the origin of first-stage larvae. First-stage larvae, used in this study to infect snails, were recovered from caribou feces collected in Newfoundland, Canada. Watson and Gill (1985) used first-stage larvae from red deer and wapiti in New Zealand. Previously, *Elaphostrongylus* sp. from red deer in Europe, maral and sika deer in Asia, and

from reindeer in Europe, were each considered separate species (*E. cervi* Cameron, 1931, *E. panticola* Liubimov, 1945, and *E. rangiferi* Mitskevich, 1958, respectively). The primary difference between them was their occurrence in different hosts (see review by Kontrimavichus *et al.* 1976). Others have suggested that the latter two species are synonyms of *E. cervi* (see review by Lankester and Northcott 1979), but agreement is not universal. Norwegian workers persist in referring to the species in reindeer as *E. rangiferi* (see Bakken and Sparboe 1973; Halvorsen *et al.* 1980, 1985; Gaudernack *et al.* 1984), and some Swedish workers feel that specimens infecting moose there represent a new species (Dr. M. Steen, Inst. Vet. Med., Uppsala, Sweden, pers. comm. with M.W.L.). The results of the present study also raise questions regarding species or strain differences in *Elaphostrongylus*, and therefore further experimental study of the infectivity of *E. cervi* from different cervid hosts would be valuable in examining the apparent differences discussed herein.

Differences in the location of adult worms, identified as E. cervi, within moose examined in different geographic locations have led Stuve and Skorping (1987) to suggest that different strains of E. cervi, and possibly different species, exist in this host. These authors reported the recovery of adult worms, which they identified as *E. cervi*, only from the epidural spaces of experimentally infected moose in southern Norway, rather than from within the leptomeninges and brain tissues of moose as reported by Lankester (1977) and Steen and Morner (1985). Further studies of elaphostrongyline adults currently considered *E. cervi* may reveal morphological differences that have gone unobserved up to the present (Kontrimavichus *et al.* 1976). Future experimental infections of guinea pigs with *E. cervi* from different hosts and locations may reveal variations in the infectivity of this species, which may

clarify uncertainties regarding speciation within the genus *Elaphostrongylus*.

It is apparent from the published literature and from the present study, that third-stage elaphostrongyline larvae, migrating through the abdominal viscera, elicit an inflammatory response in their host. Migrating larvae found in the tissues of guinea pigs (*P. tenuis*, *P. andersoni* or *P. odocoilei*) or white-tailed deer (*P. tenuis*) are usually surrounded by heavy infiltrations of eosinophils, and are often encapsulated by compact granulomata which result in the death of some larvae (Anderson and Strelive 1966; Spratt 1967; Pybus and Samuel 1984c; this study). Examination of blood samples from infected animals also generally show increased numbers of circulating eosinophils (Spratt 1967; Nettles and Prestwood 1976; Prestwood and Nettles 1977; Pybus 1983; Watson and Gill 1985; Stuve and Skorping 1987). Levels of circulating eosinophils in guinea pigs infected with P. tenuis were reported to fluctuate after 5 DPI; a reduction in circulating eosinophils was suggested to be related to the trapping and encapsulation of larvae in the tissues of the host (Spratt and Anderson 1968).

Eosinophils are a common feature in nematode infections (Goetzl and Austen 1977; Mitchell 1979; Ogilvie and DeSavigny 1982), but their function(s) has not been fully elucidated. These leukocytes may be directly parasiticidal, as well as act as mediators of tissue repair; they are thought to limit the extent of fibrotic encapsulation, wound repair and granuloma formation during parasitic infections (see review by Mitchell 1979). *In vitro* studies indicate that eosinophils can destroy the larval stages of some nematodes (see review by Ogilvie *et al.* 1980).

Dexamethasone, an anti-inflammatory glucocorticosteroid, has been

reported to have some effect upon eosinophils and other aspects of the cell-mediated immune response. In goats, treatment with dexamethasone resulted in neutrophilia, eosinopenia, and lowered lymphocyte counts in the circulating blood (Maddux *et al.* 1987). Similarly, *in vitro* studies have shown that dexamethasone causes neutrophilia, a reduced number of macrophages, and suppression of mast mucosal cell growth and differentiation (McMenamin *et al.* 1987). Cortisone, another glucocorticosteroid, was shown to delay and reduce the inflammatory response of rabbits to bacterial infection. Weston *et al.* (1973) determined that cortisol, a cortisone derivative, inhibits the cell-mediated immune responses by preventing macrophages from responding to lymphokines produced by antigen-stimulated lymphocytes. This assortment of observations provides some indication of the complexities of the immune response and the possible effects of glucocorticosteroids upon it.

Glucocorticosteroids have been used previously in experimental studies of parasitic infections in attempts to increase the number of parasites establishing in abnormal or laboratory hosts (Weinstein 1955; Coker 1956a; Cross 1960; Parker 1961). Infective larvae of *Nippostrongylus muris*, an intestinal nematode of rats, successfully developed in guinea pigs treated with cortisone (Parker 1961). Cortisone-treated guinea pigs showed a reduced inflammatory response to larvae in the tissues (Parker 1961). Without such treatment, *N. muris* larvae failed to complete their tissue migration through the skin to the lungs, and eventually to the intestine in guinea pigs (Lindquist 1950). A similar experiment showed that *Nematospiroides dubius*, a parasite of mice, developed normally in rats treated with cortisone (Cross 1960); in untreated rats, an intense inflammatory response prevented larvae from developing (Cross 1960).

Treatment of guinea pigs with dexamethasone had no apparent effect on the survival and development of the elaphostrongylines studied here. The mean number of *P. tenuis* larvae recovered from the CNS of animals treated with dexamethasone (0.4 mg per animal) was not significantly different from that recovered from control animals (given saline). No *P. andersoni* larvae were recovered from the musculature of animals given either dosage of dexamethasone (0.2 or 0.4 mg per animal). Likewise, no larvae were recovered from the CNS or musculature of any animal infected with *E. cervi*. Only one previous study examined the effect of an immunosuppressant on the development of an elaphostrongyline nematode. Pybus and Samuel (1984c) treated guinea pigs with cortisone acetate, but larvae of *P. odocoilei* were still unable to migrate or establish in this laboratory host.

Possible reasons for the apparent failure of dexamethasone to suppress the immune responses of guinea pigs, and thereby allow more elaphostrongyline larvae to establish, are difficult to assess. The dosages of dexamethasone used were within levels recommended in the "Compendium of Pharmaceuticals and Specialties" (Canadian Pharmaceutical Association, Ottawa, Ontario); these dosages were also equated with levels of cortisone used previously which enabled other parasites to establish in abnormal hosts (Cross 1960; Parker 1961). It is possible that dexamethasone does not act upon the specific host reaction(s) involved in elaphostrongyline infections. As discussed, elaphostrongyline larvae elicit an intense inflammatory response in the gut of their hosts, and dexamethasone has been shown to act upon various components of this host response. Unfortunately, the specific defence mechanisms acting against these elaphostrongyline nematodes are poorly understood and require more detailed study in order to establish conclusively

which reactions of the host are involved. Similarly, little is known still about the effect of various immunosuppressants on parasite-host interactions, and further study is required to evaluate their potential use in similar experimental infections.

LITERATURE CITED

Alibasoglu, M., D.C. Kradel and H.W. Dunne. 1961. Cerebral nematodiasis in Pennsylvania deer (*Odocoileus virginianus*). Cornell Vet. 51: 431-441.

Anderson, R.C. 1956. *Elaphostrongylus odocoilei* Hobmaier and Hobmaier, 1934, in the cranial case of *Odocoileus virginianus borealis* Miller. Can. J. Zool. 34: 167-173.

Anderson, R.C. 1963. The incidence, development, and experimental transmission of *Pneumostrongylus tenuis* Dougherty (Metastrongyloidea: Protostrongylidae) of the meninges of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. Can. J. Zool. 41: 775-792.

Anderson, R.C. 1964. Neurologic disease in moose infected experimentally with *Pneumostrongylus tenuis* from white-tailed deer. Pathol. Vet. 1: 289-322.

Anderson, R.C. 1965a. The development of *Pneumostrongylus tenuis* in the central nervous system of white-tailed deer. Pathol. Vet. 2: 360-379.

Anderson, R.C. 1965b. An examination of wild moose exhibiting neurologic signs in Ontario. Can. J. Zool. 43: 635-639.

Anderson, R.C. 1965c. Cerebrospinal nematodiasis (*Pneumostrongylus tenuis*) in North American cervids. Trans. N.Am. Wildl. Nat. Resourc. Conf. 13: 156-157.

Anderson, R.C. 1971. Neurologic disease in reindeer (*Rangifer tarandus tarandus*) introduced into Ontario. Can. J. Zool. 49: 159-166.

Anderson, R.C. 1978. Keys to genera of the superfamily Metastrongyloidea. *In:* CIH keys to the nematode parasites of vertebrates. No. 5. 40 pp. *Edited by* R.C. Anderson, A.G. Chabaud and S. Willmott. Commonwealth

Agricultural Bureaux, Farnham Royal, Bucks, England.

Anderson, R.C., M.W. Lankester and U.R. Strelive. 1966. Further experimental studies of *Pneumostrongylus tenuis* in cervids. Can. J. Zool. 44: 851-861.

Anderson, R.C. and A.K. Prestwood. 1981. Lungworms. *In:* Diseases and parasites of white-tailed deer. W.R. Davidson *et al.* (eds.). pp. 266-317. Misc. Publ. No. 7, Tall Timbers Res. St., Tallahassee, Florida.

Anderson, R.C. and U.R. Strelive. 1966. The transmission of *Pneumostrongylus tenuis* to guinea pigs. Can. J. Zool. 44: 533-540.

Anderson R.C. and U.R. Strelive. 1967. The penetration of *Pneumostrongylus tenuis* into the tissues of white-tailed deer. Can. J. Zool. 45: 285-289.

Anderson, R.C. and U.R. Strelive. 1968. The experimental transmission of *Pneumostrongylus tenuis* to caribou (*Rangifer tarandus terraenovae*). Can. J. Zool. 46: 503-510.

Bakken, G. and O. Sparboe. 1973. [Elaphostrongylosis in reindeer]. Nord. Vet. Med. 25: 203-210.

Ballantyne, R.J. and W.M. Samuel. 1984. Diagnostic morphology of the third-stage larvae of three species of *Parelaphostrongylus* (Nematoda, Metastrongyloidea). J. Parasitol. 70: 602-604.

Bird, A.F. 1971. *The structure of nematodes.* Academic Press, Inc. New York, New York. 318 pp.

Brown, T.T., Jr., H.E. Jordan and C.N. DeMorest. 1978. Cerebrospinal parelaphostrongylosis in llamas. J. Wildl. Dis. 14: 441-444.

Brunetti, O.A. 1969. Redescription of *Parelaphostrongylus* (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish Game 55: 307-316.

Bye, K. and O. Halvorsen. 1984. Isolation of *Elaphostrongylus rangiferi* from wild reindeer in Scandinavia. Vet. Rec. 115: 87.

Carpenter, J.W., H.E. Jordan and B.C. Ward. 1973. Neurologic disease in wapiti naturally infected with meningeal worms. J. Wildl. Dis. 9: 148-153.

Coker, C.M. 1956. Effects of cortisone on cellular inflammation in the musculature of mice given one infection with *Trichinella spiralis*. J. Parasitol. 42(5): 479-484.

Croll, N.A. and B.E. Matthews. 1977. *Biology of nematodes*. Halstead Press, Toronto. 201 pp.

Cross, J.H. 1960. The natural resistance of the white rat to *Nematospiroides dubius* and the effect of cortisone on this resistance. J. Parasitol. 46: 175-185.

Dew, T.L. 1988. Prevalence of *Parelaphostrongylus tenuis* in a sample of hunter-harvested white-tailed deer from a tri-county area in northeastern Wisconsin. J. Wildl. Dis. 24(4): 720-721.

Dougherty, E.C. 1945. The nematode lungworms (sub-order Strongylina) of North American deer of the genus Odocoileus. Parasitol. 36: 199-208.

Gaudernack, G., O. Halvorsen, A. Skorping and K.A. Stokkan. 1984. Humoral immunity and output of first stage larvae of *Elaphostrongylus rangiferi* (Nematoda Metastrongyloidea) by infected reindeer, *Rangifer tarandus*. J. Helminthol. 58: 13-18.

Gilbert, G.F. 1973. *Parelaphostrongylus tenuis* (Dougherty) in Maine: I - The parasite in white-tailed deer (*Odocoileus virginianus* Zimmerman). J. Wildl. Dis. 9: 136-143.

Goetzl, E.J. and K.F. Austen. 1977. Cellular characteristics of the eosinophil compatible with a dual role in host defense in parasitic infections. Am.

J. Trop. Med. Hyg. 26(6): 142-148.

Gray, J.B., W.M. Samuel, A.W. Shostak and M.J. Pybus. 1985. *Varestrongylus alpenae* (Nematoda: Metastrongyloidea) in white-tailed deer (*Odocoileus virginianus*) of Saskatchewan. Can. J. Zool. 63: 1449-1454.

Gray, J.B. and W.M. Samuel. 1986. *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) and a protostrongylid nematode in woodland caribou (*Rangifer tarandus caribou*) of Alberta, Canada. J. Wildl. Dis. 22(1): 48-50.

Halvorsen, O., J. Andersen, A. Skorping and G. Lorentzen. 1980. Infection in reindeer with the nematode *Elaphostrongylus rangiferi* Mitskevich in relation to climate and distribution of intermediate hosts. Proc. 2nd Int. Reindeer/Caribou Symp., Roros, Norway. 449- 455.

Halvorsen, O., A. Skorping and K. Hansen. 1985. Seasonal cycles in the output of first stage larvae of the nematode *Elaphostrongylus rangiferi* from reindeer, *Rangifer tarandus tarandus*. Polar Biol. 5: 49-54.

Halvorsen, O. and K. Wissler. 1983. *Elaphostrongylus* sp. (Nematoda, Protostrongylidae) and other helminths in faeces of moose in north Norway. Fauna Norv. Ser. A. 4: 37-40.

Hobmaier, A. and M. Hobmaier. 1934. *Elaphostrongylus odocoilei* n.sp., a new lungworm in black-tailed deer (*Odocoileus columbianus*). Description and life history. Proc. Soc. Exp. Biol. Med. 31: 509-514.

Karns, P.D. 1967. *Pneumostrongylus tenuis* in deer in Minnesota and implications for moose. J. Wildl. Mgmt. 31(2): 299-303.

Kistner, T.P., G.R. Johnson and G.A. Rilling. 1977. Naturally occurring neurologic disease in a fallow deer infected with meningeal worms. J. Wildl. Dis. 13: 55-58.

Kocan, A.A., M.G. Shaw, K.A. Waldrup and G.J. Kubat. 1982. Distribution of *Parelaphostrongylus tenuis* (Nematoda: Metastrongyloidea) in white-tailed deer from Oklahoma. J. Wildl. Dis. 18: 457-460.

Kontrimavichus, V.L., S.L. Delyamure and S.N. Boev. 1976. Metastrongyloidea of domestic and wild animals. *In:* Principles of nematology. Ryzhikov, K.M. (ed.). Vol. 26. pp.1-24. Iedatel'stvo "Nauka", Moscow, USSR.

Lankester, M.W. 1974. *Parelaphostrongylus tenuis* (Nematoda) and *Fascioloides magna* (Trematoda) in moose of southeastern Manitoba. Can. J. Zool. 52: 235-239.

Lankester, M.W. 1976. A protostrongylid nematode of woodland caribou and implications in moose management. Proc. North Am. Moose Conf. Wkshp. 12:173-190.

Lankester, M.W. 1977. Neurologic disease in moose caused by *Elaphostrongylus cervi* Cameron 1931 from caribou. Proc. North Am. Moose Conf. Wkshp. 13: 177-190.

Lankester, M.W. and P.L. Hauta. 1989. *Parelaphostrongylus andersoni* (Nematoda: Elaphostrongylinae) in caribou (*Rangifer tarandus*) of northern and central Canada. Can. J. Zool. (in press).

Lankester, M.W. and T.H. Northcott 1979. *Elaphostrongylus cervi* Cameron 1931 (Nematoda: Metastrongyloidea) in caribou (*Rangifer tarandus caribou*) of Newfoundland. Can. J. Zool. 57(7): 1384-1392.

Lindquist, W.D. 1950. Some abnormal host relationships of a rat nematode, *Nippostrongylus muris*. Am. J. Hyg. 52: 22-41.

Mackerras, M.J. and D.F. Sandars. 1955. The life history of the rat lungworm *Angiostrongylus cantonensis* (Chen) (Nematoda: Metastrongyloidea). Aust. J. Zool. 3: 1-25; *from:* Spratt 1967.

Maddux, J.M. and K.S. Keeton 1987. Effects of dexamethasone, levamisole, and dexamethasone-levamisole combination on neutrophil function in female goats. Am. J. Vet. Res. 48(7): 1114-1119.

Mason, P.C., N.R. Kiddey, R.J. Sutherland, D.M. Rutherford and A.G. Green. 1976. *Elaphostrongylus cervi* in red deer. N.Z. Vet. J. 24: 22-23.

Mason, P.C. and H.J.F. McAllum. 1976. *Dictyocaulus viviparus* and *Elaphostrongylus cervi* in wapiti. N.Z. Vet. J. 24: 23.

McMenamin, C.C., E.A. Gault and D.M. Haig. 1987. The effect of dexamethasone on growth and differentiation of bone marrow derived mucosal vast cell *in vitro*. Immunology 62(1): 29-34.

Mitchell, G.F. 1979. Effector cells, molecules and mechanisms in host-protective immunity to parasites. Immunology 38: 209-224.

Nettles, V.F. and A.K. Prestwood. 1976. Experimental *Parelaphostrongylus andersoni* infections in white-tailed deer. Vet. Pathol. 13: 381-393.

Nettles, V.F., A.K. Prestwood, R.G. Nichols and C.J. Whitehead. 1977a. Meningeal worm-induced neurologic disease in black-tailed deer. J. Wildl. Dis. 13: 137-143.80

Nettles, V.F., A.K. Prestwood and R.D. Smith. 1977b. Cerebrospinal parelaphostrongylosis in fallow deer. J. Wildl. Dis. 13: 440-444.

Nichols, D.K., R.J. Montali, L.G. Phillips, T.P. Alvarado, M. Bush and L. Collins. 1986. *Parelaphostrongylus tenuis* in captive reindeer and sable antelope. J. Am. Vet. Med. Assoc. 188(6): 619-621.

Ogilvie, B.M. and D. DeSavigny. 1982. Immune response to nematodes. *In:* Immunology of parasitic infections. S. Cohen and K.S. Warren (eds.). 2nd edition. Blackwell Scientific Publications Ltd. Oxford, UK. pp. 715-757.

Ogilvie, B.M., M. Philipp, M. Jungery, R.M. Maizels, M.J. Worms and R.M.E. Parkhouse. 1980. The surface of nematodes and the immune response of the host. *In*: The host-invader interplay. H. van den Bossche (ed.). pp. 99; *from*: Ogilvie and DeSavigny 1982.

Parker, J.C. 1961. Effect of cortisone on the resistance of the guinea pig to infections with the rat nematode *Nippostrongylus brasiliensis*. Exp. Parasitol. 11: 380-390.

Platt, T.R. and W.M. Samuel. 1978a. A redescription and neotype designation for *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea). J. Parasitol. 64: 226-232.

Platt, T.R. and W.M. Samuel. 1978b. *Parelaphostrongylus odocoilei*: life cycle in experimentally infected cervids including the mule deer, *Odocoileus h. hemionus*. Exp. Parasitol. 46(2): 330-338.

Prestwood, A.K. 1972. *Parelaphostrongylus andersoni* sp.n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). J. Parasitol. 58: 897-902.

Prestwood, A.K. and V.F. Nettles. 1977. Repeated low-level infection of white-tailed deer with *Parelaphostrongylus andersoni*. J. Parasitol. 63(6): 974-978.

Prestwood, A.K., V.F. Nettles, and F.E. Kellogg. 1974. Distribution of muscleworm, *Parelaphostrongylus andersoni*, among white-tailed deer of the southeastern United States. J. Wildl. Dis. 10: 404-409.

Pursglove, S.R. 1977. Helminth parasites of white-tailed deer (*Odocoileus virginianus*) from New Jersey and Oklahoma. Proc. Helm. Soc. Washington 44:107-108.

Pybus, M.J. 1983. *Parelaphostrongylus andersoni* Prestwood 1972 and *P.*

Odocoilei(Hobmaier and Hobmaier 1934) (Nematoda: Metastrongyloidea) in two cervid definitive hosts. Ph.D. Thesis. University of Alberta, Edmonton, Alberta.

Pybus, M.J., W.J. Foreyt and W.M. Samuel. 1984. Natural infections of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in several hosts and locations. Proc. Helminthol. Soc. Wash. 51(2): 338-340.

Pybus, M.J. and W.M. Samuel. 1980. Pathology of the muscle worm, *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea) in moose. Proc. N. Am. Moose Conf. Workshop 16: 152-170.

Pybus, M.J. and W.M. Samuel. 1981. Nematode muscleworm from white-tailed deer of southeastern British Columbia. J. Wildl. Mgmt. 45(2): 537-542.

Pybus, M.J. and W.M. Samuel. 1984a. *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) and *P. odocoilei* in two cervid definitive hosts. J. Parasitol. 70: 507-515.

Pybus, M.J. and W.M. Samuel. 1984b. Lesions caused by *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea) in two cervid hosts. Vet. Pathol. 21: 425-431.

Pybus, M.J., and W.M. Samuel. 1984c. Attempts to find a laboratory host for *Parelaphostrongylus andersoni* and *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae). Can. J. Zool. 62: 1181-1184.

Pybus, M.J. and H. Shave. 1984. *Muellarius capillarius* (Mueller, 1889) (Nematoda: Protostrongylidae): an unusual finding in Rocky Mountain bighorn sheep (*Ovis canadensis* Shaw) in South Dakota. J. Wildl. Dis. 20(4): 284-288.

Roneus, O. and M. Nordkvist. 1962. Cerebrospinal and muscular nematodiasis (*Elaphostrongylus rangiferi*) in Swedish reindeer. Acta. Vet. Scand. 3:

201-225.

Samuel, W.M. and J.C. Holmes. 1974. Search for elaphostrongyline parasites in cervids from Alberta. Can. J. Zool. 52: 401-403.

Smith, H.J., R.M. Archibald and A.H. Corner. 1964. Elaphostrongylosis in maritime moose and deer. Can. Vet. J. 5: 287-296.

Spratt, D.M. 1967. The guinea pig as an experimental host of the meningeal worm (Pneumostrongylus tenuis Dougherty, 1945) of white-tailed deer. M.Sc. Thesis, University of Toronto, Toronto, Ontario.

Steen, M. and T. Morner. 1985. [Brainworm - a new serious disease of wild game]. Svensk. jakt. 7/8: 601-603; from: Stuve and Skorping 1987.

Stuve, G. and A. Skorping. 1987. Experimental Elaphostrongylus cervi infection in moose (Alces alces). Acta Vet. Scand. 28: 165-171.

Thurston, D.R. and R.C. Strout. 1978. Prevalence of meningeal worm (Parelaphostrongylus tenuis) in white-tailed deer from New Hampshire. J. Wildl. Dis. 14: 89-96.

Trainer, D.O. 1973. Caribou mortality due to meningeal worm (Parelaphostrongylus tenuis). J. Wildl. Dis. 9: 376-378.

Tyler, G.V., C.P Hibler and A.K. Prestwood. 1980. Experimental infection of mule deer with Parelaphostrongylus tenuis. J. Wildl. Dis. 16(4): 533-540.

Watson, T.G. and J.M. Gill. 1985. The experimental infection of guinea pigs with the tissue worm of deer - Elaphostrongylus cervi. New Zealand Vet. J. 33(6): 81-83.

Wienstein, P.P. 1955. The effect of cortisone on the immune response of the white rat to Nippostrongylus muris. Am. J. Trop. Med. Hyg. 4: 61-74.

Weston, W.L., H.N. Claman and G.G. Krueger. 1973. Site of action of cortisol in cellular immunity. J. Immunol. 110: 880.

Woolf, A., C.A. Mason and D. Kradel. 1977. Prevalence and effects of *Parelaphostrongylus tenuis* in a captive wapiti population. J. Wildl. Dis. 13: 149-154.