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THE LIFE CYCLE AND SYSTEMATICS OF [illegible]

(NEMATODA:METASTRONGYLOIDEA), A PARASITE OF MULE DEER

[illegible], WITH SPECIAL REFERENCE

TO THE MOLLUSCAN INTERMEDIATE HOST

by

THOMAS REID PLATT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1978

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The life cycle and systematics of *Parelaphostrongylus odocoilei* (Nematoda:Metastrongyloidea), a parasite of mule deer (*Odocoileus hemionus hemionus*), with special reference to the molluscan intermediate host" submitted by Thomas Reid Platt, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

W. M. Samuel
Supervisor

A. N. Spencer

J. Ralph Lichtenfels
External Examiner

Date Feb. 2, 1978

ABSTRACT

Parelaphostrongylus odocoilei (Hobmaier and Hobmaier 1934) Boev and Schul'ts 1950, is described from the Rocky Mountain mule deer (*Odocoileus h. hemionus*) in Alberta, Canada. A key to the males of the genus *Parelaphostrongylus* is provided. The first- and third-stage larvae of *P. odocoilei* are described in detail for the first time.

The life cycle of *P. odocoilei* was completed in mule deer, black-tailed deer (*Odocoileus hemionus columbianus*) and moose (*Alces alces*). A mean prepatent period of 55 days was established for *P. odocoilei* in the mule deer. The duration of the prepatent period is inversely related to the size of the infective inoculum. A mean prepatent period of 64 and 70 days was established for this parasite in black-tailed deer (*O. h. columbianus*) and moose (*Alces alces*), respectively. Experimentally infected white-tailed deer (*O. virginianus*) failed to become patent.

Larval production of *P. odocoilei* in mule deer was approximately 5 times higher than previous reports in the literature for other elaphostrongyline nematodes. Duration of patency has not been firmly established; however, two animals passed larvae for over 1 year. Larval production, duration and intensity, was reduced in black-tailed deer and moose. On the basis of these factors, *O. h. hemionus* is considered the primary definitive host of *P. odocoilei*.

Seven of 15 species of terrestrial molluscs were naturally infected with *P. odocoilei* in the vicinity of Jasper, Alberta. They

are: [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] and [illegible]. [illegible] and *H. falcata* were considered the primary intermediate hosts of *P. odocoilei* in this area. Although the overall prevalence of infections in molluscs was low (<2%), part of the wintering area of the Townsite mule deer herd was identified as a focus of infection. The prevalence of infection in *H. [illegible]* in this area was 19%. Peak prevalence of infective larvae of *P. odocoilei* occurred in late August, coinciding with the return of mule deer to the wintering area. Possible factors delimiting a focus of infection or "hot spot" are discussed.

Mode of entry and development of larval *P. odocoilei* were studied experimentally in several gastropods, primarily *Triodopsis multilineata* (Say). First-stage larvae of *P. odocoilei* gained entry into susceptible molluscs primarily by direct penetration, although entry via ingestion occurred. The host-reaction of *Triodopsis multilineata* to developing larvae was documented and compared to previous studies of this general phenomenon. The reaction is biphasic: an initial influx of amoebocytes, followed by the formation of fibrotic elements that delineate the cyst.

Larval molts occurred at 8 and 17 days post-infection at 18°C in *T. multilineata*. A maturation period of approximately 5 days was required for third-stage larvae to become infective.

The evolutionary relationships of the members of the Elaphostrongylinae are analyzed in the framework of phylogenetic systematics. Two basic lineages are identified: a muscle dwelling group

(*P. odocoilei* and *P. andersoni*) and a CNS lineage (*P. tenuis* and *P. pratti*). Evidence is presented to indicate that the Elaphostrongylinae are of Nearctic origin, contrary to speculation by previous authors, and *P. pratti* is a recent immigrant to Eurasia.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my advisor, Dr. William M. Samuel, for his confidence, encouragement, assistance and friendship during this project. I also wish to thank the members of my advisory committee: Drs. J. Holmes and A. Spencer, and particularly Dr. G. Ball, for their advice and criticism.

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Finally, I wish to thank my wife, Kathy, for her understanding and love. Her support during the past four years has been an invaluable commodity. Repayment will take a lifetime.

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SECTION I

GENERAL INTRODUCTION

Elaphostrongyline nematodes comprise a small but important group of lungworms (Protostrongylidae) in cervids. However, they received scant attention in North America until Anderson (1961) demonstrated that *Parelaphostrongylus tenuis* (Dougherty and Goble, 1946) was the causative agent of "moose sickness." Once the role of this elaphostrongyline was established conclusively as an agent of epizootiologic importance many papers followed (see Anderson 1968, 1971a; Anderson and Prestwood 1978 for reviews). In eastern North America meningeal worm was shown to cause cerebrospinal nematodiasis in a variety of ungulates including wapiti (*Cervus elaphus canadensis*), reindeer (*Rangifer tarandus tarandus*), domestic sheep and goats (Anderson and Strelive 1968; Anderson 1971b; Alden *et al.* 1975; Woolf *et al.* 1977) and, as previously mentioned, moose (*Alces alces*).

In the East, the discovery of *Parelaphostrongylus andersoni* Prestwood 1972, in white-tailed deer of the southeastern United States, resulted in a more precise evaluation of the ecological (Anderson 1972), geographical (Prestwood and Smith 1969; Prestwood *et al.* 1974) and pathological (Nettles and Prestwood 1976) relationships of the members of the genus *Parelaphostrongylus*.

In the West, less progress has been made. The only elaphostrongyline described from the West to date, *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier 1934a), from Columbian black-tailed deer, is the type species of the genus. It has only been reported twice in the literature (Hobmaier and Hobmaier 1934; Brunetti 1969). Both cases

were from deer in northwestern California and were limited primarily to morphological descriptions of adult worms. Brunetti (1969) described the pathological effects of eggs and first stage larvae on the lungs of naturally and experimentally infected animals. Life cycle data presented by these authors were limited and, in some instances, contradictory. General surveys of the helminths of deer of western North America (e.g. Cowan 1951; Worley and Eustace 1972) were not conducted utilizing techniques that would have demonstrated the presence of these worms in the musculature.

In Alberta, Samuel and Holmes (1974) examined deer fecal pellet groups and heads of white-tailed deer, mule deer and moose from southern Alberta for lungworm larvae and adult worms, respectively. They found spined larvae "indistinguishable from those of *P. tenuis*" in 19 deer pellet groups from the foothills region of Alberta, which is primarily mule deer habitat. Samuel and Holmes offered an hypothesis that countered the suggestion of Bindernagel and Anderson (1972): i.e., that *P. tenuis* is spreading westward in the Aspen Parkland. They stated that "...some ecological feature...currently limits its [*P. tenuis*] populations in the west." Although not implicitly stated by these authors, their hypothesis demands the presence of an alternate parasite of mule deer in western Alberta.

It was against this background that the present study was designed. The objectives were:

1) to locate the adult worm of an apparently unidentified elaphostrongyline parasite of mule deer;

2) using morphologic data of adult as well as larval stages, if

applicable, to assess the taxonomic position of this worm;

5) to complete the life cycle of this parasite experimentally, and determine the length of the prepatent period, patent period and the intensity of larval output;

4) to determine the extent of host specificity of this worm in a few native cervids;

5) to determine the natural intermediate host(s) of this parasite in Jasper, Alberta, and assess the prevalence of infection in the intermediate host on a seasonal basis;

6) to determine the route of penetration, migration, site selection and length of development of the larvae in experimentally infected intermediate hosts;

7) to observe the reaction, if any, of the intermediate host to the developing larvae of this parasite, and

8) utilizing the above information and the current literature, to determine the phylogenetic relationships of the species comprising the nematode subfamily Elaphostrongylinae and their evolutionary relationship with the intermediate and definitive hosts.

SECTION II

TAXONOMY

A. Introduction

"Animals cannot be discussed or treated in a scientific way until some taxonomy has been achieved," (Simpson 1945). The primary objective of this research was to identify the alternate parasitic nematode implied by the work of Samuel and Holmes (1974) parasitizing mule deer in Alberta. Captive animals were experimentally infected with infective larvae obtained from experimentally infected snails (see Section III). The infection was originally derived from a naturally infected male mule deer in Jasper, Alberta. Worms found at necropsy were identical to *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier 1934) Boev and Schul'ts 1950.

Parelaphostrongylus odocoilei has occupied a central position in the taxonomic problems involving the elaphostrongyline nematodes. The problem has focused primarily on the infrequent reports of this worm in the literature and the incomplete nature of the descriptions and figures offered by earlier workers. As the type species, *P. odocoilei* sets the standard for the genus *Parelaphostrongylus*. Hence, any change in the taxonomic status of this worm due to an incomplete understanding of its form will have, and unfortunately has had, an effect on the nomenclature and disbursement of the taxa comprising the Elaphostrongylinae.

As I have indicated, *P. odocoilei* is the most poorly known of the species comprising the genus. Although *P. odocoilei* has been recently redescribed (Brunetti 1969), the identification of this worm from a

new host (*O. h. hemionus*) and such widely separated areas as California and Alberta requires a re-evaluation of the species as well as a comprehensive comparison with the other members of the genus.

Attempts to find adult worms in naturally infected mule deer from near Jasper National Park, Alberta, were unsuccessful (Samuel unpublished). Therefore an experimental approach was adopted for the present study.

B. A Review of Generic Level Taxonomy

The first elaphostrongylid described in the literature was *Elaphostrongylus cervi* Cameron 1931. This nematode was described from the dorsal musculature of the european elk, *Cervus elaphus* by Cameron (1931). Shortly thereafter, Hobmaier and Hobmaier (1934a) described *Elaphostrongylus odocoilei* from the dorsal musculature of the Columbian black-tailed deer (*O. hemionus columbianus*) in northwestern California. Dougherty (1945), in a review of the lungworms parasitizing *Odocoileus*, suggested that differences in the structure of the gubernaculum of the two species might preclude placing them in the same genus. He concluded, however, that additional studies of *E. cervi* were necessary before such a determination could be rendered. At the same time Dougherty (1945) described *Pneumostrongylus tenuis* as a new species of lungworm on the basis of a single male specimen found in the lung of a white-tailed deer (*O. virginianus*) from New York.

Boev and Schul'ts (1950) erected the genus *Parelaphostrongylus* for *E. odocoilei* on the basis of the split nature of the gubernacular corpus. This was done on the basis of observations of these and other Russian workers; on the gubernacular structure of *Elaphostrongylus* spp.

from the USSR. Schul'ts (1951) erected the genus *Odocoileostrongylus* for *Pneumostrongylus tenuis*. This was done on the basis of a literature review and not on observations of actual material. This genus was placed in the Capreocaulinae by Schul'ts (1951).

Whitlock (1952) described *Neostrongylus cervi tenuis* as a new genus and species of nematode from the CNS of domestic sheep. Anderson (1956) offered a redescription of *E. odocoilei* from the CNS of white-tailed deer in Ontario although he was certainly referring to *P. tenuis*. After examining specimens provided by Anderson, from deer, and recovering additional cerebrospinal worms from sheep, Whitlock (1959) reduced *N. cornellensis* and *E. odocoilei* (sensu Anderson 1956) to synonyms of *Elaphostrongylus tenuis*. He removed *E. tenuis* from the genus *Pneumostrongylus* on morphological grounds but refused to accept *Odocoileostrongylus* Schul'ts 1951 for extremely superficial reasons. Anderson (1962a) continued to recognize *Pneumostrongylus* Monnig 1932 for *P. tenuis* on the grounds that a close relationship existed between *Pneumostrongylus tenuis* and *Parelaphostrongylus odocoilei*, and until that issue was resolved, he preferred a more conservative approach.

Boev (1968) declared *P. odocoilei* a *species inquirendum*, thereby eliminating the genus *Parelaphostrongylus* as a valid taxon. In addition, he transferred *Odocoileostrongylus* from the subfamily Capreocaulinae to the Elaphostrongylinae. Brunetti (1969) re-described *P. odocoilei* from the dorsal musculature of the Columbian black-tailed deer (*O. h. columbianus*) in north central California. Pryadko and Boev (1971) accepted the redescription of *P. odocoilei* and reestablished the genus *Parelaphostrongylus* as a valid taxon. They also formally

recognized the relationship of *P. tenuis* and reduced *Parelaphostrongylus* Schul'ts 1951 to a junior synonym of *Pneumostrongylus* Boev and Schul'ts 1950, on the basis of priority. Thus *Pneumostrongylus* contained two species: *P. odocoilei* (Hobmaier and Hobmaier 1934) and *P. tenuis* (Doutherty 1945). Anderson (1972) recognized the preceding changes, thus stabilizing the nomenclature. Prestwood (1972) added a third species, *P. andersoni*, from the dorsal musculature of the white-tailed deer, in the southeastern United States.

The recent discovery of *P. odocoilei* in Alberta (this study) has initiated new interest in the systematics of this genus. *Parelaphostrongylus odocoilei* has been re-described on the basis of specimens from Alberta and California and a neotype specimen has been designated for this species (Platt and Samuel 1978).

C. Materials and Methods

Adult nematodes were collected from mule deer fawns experimentally infected as outlined in Section III. Worms were killed in hot glycerine-alcohol (95 parts 70% ETOH and 5 parts pure glycerine) and cleared by the daily addition of pure glycerine, while allowing gradual evaporation of the alcohol. Nematodes were examined as temporary mounts in glycerine. *En face* mounts were prepared according to the method of Anderson (1958) as modified by Hobbs (1976). The posterior portions of several male worms were stored in glycerine-alcohol and then cleared and examined in lactophenol-beechwood creosote (1:1) to aid in determining the nature of the male reproductive structures.

Through the kindness of Dr. O. A. Brunetti, I had the opportunity of examining specimens of *P. odocoilei* collected from the type host

(*Odocoileus hemionus hemionus*) in California. These worms arrived in a dilute formalin solution. They were transferred to glycerine-alcohol and subsequently cleared and examined as temporary mounts in lactophenol-beechwood creosote.

First stage larvae were collected via the Baermann technique (p. 37). Third stage larvae were obtained by the artificial digestion of experimentally infected snails (*Deroceras reticulatum*) (p. 37). All larvae were killed in hot glycerine-alcohol, cleared and examined in lactophenol-beechwood creosote.

Measurements were made with the aid of an ocular micrometer, drawing tube and measuring wheel. Drawings were made with the aid of a drawing tube. All measurements are in micrometers (μm) unless otherwise indicated.

Larval stages of *P. odocoilei* were prepared for scanning electron microscopy (SEM) by dehydration of larvae through a series of graded alcohols to 100% ETOH. The specimens were then moved through a graded series of 100% ETOH and amyl acetate, until 100% amyl acetate was attained. Specimens were critical point dried, coated with gold and carbon and examined on a Cambridge Stereoscan S4 scanning electron microscope.

D. Redescription

Adult (Figs. 1-10, 16-19; Table I)

Parelaphostrongylus odocoilei (Hobmaier and Hobmaier 1934) Boev and Schul'ts 1950.

Synonym: *Elaphostrongylus odocoilei* Hobmaier and Hobmaier 1934, nec Anderson 1956.

Elaphostrongylinae: with the characters of the genus as amended by Pryadko and Boev (1971).

General description: Worms long and thread-like. Living specimens opaque white with intestine a distinct, contrasting black, probably from ingestion of hematin. Anterior end (Fig. 1) with thickened oral plate and thickly cuticularized. Asymmetrical stoma. Mouth surrounded by outer circle of four large papillae and inner set of six perityls, each possessing a small, centrally located papilla. Amphids are lateral (Fig. 2), having appearance of large pits. Esophagus club-shaped, not divided into muscular and glandular portions. Nerve ring located near opening of excretory pore (Fig. 1) approximately 1/5-1/7 the length of the esophagus from anterior end. Terminal excretory duct heavily cuticularized. Teguminal sheath absent.

Male: Delicate worm 18-35 mm long, 108-156 in maximum width. Esophagus muscular 565-795 long and 58-87 wide at the base, slightly anterior to the esophageal-intestinal junction. Excretory pore 56-97 and nerve ring 68-94 from anterior end. Nerve ring usually located posterior to excretory pore.

Bursa simple (Fig. 3), 100-117 wide by 79-94 long, not divided into lobes. Ventroventral and ventrolateral rays short, directed anteriorly and joined along the proximal 1/2 of their length. Anteriolateral, mediolateral and dorsolateral rays thick and arise from common stem. Anteriolateral separate from the other lateral rays. Mediolateral and dorsolateral are longest rays and are joined along the proximal 1/2 of their lengths. Externodorsal separate. Dorsal ray (Fig. 8) short, thick with two terminal stems, one of which (or both)

Figures 1-10. Morphology of adult [illegible].

Fig. 1. Anterior end ♀, lateral.

Fig. 2. [illegible], ♀.

Fig. 3. Bursa ♂, ventral.

Fig. 4. Spicules ♂, ventro-lateral.

Fig. 5. Spicules and gubernaculum ♂, ventral.

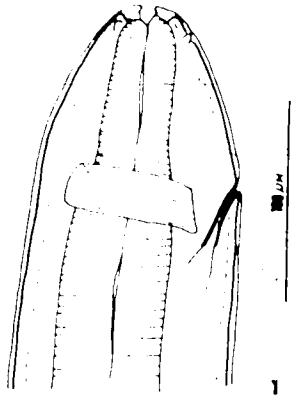
Fig. 6. Spicules and gubernaculum, cross section.

Fig. 7. Posterior ♂, lateral.

Fig. 8. Dorsal ray ♂, dorsal.

Fig. 9. Posterior ♀, lateral.

Fig. 10. Posterior ♀, ventral.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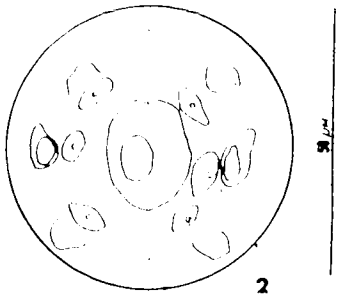
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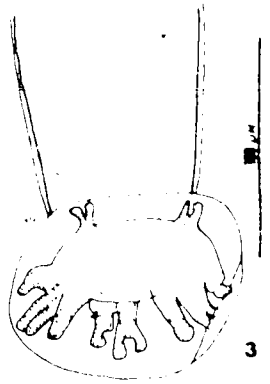
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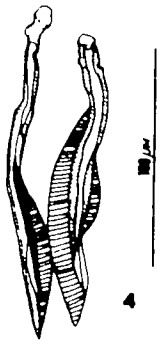
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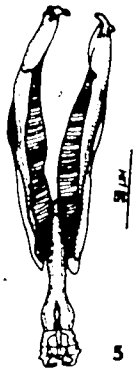
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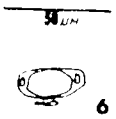
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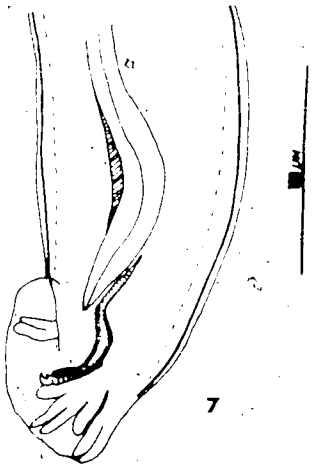
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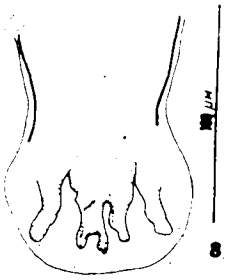
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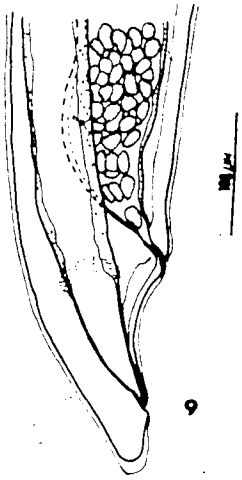
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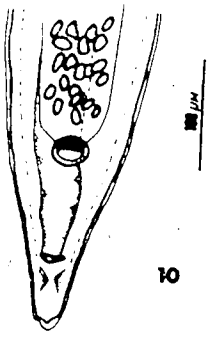
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TABLE I. COMPARATIVE MEASUREMENTS OF ADULT *PARELAPHOSTRONGYLUS ODOCOILEI* (HOBMAIER AND HOBMAIER 1934) FROM VARIOUS SOURCES

Character	Hobmaier and Hobmaier 1934a	Brunetti 1969	This study*
Males:			
Length	35 mm	28.2 (23.8-32.6)**	23 (18-26)**
Width	150	115.5 (108.5-122.5)	147 (138-156)
Esophagus			
Length	670	723 (675-795)	655 (565-717)
Width	---	78.8 (70-87.5)	70 (58-85)
Nerve ring from anterior end	---	90.1 (87.5-92.8)	88 (68-94)
Excretory pore from anterior end	---	77 (70-82.5)	75 (56-94)
Spicule length	144	163.2 (157.5-175)	149 (132-170)
Gubernaculum			
Total length	---	---	98 (73-112)
Corpus length	---	81.4 (75.3-87.5)	86 (65-103)
Corpus width	---	17.5	22 (21-24)
Crura length	---	19.4 (18-21)	24 (21-25)
Crura width	---	8.8	12 (10-14)
Bursa			
Length	84	78.8	89 (80-94)
Width	100	5	108 (103-112)

(Continued)

Table I (Continued)

Character	Hobmaier and Hobmaier 1934a	Brunetti 1969	This study*
Females:			
Length	55 mm	49.5 (44.5-56.2)	44 (39-48)
Width	165	183.3 (154-217)	163 (141-179)
Esophagus			
Length	670	878.5 (788-1050)	627 (588-658)
Width	---	84 (70-96.3)	70 (65-76)
Nerve ring from anterior end	---	85.2 (64.8-103.25)	92 (79-106)
Excretory pore from anterior end	---	88.4 (75-112.5)	78 (71-82)
Vulva from posterior end	144	160.1 (110-191)	178 (161-194)
Anus from posterior end	40	(40-61.25)	48 (44-65)
Length of ovejector	1 mm	1244.4 (1140-1508)	981 (823-1058)

*Worms recovered from experimentally infected mule deer (*O. h. hemionus*) in Alberta.
**Mean followed by the range in parentheses.

is bifurcate. Terminal stems vary from two separate bifid structures to trident appearance. No rays reach margin of bursa.

Spicules complex (Figs. 4, 5), equal and similar, 152-175 long, with proximal swelling (capitulum) and distally twisted into a spoon-shaped formation. Body highly sclerotized and bent medially at the midpoint. Dorsal and ventral pectinate alae present forming a closed tube when viewed in cross section (Fig. 6).

Gubernaculum (Figs. 5, 7) 73-112 long, composed of corpus and paired crura; capitulum absent. Corpus 65-103 long by 17-24 wide, distal portion highly sclerotized and split along 1/3 of its length. Proximal portion granular when viewed from dorsal or ventral aspect, but sclerotized in lateral view. Crura are situated dorsolaterally on distal end of corpus; heavily sclerotized, 18-26 long by 9-14 wide. Crura possess 4-6 bluntly-rounded externolateral projections.

Female: Slender, 39-56 mm long, bluntly tapered at both ends, 141-217 in maximum width. Esophagus muscular 588-1050 long by 65-96 wide. Excretory pore prominent 71-112 from anterior end; nerve ring 65-106 from anterior end.

Vulva 110-194 from posterior end. Ovejector 0.8-1.5 mm long. Anus 40-65 from posterior end; cuticle thickened (Fig. 9). Single pair of sessile papillae located ventrally, midway between the anus and posterior end (Fig. 10).

Hosts: *Odocoileus hemionus columbianus* (Type host).
Odocoileus hemionus californicus
Odocoileus hemionus hemionus

Location: Dorsal skeletal musculature; occasionally in lungs and circulatory system.

Locale: Coast Range, California (Type locality)

Butte County, California

West central Alberta, Canada

First-Stage Larvae (Figs. 11, 14; Table II)

Generally active in Baermann fluid. When inactive, generally exhibit the "C" shape illustrated in Fig. 11. Upon fixation, illustrated position predominates. Gently tapered anteriorly and posteriorly. Maximum width attained at or slightly posterior to esophageal bulb. Buccal capsule heavily sclerotized. Nerve ring located 5/8 to 2/3 length of esophagus from anterior end. Excretory pore prominent, emptying at level of nerve ring. Excretory canal sclerotized. Esophagus expanded anterior to nerve ring and ending in a pronounced esophageal bulb. Intestinal tract granular, terminating in a sclerotized rectum. Genital primordium prominent, located ventrally, 2/3 of body length from anterior end. Tail possesses a prominant hump ventrally, terminating in a sharp point. A prominent spine located dorsally on tail and directed posteriad. Lateral alae present, extending almost entire length of body (Fig. 14).

Third-Stage Larvae (Figs. 12, 13, 15; Table III)

Generally very active in artificial digest, tightly coiled when inactive and assume a characteristic "C" shape upon fixation (Fig. 12). Stout worms, tapering at both ends. Maximum width attained at midbody. Buccal capsule with thick, sclerotized walls (Fig. 13). Esophagus, excretory pore and nerve ring located as in first-stage larvae.

Figure 11. First-stage larvae of *Paretaphoetron quiae adoorlei*.

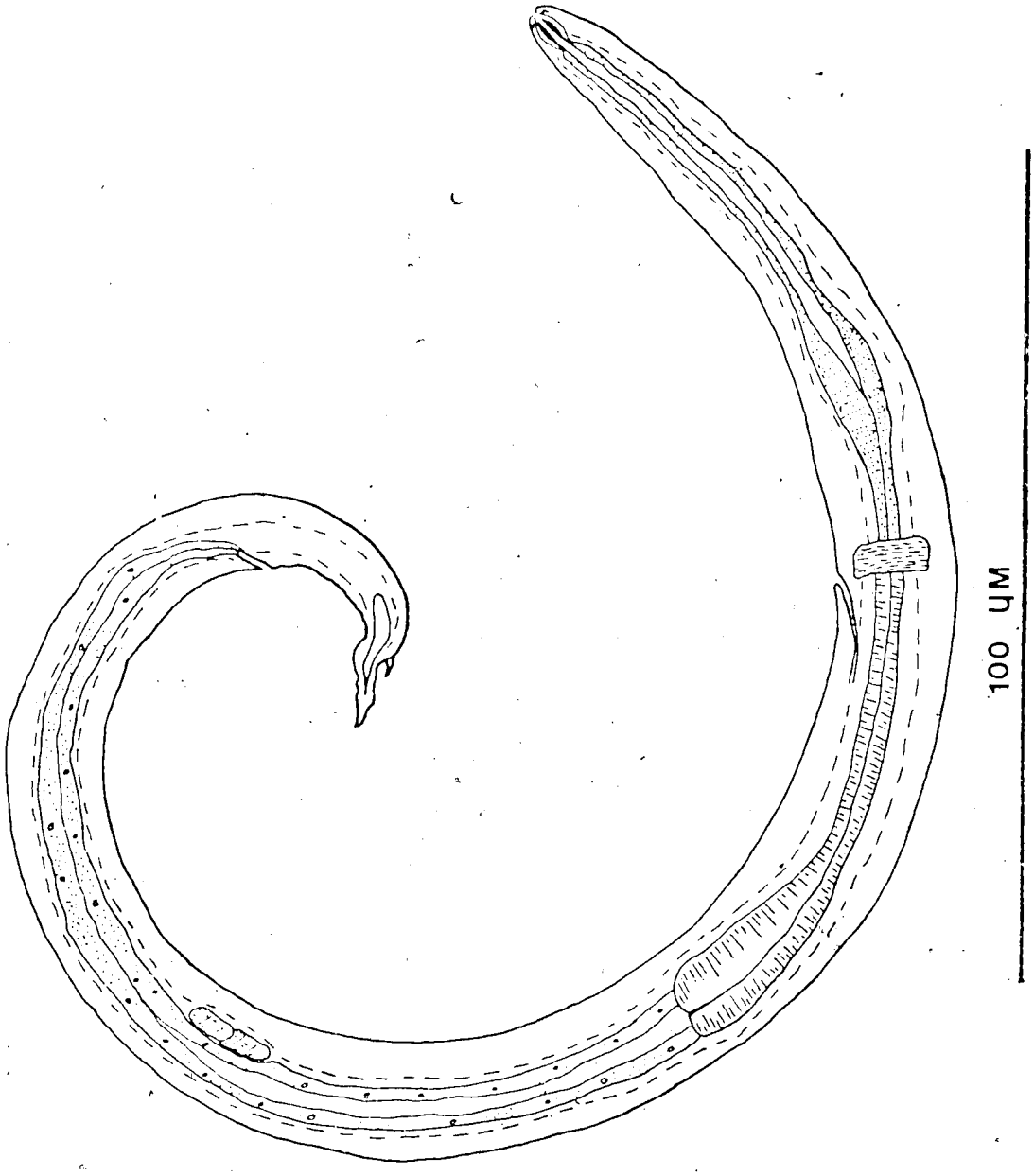

100 µM

TABLE II. COMPARATIVE MEASUREMENTS OF FIRST-STAGE LARVAE OF ELAPHOSTRONGYLINE NEMATODES FROM VARIOUS SOURCES

	P. odocoilei (Hobmaier and Hobmaier 1934a)	*P. odocoilei* (this study)	*P. tenuis* (Anderson 1963)	*P. andersoni* (Prestwood 1972)	*P. depressi* (Panin 1964a)
n =	?	25	10	10	?
Length	378	330 (286-361)*	348 (310-380)*	351 (308-382)*	352-425
Width	17	20 (18-23)	18 (16-19)	17 (17-18)	19-22
Nerve Ring	--	85 (62-106)	94 (80-112)	94 (66-109)	--
Excretory Pore	98	84 (68-98)	94 (80-112)	94 (66-109)	101-117
Esophagus	166	159 (134-179)	165 (132-181)	175 (163-185)	165-190
Genital Primordium	--	212 (178-237)	224 (210-246)	234 (216-249)	--
Anus	40	36 (30-42)	32 (29-41)	32 (27-36)	34-42

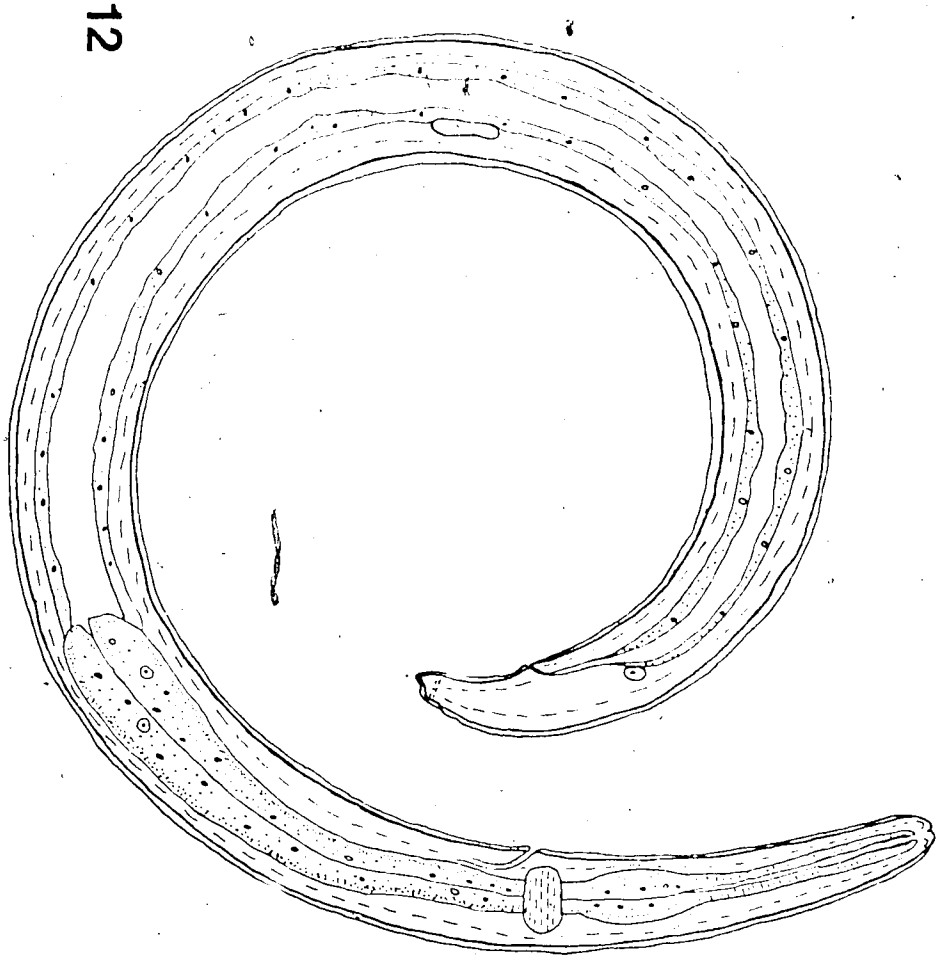
*Mean (Range)

Figures 12 and 13. Infective larvae (L3) of *Parelaphostrongylus odocoilei*.

Fig. 12. Whole mount.

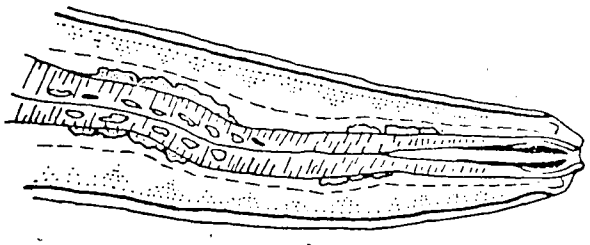
Fig. 13. Anterior end.

12



13

100 µM



50 µM

TABLE III. COMPARATIVE MEASUREMENTS OF THIRD-STAGE LARVAE OF ELAPHOSTRONGYLINE NEMATODES FROM VARIOUS SOURCES

	P. odocoilei		*P. tenuis*	*P. andersoni*	*E. pearsi*
	(Hobmaier and Hobmaier 1954a)	(this study)	(Anderson 1963)	(Prestwood 1972)	(Panin 1964a)
n =	?	25	10	10	?
Length	624	734 (562-855)*	971 (900-1080)	1019 (966-1200)*	910-1100
Width	47	37 (32-43)	42 (36-45)	36 (33-36)	42-50
Nerve Ring	--	101 (89-118)	114 (100-125)	117 (100-133)	98-105
Excretory Pore	100	104 (95-125)	133 (122-149)	147 (100-155)	134-137
Esophagus	218	216 (212-327)	352 (300-400)	358 (300-420)	508-536
Genital Primordium	386	456 (358-558)	629 (569-707)	681 (600-800)	504-527
Anus	40	34 (29-43)	35 (31-45)	35 (33-45)	53-61

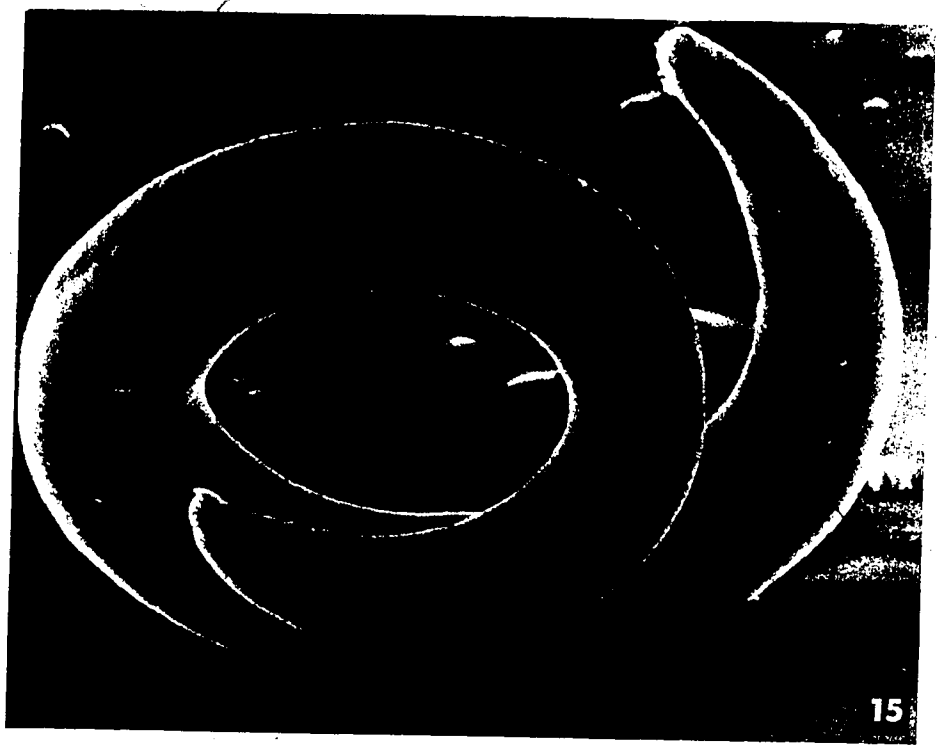
*Mean (Range)

Figures 14 and 15. Scanning electron micrographs of the larvae of *Parelaphostrongylus odocoilei*.

Fig. 14. SEM of first-stage larvae. Note the wide, thin, lateral alae.

Fig. 15. SEM of the infective larvae (L3). Note the broad, rugous alae.


14


15

Esophageal gland nuclei prominent at posterior end of esophagus. Intestinal tract open, devoid of granular material. Genital primordium prominent. Rectum sclerotized. Tail bluntly rounded, with small rudiment of dorsal spine present. Lateral alae present, but of different form from the L1. Alae broad, flat with serrate appearance (Fig. 15).

E. Discussion

The larval stages of *P. odocoilei* are practically unknown. The Hobmaiers (1934a) figured a first stage larva with a dorsal spine and gave measurements for a single specimen. Brunetti (1969) also figured an L1 with a dorsal spine and stated that the "first stage larvae average 367.5 μ in length." Although descriptions of the first stage larvae are incomplete, the measurements do fall within the ranges of the first stage larvae of *P. odocoilei* in this study (Table II).

It would be helpful if the members of the Elaphostrongylinae could be identified at the species level on the basis of larval characteristics. A comparison of the L1's of the subfamily (Table II) is indicative of the similarities of the first stage larvae. Ranges overlap to the extent that separation of species on the basis of metric analysis would be impossible at this time. Examination of non-metric characters, i.e., buccal capsule, esophagus, and shape of the tail, failed to reveal specific differences at the light microscope level.

The infective larvae of *P. odocoilei* have not been described. The Hobmaiers (1934a) provided measurements and a diagram for a pre-infective L3 (Gerichter 1948). The presence of lipid-like granules figured in

their illustration (Hobmaier and Hobmaier 1951a) precludes the possibility of the larvae being infective (see Section VI) and it is therefore not comparable to the infective forms of *P. odocoilei* described from this study.

The infective stage of *P. odocoilei* is slightly smaller in overall body length and esophageal length than *P. tenuis* and *P. andersoni* (Table III). The anus of *P. odocoilei* is located farther from the posterior end than it is in the other species. In view of the reduced specificity of these nematodes at the level of the intermediate host and the possibility of host-induced variation at this level, as demonstrated for other parasitic organisms (Blankespoor 1974), attempts to identify species on the basis of metric analysis of L3's (Prestwood 1972) may be premature. SEM has assisted in verifying the presence of broad, rugous alae in *P. odocoilei* (Fig. 15) similar to those reported for *P. tenuis* by Anderson (1963) and for *P. andersoni* by Prestwood (1972).

Prestwood (1972) also described a small "swelling on the dorsal surface immediately anterior to the apex" of the tail of the L3 of *P. andersoni*. She considered this character a means of separating *P. andersoni* and *P. tenuis*. A similar structure is present on the infective larvae of *P. odocoilei* (Fig. 12). This structure is interpreted as a rudiment of the dorsal spine of the L1. A re-examination of the larvae of *P. tenuis* may reveal the presence of a similar structure.

Ash (1970) defined morphological criteria for the specific identification of the infective larvae of several taxa of rat lungworms of the subfamily Angiostrongylinae (Metastrongyloidea). A similar situation does not exist within the Elaphostrongylinae. Detailed

comparative analysis using SEM, immunologic and/or serologic techniques, however, may provide criteria for subsequent differentiation in the future.

The foregoing description of *P. odocoilei* differs somewhat from the previous redescription (Brunetti 1969). Several morphometric characters (Table , such as the length of the esophagus of the female, are longer in the present material. The majority of measurements are identical or overlap those of previous workers. The differences that do occur are not considered important at the species level. Methods of specimen preparation by the Hobmaiers and Brunetti are unknown and might have affected metric analysis. Comparison of the worms found in the present study with specimens provided by Dr. Brunetti, collected from the type host in California, provided additional support for my contention that these worms are conspecific (Platt and Samuel 1978).

The location of the dorsal ray of *P. odocoilei*, as herein described, has not been noted in previous descriptions of this species (Hobmaier and Hobmaier 1934a; Dougherty 1945; Brunetti 1969). I feel this is more a problem of interpretation than an actual morphological deviation. Hobmaier and Hobmaier (1934a) referred to the dorsal ray as "[the dorsal ray] is divided in 2 stems and each stem in 2 branches [that] are equal in length, but the internal branch is more slender than the external branch." Dougherty (1945) confirmed the unusual split nature of the dorsal ray in *P. odocoilei*. Brunetti (1969) more accurately described the actual situation, stating, "the dorsal ray is mound-like and variable in structure." As I have demonstrated (Fig. 8), the dorsal ray is a cushion-like structure similar to that of *P. tenuis* as figured by

Dougherty (1945) and *P. andersoni* as described by Prestwood (1972). The primary difference is that the mound in *P. odocoilei* is situated in a more dorsal position than in *P. tenuis* or *P. andersoni*, leaving only the branches and stems visible in a ventral perspective in the specimens examined by the Hobmaiers (1934a). Careful examination of the dorsal surface revealed that the dorsal ray is located slightly dorsad on the worm and the highly variable branches and stems (Brunetti 1969; this study Fig. 19) are terminal appendages of that structure.

Comparison of *P. odocoilei* and the other species of the genus must be based upon the accessory reproductive structures of the male. This is because the larvae cannot be reliably identified, the females, as described to date, show no consistent morphological differences, and the extreme overlap in the majority of measurements precludes their usefulness for taxonomic purposes.

Anderson (1956) initially identified specimens of *P. tenuis* from the CNS of white-tailed deer in Ontario as *Elaphostrongylus (= Parelaphostrongylus) odocoilei* on the basis of the similarity of the male reproductive structures. There is no question that the three species that constitute the genus *Parelaphostrongylus* are closely allied forms. At the present time, however, males of these species, although similar in general appearance, are readily identifiable. A comparative examination of the male reproductive structures is, therefore, deemed warranted.

Spicules of the three species are distinct. Dougherty (1945) described the spicules of *P. tenuis* as "0.195 mm long with two strongly developed striated alae and a longitudinal slit in the first part of the distal half of the lamina." The description given by Anderson (1956)

confirmed Dougherty's observations, with the exception that no mention was made of the longitudinal slit in the distal lamina. Whitlock (1959) dismissed the presence of a split as an optical illusion. I have examined specimens of *P. tenuis* and confirm the presence of a split, or preferably a foramen (Fig. 16a) in the distal half of the spicule. The spicules of *P. tenuis* also lack a capitulum, set off from the body by a well defined proximal constriction. The spicules of *P. odocoilei* (Figs. 4, 5) possess a well defined proximal constriction and lack a foramen in the lamina. The spicules of *P. anderson[illegible]* are knob-like proximally, lack a foramen and are bifurcate at the distal tip (Fig. 16b).

The gubernaculum of *P. odocoilei* is distinctive in that the corpus is split along the proximal 1/3 of the shaft (Fig. 5). This species also possesses large, paired crura. The corpus of the gubernaculum of *P. tenuis* is similar in shape to that of *P. odocoilei*, although somewhat smaller (Fig. 17a). The corpus is not divided (Dougherty 1945; Anderson 1956). The crura are paired and possess 4 to 6 knob-like projections, as does *P. odocoilei*. The gubernaculum of *P. andersoni* is distinct (Prestwood 1972) in that the corpus is small, triangular, and not divided (Fig. 17b). The crura are extremely small.

The dorsal ray of *P. odocoilei* is variable, but of a basically different form from that of the other two species. The base of the ray is broad and located in an extreme dorsal position (Fig. 8). The ray gives rise to two terminal projections, or stems. The stems may bifurcate or not, giving rise to a number of different forms (Fig. 19). The stems are similar in size. The dorsal ray of *P. tenuis* is also variable (Anderson 1956). The extremely broad base of this ray is more ventral

Figures 16-18. Male reproductive structure of *Parelaphostrongylus* spp.

Fig. 16. Spicules of *Parelaphostrongylus* spp.

a. *P. tenuis* (from Dougherty 1945)

b. *P. andersoni* (from Prestwood 1972)

Fig. 17. Gubernacula of *Parelaphostrongylus* spp.

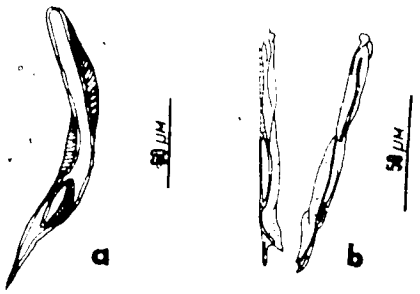
a. *P. tenuis* (from Dougherty 1945)

b. *P. andersoni* (from Prestwood 1972)

Fig. 18. Bursa of *Parelaphostrongylus* spp.

a. *P. tenuis* (from Dougherty 1945)

b. *P. andersoni* (from Prestwood 1972)



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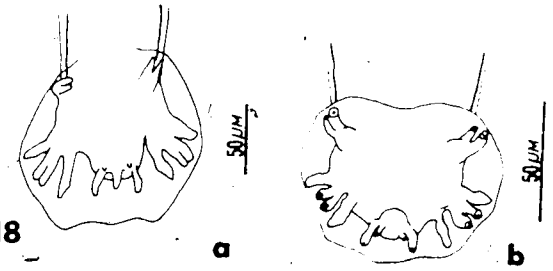

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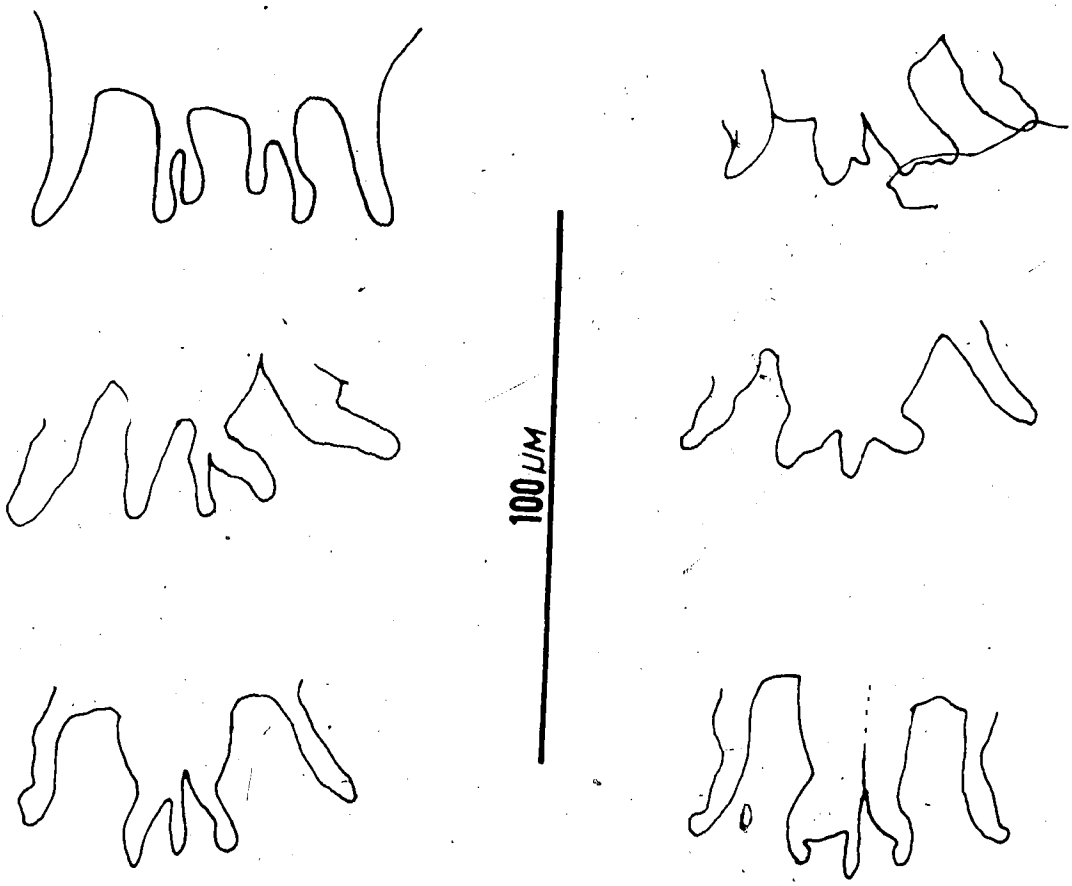
Figure 19. Variability of the dorsal ray of *Parelaphostrongylus odocoilei* from experimentally infected mule deer (*Odocoileus h. hemionus*).



19

in position than that of *P. odocoilei*. The base then gives rise to a variable number (2 to 6) of ventral projections (Fig. 18a). The dorsal ray of *P. andersoni* (Fig. 18b) is similar to that of *P. tenuis* but has four (2 large and 2 small) ventral projections from the base. The extent of variability of the dorsal ray of *P. andersoni* has not been reported.

F. Key to the Males of *Parelaphostrongylus*

1(2) Gubernaculum reduced, less than 52 in total length; corpus undivided. Crura chitinized, lacking large, lateral projections. Spicules divided distally to form a bifid tip. Parasites of the dorsal musculature of *O. virginianus* ..... *P. andersoni*.

2(1) Gubernaculum large, greater than 70 in total length, corpus divided or undivided. Crura lateral to corpus, possessing 4 to 6 lateral projections. Spicules not divided at the distal tip ..... 2.

3(4) Corpus of the gubernaculum undivided. Spicules with a for in the mid-region of the shaft. Parasites in the CNS of cervids ..... *P. tenuis*.

4(3) Corpus of gubernaculum divided along the proximal 1/3. Spicules lacking a foramen. Parasites of the dorsal musculature of the mule deer, *O. hemionus* subspp. ..... *P. odocoilei*.

SECTION III

EXPERIMENTAL LIFE CYCLE IN CERVIDS

A. Introduction

Parasitic organisms are continually faced with the problem of transferring infective forms from host to host. If the life cycle is heteroxenous, a second host species, usually in a different phylum, is incorporated as an intermediate host. The life cycle of the elaphostrongyline nematodes involves a cervid definitive host and a terrestrial mollusc as the intermediate host.

Basic aspects of the life cycle of the elaphostrongylines, such as the length of the prepatent period, duration of patency and the dynamics of larval production are poorly known for the majority of the members of the subfamily. In some instances data published in the literature are contradictory.

The meningeal worm, *Parelaphostrongylus tenuis*, is the most studied member of the group. Anderson (1963) completed the life cycle of this species in the normal definitive host, white-tailed deer, and established a prepatent period of 83 to 91 days for *P. tenuis* in that host species (Anderson 1965). The duration of patency and a quantitative assessment of larval production have not been documented for the meningeal worm.

The aforementioned aspects of the life cycle of *P. andersoni* have been well documented. The prepatent period of *P. andersoni* in the white-tailed deer is 58 to 67 days (Prestwood 1972; Nettles and Prestwood 1976). Although the duration of patency has not been firmly established for this species, larval production has been monitored for over one year in at least a single animal. Experimental infections of cervids

other than white-tailed deer, with *P. odocoilei*, have not been attempted. Nettles *et al.* (1977) have speculated that this species can infect a whitetail x blacktail cross.

The life cycle of *P. odocoilei* has been completed experimentally in the California mule deer (*Odocoileus hemionus californicus*) by Hobmaier and Hobmaier (1934a) and Brunetti (1969). Hobmaier and Hobmaier (1934a) reported a prepatent period of 4 to 5 months, while Brunetti (1969) reported approximately 2-1/2 months. Although neither of these workers quantitatively assessed larval production, Brunetti (1969) determined the patent period of *P. odocoilei* to be 6 months. The course of infection of *P. odocoilei* in other cervids is unknown.

The objectives of the first portion of the current study were to determine the length of the prepatent period, duration of patency and to quantitatively document larval production of *P. odocoilei* in the mule deer, *O. h. hemionus*. The second objective was to determine the ability of *P. odocoilei* to establish a patent infection in selected cervids and the effect of an alternate host on the prepatent period, duration of patency and larval production.

B. Materials and Methods

The following taxa of cervids were used in this study: mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*O. h. columbianus*), white-tailed deer (*O. virginianus dacotensis*) and moose (*Alces alces andersoni*). Most animals were obtained as neonatal fawns from field personnel of the Alberta Fish and Wildlife Division and the Fish and Game Branch of British Columbia. Dr. R.M.F.S. Sadleir of Simon Fraser

University, British Columbia, kindly provided black-tailed deer fawns from a captive herd maintained at that institution. A complete list of animals infected, with background information, is given in Appendix I.

All animals were raised and maintained in conditions free of elaphostrongylines at the University of Alberta Vivarium, Ellerslie, Alberta. The animals were maintained indoors with limited access to an outside pen until weaning. After weaning, animals that had not received infective larvae were placed in outdoor pens (0.14 hectares) that had not been used previously by ruminants. Fawns were bottle fed for approximately 3 months, until weaning. After weaning they received deer pellets (Northwest Feeds, Edmonton, Alberta), hay and grain. Moose were given similar foods, but also received aspen leaves and branches and other material as browse. Fecal examinations were performed on each animal prior to experimentation. All were negative for lungworm infections.

Infective larvae of *P. odoco[illegible]i*, used in all trials with the exception of MD 4, were obtained from experimentally infected *Triodopsis multilineata* (Say) (Pulmonata:Stylommatophora). First stage larvae (L1) used to infect *T. multilineata* were obtained from two sources. Mule deer 1, 2 and 3 received L3's originating from a naturally infected mule deer buck from Jasper, Alberta. The remaining infections were derived from L1's isolated from MD 1. MD 4 received 6 and 8 infective larvae on consecutive days, obtained from two naturally infected *Euconulus fulvus* (Pulmonata:Stylommatophora), collected in Jasper, Alberta.

Infective larvae were obtained by artificially digesting *T. multilineata* in a standard digestion medium (0.6 gm pepsin and 0.7 ml HCl/100 ml distilled water). Snails were removed from the shell, minced with a fine scissors and placed in a test tube with approximately 10 ml of digestion fluid. The test tubes were then incubated in a water bath at 37°C ± 1°C for three hours. The test tubes were centrifuged at 1500 RPM for 4 minute Two to three ml of supernatant and the cellular plug were retained for examination. L3's were counted and placed in physiological saline prior to infecting deer. Deer were infected before weaning with L3's in a small quantity of milk. This technique was used for MD 1, 3, 4 and 7; BT 1 , 18 and 22; WTD 17 and 18; and Moose 9. The remaining animals were infected after weaning using a slightly different technique. Larvae, in saline, were placed in a 20 ml syringe. The animal was restrained and the syringe was slowly emptied into the rear of the mouth while the animal was forced to "drink". A partial syringe of saline was then administered to ensure removal of all larvae. All animals were less than 6 months of age when infected. Infected animals were held outdoors until 30 days postexposure (PE). Thereafter, they were held indoors, in individual pens with cement floors.

Fecal examinations were initiated approximately 40 days PE and continued on ily basis for 1 to 2 months after patency (PP), then weekly until the animal died or was killed.

Fecal examinations were done using the Baermann technique, following the suggestions of Todd *et al.* (1970). Fecal material was collected fresh and generally examined the following day. If examination was delayed, feces were stored in plastic bags and refrigerated at 4°C.

Fecal pellets were weighed to the nearest 0.1 gm and placed on a double layer of coarse cheesecloth. The cheesecloth was placed on a platform of hardware cloth in a 25.4 cm diameter funnel. A standard amount of tap water, 650 ml, sufficient to cover the feces, was then added. The suspension was allowed to stand a minimum of 8 hours, but more commonly overnight (= 12 hours).

Two methods were employed for the examination of the Baermann fluid. The first, and most commonly used, included drawing off 100 ml of fluid through the clamped rubber hose at the bottom of the funnel into a 150 ml beaker. The fluid was bubbled and mixed in an "X" motion to assure an even distribution of larvae in solution. A 5 ml aliquot was removed with a pipette, placed in a counting chamber and allowed to stand for several minutes. Larvae were counted and the process repeated. If there was a difference of 10% or less between the two counts, they were averaged and the mean was used to calculate the number of larvae per gram of feces. If the counts differed by more than 10% a third aliquot was taken and the mean of all three readings was used in subsequent calculations. In heavy infections the procedure was modified so that only a 1 ml sample was used.

A second, more sensitive, method was a modification of the first. One hundred ml of Baermann fluid were divided between two 50 ml centrifuge tubes. The tubes were centrifuged at 1500 RPM for 6 to 8 minutes. The top 45 ml of supernatant were discarded and the remaining 5 ml were examined for larvae as previously described. This method was employed prior to and in the early stages of patency. In addition, it was used if the first method failed to detect larvae in an animal that

was positive prior to that time. Results are expressed as the number of larvae/gram of feces (wet weight) or LPG.

On a single occasion, 17 days PP, the larval production of MD 3 was monitored over a 24 hour period. All fecal deposits were collected individually during that period, weighed and the time of deposition recorded. The feces were frozen and examined as described above.

C. Results

Mule Deer

Duration of the prepatent period varied from 49 to 52 days in five deer fawns that received from 100 to 330 infective larvae (Table IV). Nettles and Prestwood (1976) considered that 300 L3's of *P. odocoeni* in white-tailed deer was a "moderate dose."

Larval production in these animals increased logarithmically for the first three to four weeks of patency (Fig. 20). MD 3 was killed one month PP, following the log phase of larval production, and was examined for adult worms. Peak larval production in MD 3 (11 096 LPG) occurred 25 days after the initiation of patency. MD 1 had a peak larval production of 14 580 LPG, 35 days PP. MD 7 and 8 had peak larval outputs of 13 250 and 950 LPG, at 30 and 19 days PP, respectively. Larval production in MD 8 fell off sharply from four to six weeks of patency but recovered to previous levels during the seventh week of infection (Fig. 20). MD 2 died on the first day of patency, following several days of anorexia, listlessness and scouring, accompanied by severe weight loss. MD 1 exhibited similar symptoms prior to being killed.

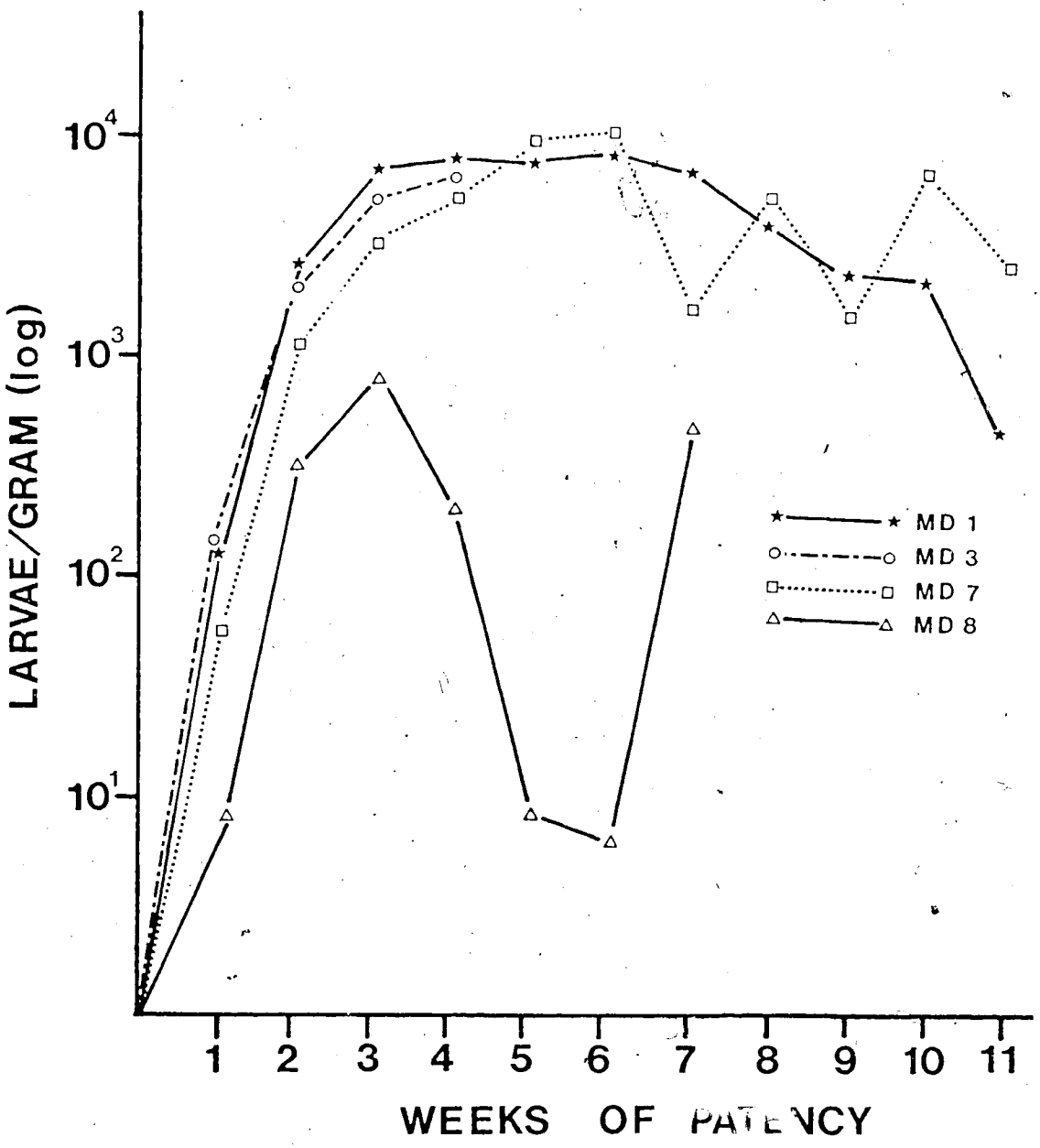
TABLE IV. RESULTS OF THE EXPERIMENTAL INFECTIONS OF CERVIDS WITH *PARELAPHOSTRONGYLUS ODOCOILEI*

	Deer Number	Size of Infective Inoculum	Length of Prepatent Period*	Maximum LPG*	Time of Patency of Maximum LPG
Mule Deer (8):	4	14	62	720	25 weeks
	6	25	54	713	17 days
	5	50	55	2260	10 weeks
	8	100	51	955	19 days
	7	175	51	13250	30 days
	2	318	50	-	--
	1	322	52	14580	34 days
	3	334	49	11096	25 days
Black-tailed Deer (6):	2	50	62	464	20 days
	7	50	72	113	10 days
	230	50	59	667	17 days
	18	300	58	235	12 days
	15	500	68	5	6 weeks
	22	500	60	235	21 days
Moose (2):	9	200	68	4	10 weeks
	1	750	72	11	15 weeks
White-tailed Deer (5):	14	50	--	--	--
	15	50	--	--	--
	17	60	--	--	--
	5	332	--	--	--
	18	500	--	--	--

*LPG - Larvae per gram of feces, wet weight.

ays

Figure 20. Numbers of larvae recovered and the duration of patency for mule deer experimentally infected with "moderate" numbers of *Parelaphostrongylus odocoilei*.


LARVAE/GRAM (log)
10^4
10^3
10^2
10^1
1 2 3 4 5 6 7 8 9 10 11
WEEKS OF PATENCY
MD 1
MD 3
MD 7
MD 8

The length of the prepatent period varied from 54 to 62 days in three mule deer fawns that received from 14 to 50 L3's (Table IV). Larval production increased logarithmically for the first three to five weeks of patency (Fig. 21). Peak production, however, was considerably below the levels of moderately infected animals. Larval production in MD 4, which received 14 L3's, remained below that of MD 5, which received 50 L3's, until 35 weeks PP. Both animals are producing moderate numbers of larvae one year after the initial infection. Peak larval production (720 LPG for MD 4 and 2260 LPG for MD 5) occurred 24 and 10 weeks into the patent period, respectively. MD 6, which received 25 L3's as an infective inoculum, had values of larval output intermediate (715 LPG at 17 days PP) to MD 4 and 5 during the first seven weeks of patency.

MD 3 defecated 26 times in a 24 hour period, totaling 949 grams of feces. Larval output was erratic (Fig. 22) ranging from a minimum of 1274 LPG at 4:25 hrs, to a maximum of 5340 LPG at 22:35 hrs, approximately a 4-fold difference. Analysis of larval output using moving averages suggests a decline in output from 00:00 to 06:00 hrs. Mean output was 36.1 gm/defecation, with an average of 3442 LPG. Larval production during the 24 hour period was estimated at 3 220 000.

Black-tailed Deer

The prepatent period of *P. odocoilei* in *O. h. columbianus* ranged from 58 to 72 days in six black-tailed deer that received from 50 to 500 infective larvae (Table IV). Larval production in blacktails increased logarithmically for the first two to three weeks of patency and then declined sharply from four to seven weeks (Figs. 23, 24). BT 7, 18 and 23D dropped to less than 1 LPG by four to seven weeks PP, with occasional

Figure 21. Number of larvae recovered and the duration of patency for mule deer experimentally infected with "low" numbers (50 or less) of *Parelaphostrongylus odocoilei*.

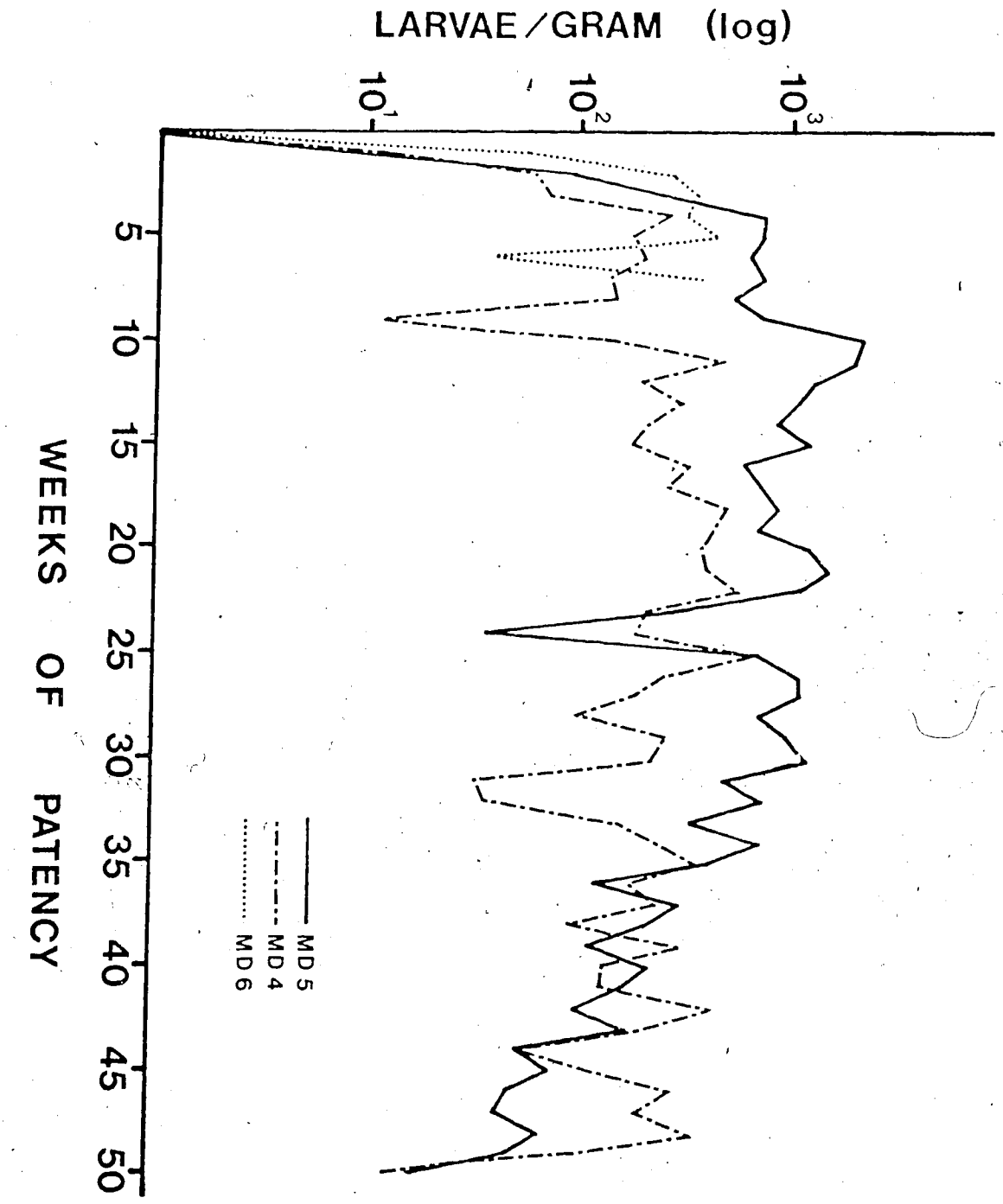

LARVAE/GRAM (log)
10^1
10^2
10^3
5
10
15
20
25
30
35
40
45
50
WEEKS OF PATENCY
MD5
MD4
MD6

Figure 22. Numbers of larvae (L1) recovered from MD 3 in a single 24 hour period (15-16 December 1975). Upper figure is a 3-point moving average analysis of larval production in the lower figure.

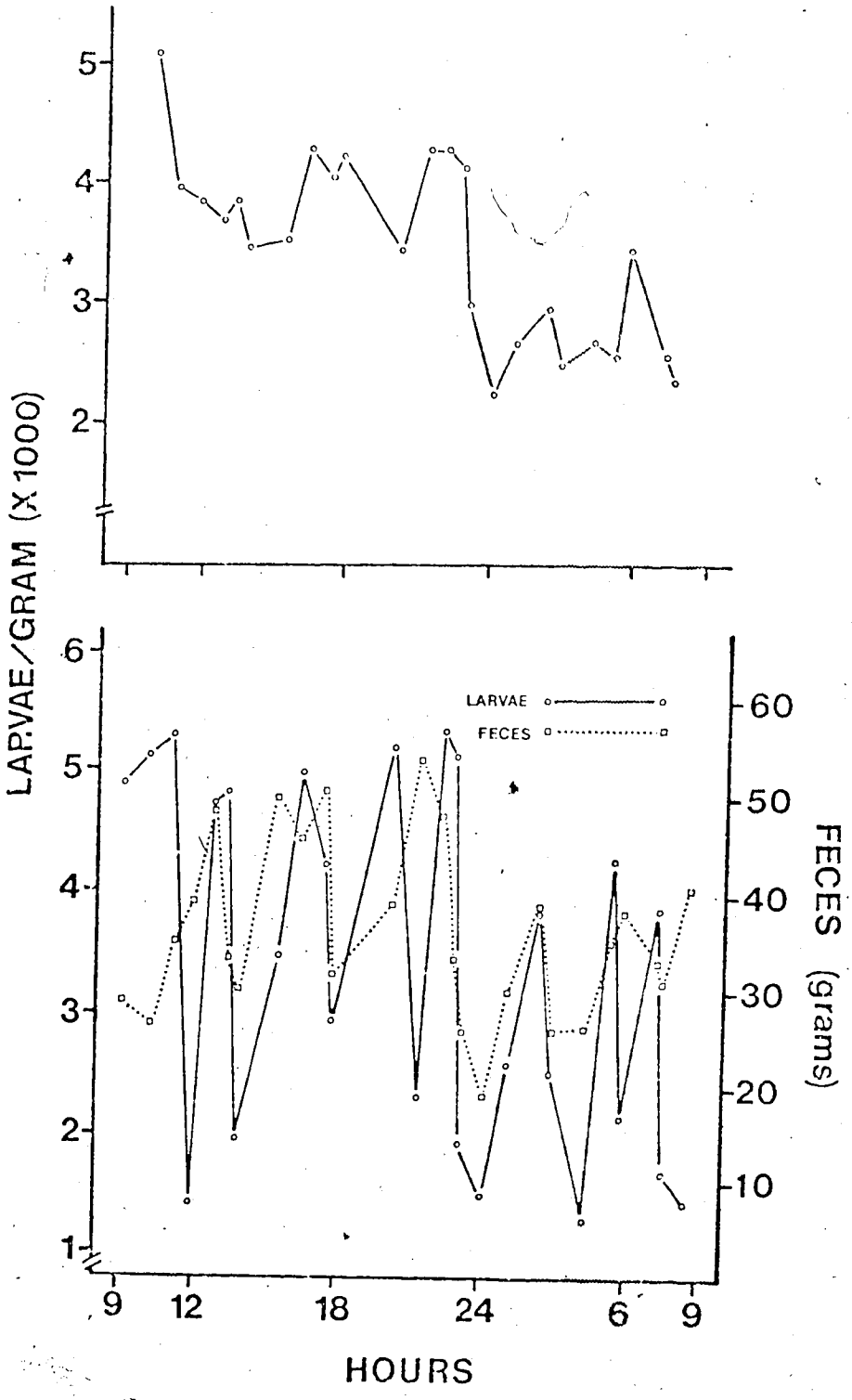

LARVAE/GRAM (X 1000)
5
4
3
2
6
5
4
3
2
1
LARVAE
FECES
60
50
40
30
20
10
FECES (grams)
9
12
18
24
6
9
HOURS

Figure 23. Numbers of larvae recovered and the duration of patency for black-tailed deer 7, 18 and 230, experimentally infected with *Parelaphostrongylus odocoilei*.

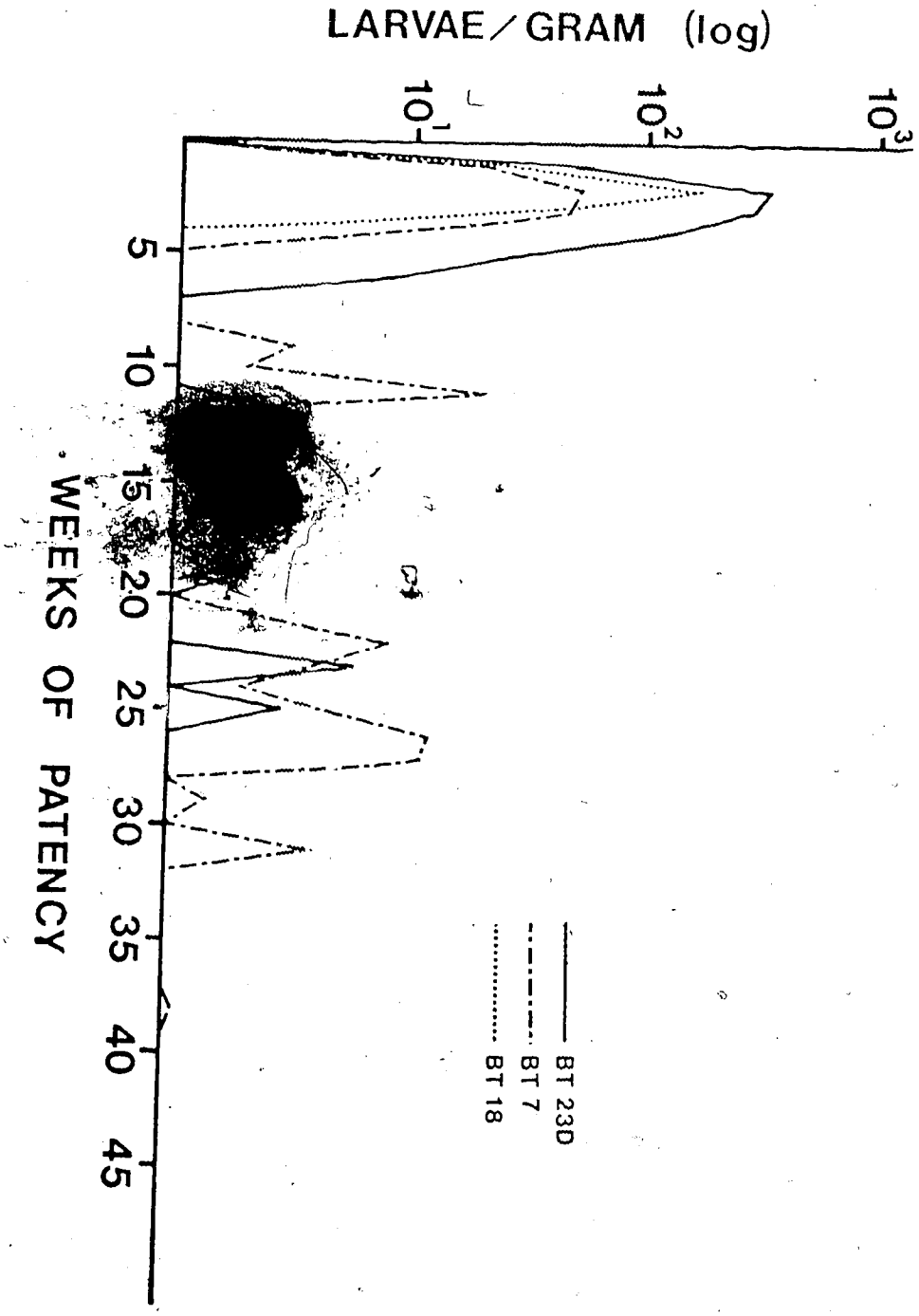
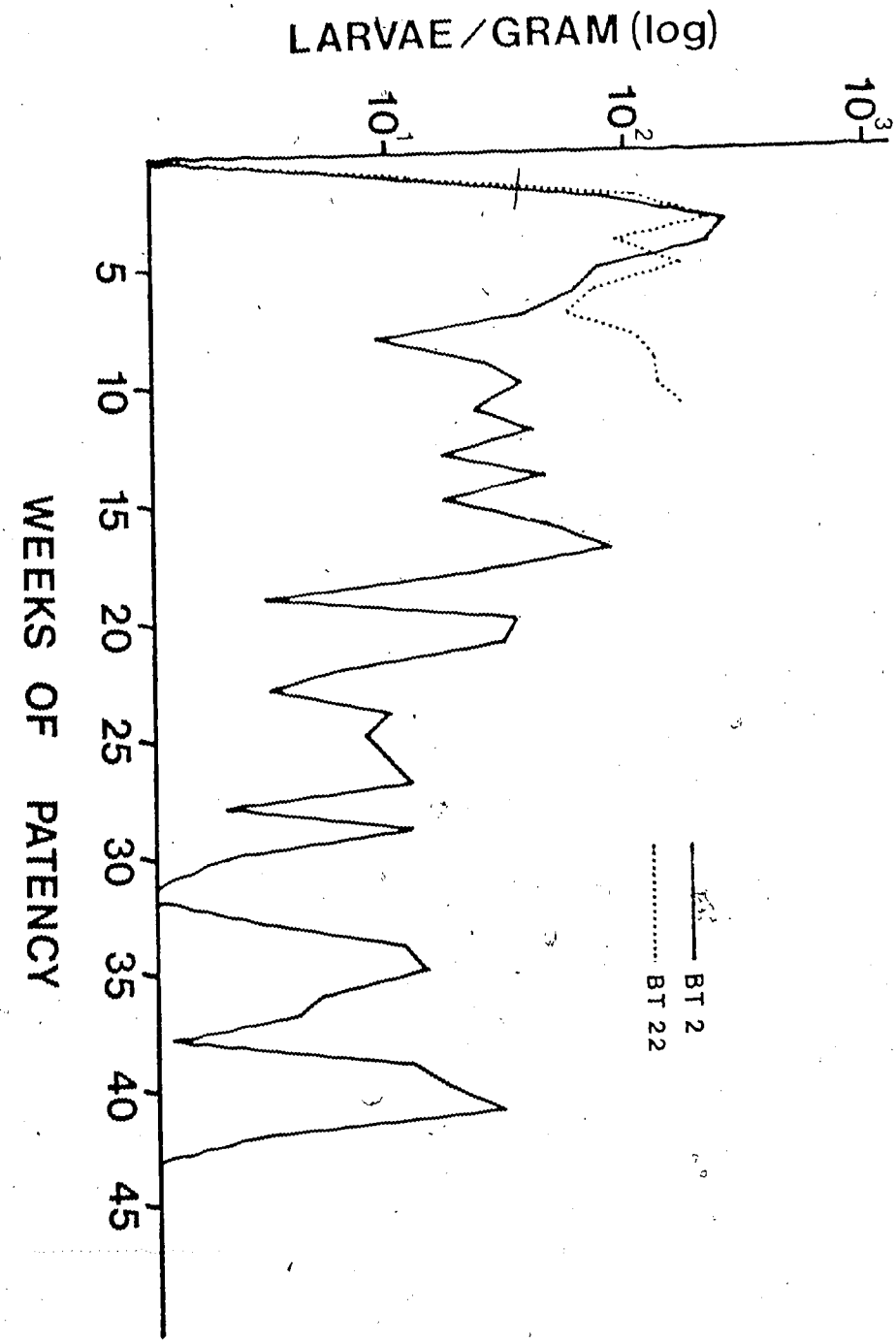

LARVAE / GRAM (log)
10^{1}
10^{2}
10^{3}
5
10
15
20
25
30
35
40
45
WEEKS OF PATENCY
BT 23D
BT 7
BT 18

Figure 24. Numbers of larvae recovered and the duration of patency for black-tailed deer 2 and 22, experimentally infected with *Parelaphostrongylus odocoilei*.


LARVAE / GRAM (log)
10^1
10^2
10^3
5
10
15
20
25
30
35
40
45
WEEKS OF PATENCY
BT 2
BT 22

recurrences of larval production interspersed with periods of reduced larval output (Fig. 23). BT 2 and 22, although having drops in larval output at approximately one month PP, maintained a relatively high, though erratic, level of larval production for the duration of patency. Peak larval output for BT 7, 18 and 23D was 115, 255 and 667 LPG at 10, 12 and 17 days PP, respectively. Peak output for BT 2 and 22 was 164 and 235 LPG at 20 and 21 days PP. BT 15 represents an anomaly. Larval output was very low (< 1 LPG) during the majority of the patent period and never rose above 5 LPG during more than two months of patency.

Moose

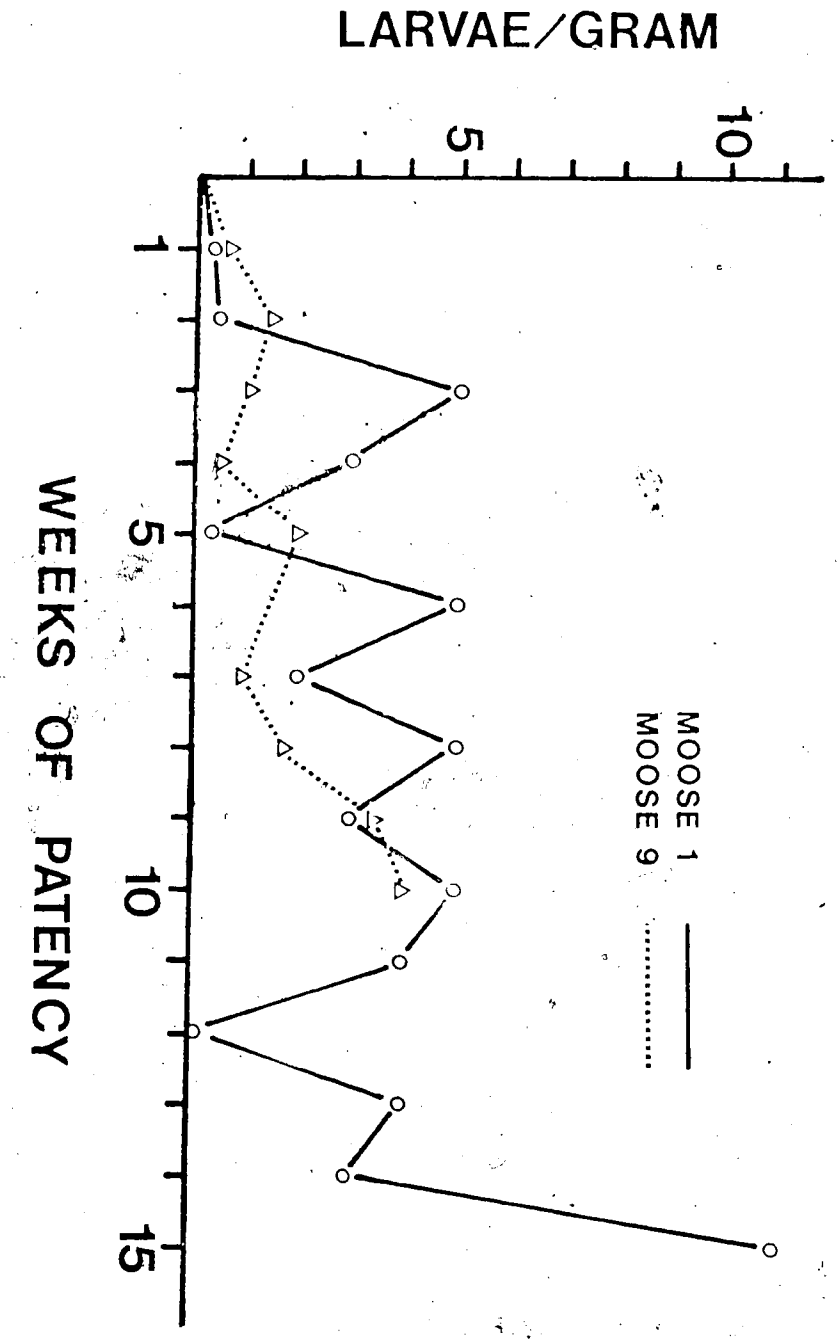
The prepatent period of *P. odocoilei* in the two moose infected in this study was 68 and 72 days. Larval output in Moose 1 was low initially (< 1 LPG) and fluctuated irregularly until the animal was killed 15 weeks PP. Larval production in Moose 9 was also low (Fig. 25) but was not as erratic as Moose 1. Peak larval output was 15 and 4 LPG for Moose 1 and 9 at 15 and 10 weeks PP, respectively.

At necropsy a number of green, caseous lesions, similar to those described by Nettles and Prestwood (1976) in white-tailed deer heavily infected with *P. andersoni*, were observed in the *longissimus dorsi* of Moose 1. A single, intact, male *P. odocoilei* was removed from one of these lesions. Similar lesions were not observed in any of the other animals examined.

White-tailed Deer

Five white-tailed deer were given from 50 to 500 infective larvae of *P. odocoilei*. None of these trials resulted in a patent infection

Figure 25. Numbers of larvae recovered and the duration of patency for moose 1 and 9, experimentally infected with *Parelaphostrongylus odocoilei*.


LARVAE/GRAM
5
10
1
5
10
15
WEEKS OF PATENCY
MOOSE 1
MOOSE 9

(Table IV). Fecal examinations were continued as long as 6 months past the initial exposure.

D. Discussion

The mean prepatent period, 63 days for *P. odocoilei* in *O. h. columbianus*, confirms in part the previous report of a prepatent period of approximately 2-1/2 months for this worm in the same host (Brunetti 1969). A prepatent period of 4 to 5 months for *P. odocoilei* in black-tailed deer (Hobmaier and Hobmaier 1934a) must be viewed with some suspicion.

The mean prepatent period of *P. odocoilei* in *O. h. hemionus* ($\bar{X}$ = 53 days) is significantly shorter than in black-tailed deer ($P < .05$; $t_{.05}[7] = 3.499$; $t_s = 7.919$) and indicates that the mule deer is the preferred host of this parasite. It may be argued that this reflects strain differences as the worms used in these trials were of mule deer origin. Experimental infections of *O. h. columbianus* with *P. odocoilei* of blacktail origin (Brunetti 1969) and infective larvae of blacktail origin (Vancouver Island) assumed to be those of *P. odocoilei*, however, have resulted in extended prepatent periods (Samuel unpubl.).

Peak larval output, as a measure of fecundity, was higher in mule deer that received identical or lower numbers of infective larvae (MD 4 and 5; Fig. 21) than Columbian black-tailed deer. Although it is difficult to evaluate differences in the duration of larval output between the two subspecies of host, the intensity of sustained larval production was low in *O. h. columbianus*, occasionally dropping to zero (Fig. 23). It is generally recognized that parasitic organisms have a

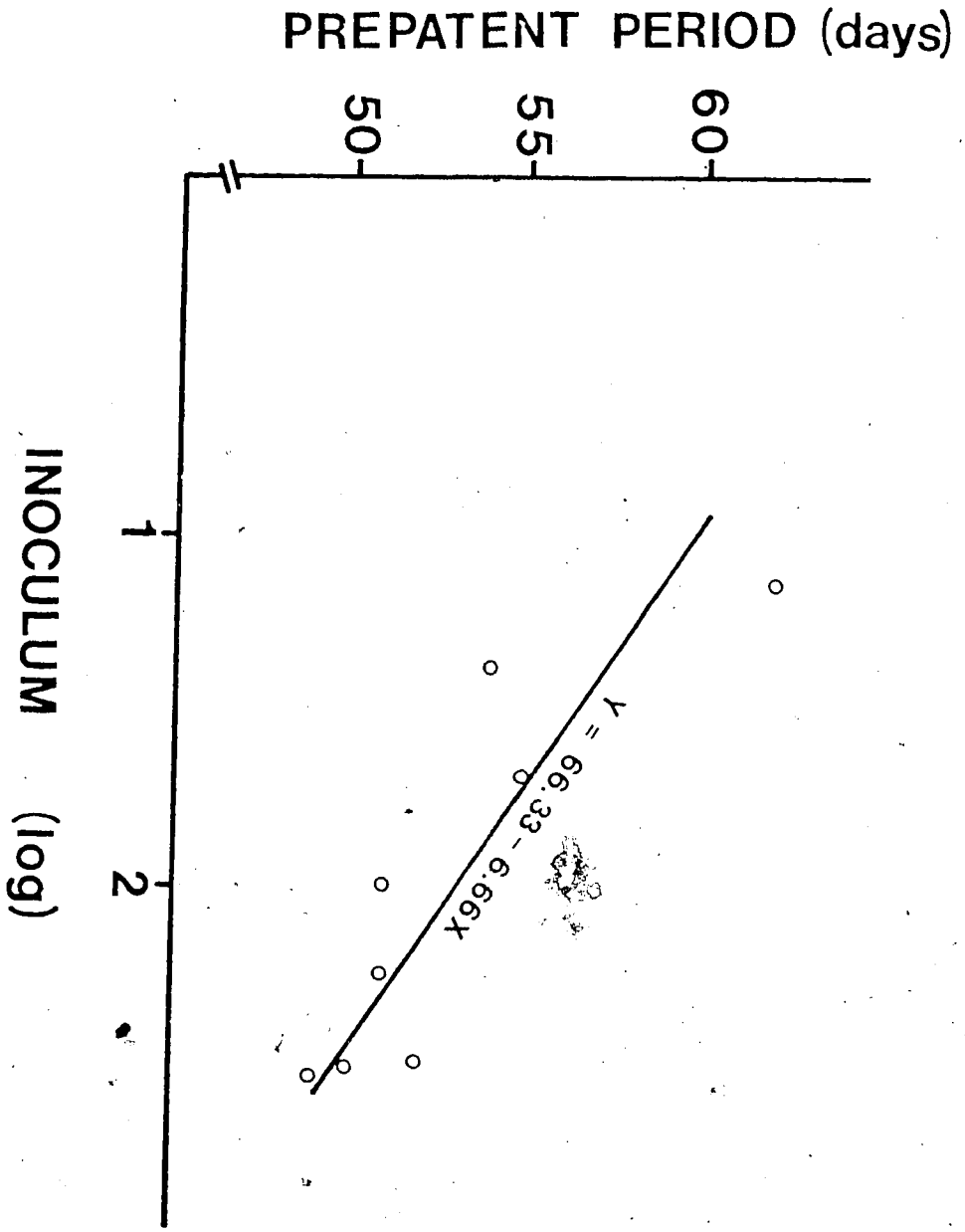
shorter prepatent period and higher reproductive potential in the preferred host (Croll 1973). Increased length of prepatency, reduced larval production, both peak and sustained, of *P. odocoilei* in *O. h. columbianus* and *Alces alces* indicates a reduction in host-parasite compatibility and points to *O. h. hemionus* as the primary definitive host of this parasite.

The prepatent period of elaphostrongyline nematodes appears to vary within relatively narrow limits. Anderson (1965) reported a prepatent period for *P. tenuis* in white-tailed deer of 83 to 92 days. Nettles and Prestwood (1976) established a period of 56 to 67 days for *P. andersoni*, also in white-tailed deer. The prepatent period of *P. odocoilei*, as indicated above, shows a similar variation.

The prepatent period of *P. odocoilei* in mule deer shows a significant negative relationship with the size of the infective inoculum (Fig. 26). This is indicative of a logistics problem encountered by dioecious parasites at low densities (Kennedy 1976). An increased search period between males and females would logically result in a prolonged prepatent period. A threshold of approximately 100 infective larvae is necessary to obtain the minimum prepatent period (Table IV).

No comparison can be made of this phenomenon with other elaphostrongylines because the number of infective larvae administered in other studies is unknown (Anderson 1963) or the inocula are uniformly high (Nettles and Prestwood 1976). The variation in the prepatent period of other species must be attributed to differences in larval viability and/or differential establishment rates in an individual host. A similar

Figure 26. Regression analysis of the inoculum size versus the duration of the prepatent period in mule deer experimentally infected with *Parelaphostrongylus odocoilei*.


PREPATENT PERIOD (days)
50
55
60
INOCULUM (log)
1
2
Y = 66.33 - 6.66X

situation was not observed for *P. odocoilei* in black-tail deer ($P > 0.5$, $F_s = 0.14$).

The prepatent period of *P. odocoilei* is similar to that of *P. andersoni* in white-tailed deer. The prepatent periods of the other elaphostrongylines, that are known to include a migration in the CNS: *P. tenuis*, 83-92 days (Anderson 1965) and *E. cervi*, 3 to 5 months (Mitskivich 1960, 1964), are considerably longer. The migratory routes of the muscleworms (*P. odocoilei* and *P. andersoni*) in their respective definitive hosts are unknown and at this time there is no reason to postulate a sojourn in the CNS for these species. None of the cervids experimentally infected with *P. odocoilei* in this study displayed symptoms characteristic of CNS involvement as described by Anderson (1964, 1965, 1971b) for cervids infected with *P. tenuis*. The longer prepatent period of *P. tenuis* and *E. cervi* may be the result of the CNS migration, which has either been lost or never acquired in the evolution of the muscleworm species.

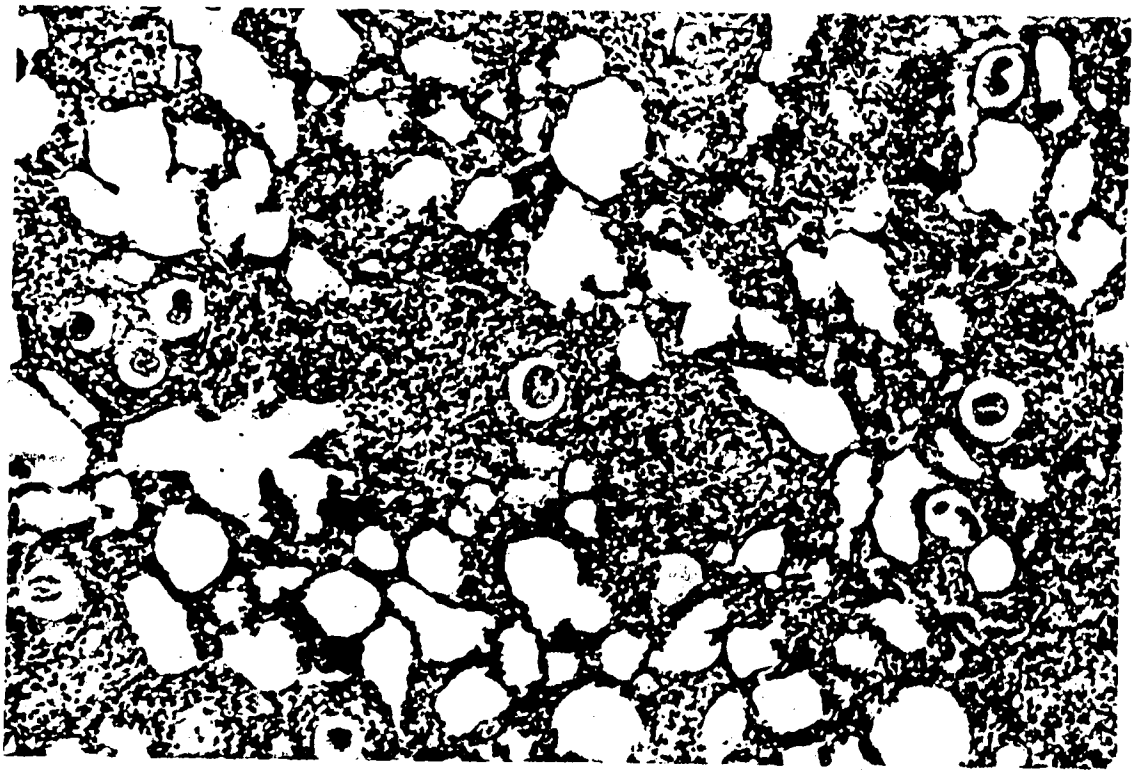
Larval production by *P. odocoilei* in mule deer is similar in many respects to that recorded for *P. andersoni* in white-tailed deer (Nettles and Prestwood 1976). Larval output for both species shows a logarithmic increase, followed by a plateau phase and an irregular, but slow, decline of long duration (i.e. > 1 year). A similar situation was described (Panin 1964b) for *Elaphostrongylus panticola (= E. cervi panticola)* in the maral deer (*Cervus elaphus maral*). The initial rise in larval production was not as rapid in that species as has been documented for *Parelaphostrongylus* spp.; however, the patent period appeared to be of similar duration. This prolonged period of larval production, a common

characteristic of protostrongylid nematodes (Rose 1959; Kassai 1962), is a K-selected feature in organisms generally considered to be r-selected (see Esch *et al.* 1977 for a review). Prolonged larval production in parasitic organisms has recently been interpreted as a natural response to an extremely favorable and stable habitat (Jennings and Calow 1975) rather than a response to the "perils of parasitism."

Peak larval output of *P. odocoilei* in mule deer that received approximately 300 L3's was six times higher than *P. andersoni* or *E. cervi* in their natural definitive hosts (Panin 1964b; Nettles and Prestwood 1976) receiving similar numbers of L3's as an infective inoculum. Nettles and Prestwood (1976) estimated that a white-tailed deer heavily infected with *P. andersoni* passed 3/4 million larvae per day. Applying their estimate of fecal production (300 gm/day) to MD 1 of the present study, which had the highest daily total production of larvae (14 000 LPG), results in an estimate of 4.2 million larvae/day! Reasons for a higher level of larval production of *P. odocoilei* are unknown. This may be related, however, to host age, sex, immune condition or other nongenetic factors (Kennedy 1976) rather than a higher innate fecundity of *P. odocoilei*.

A daily trend in larval output is suggested by moving average analysis (Fig. 22). Peaks in larval output occurred at 2 to 4 hour intervals throughout the day. The total number of defecations (26) by MD 3 during this period is consistent with previously recorded values for mule deer fawns (Smith 1964). Total larval output of *P. odocoilei* in this animal was 3.2 million larvae/day, which is similar to that estimated for MD 1 which was made on the basis of peak larval production.

Figure 27. Histologic section of the lung of a mule deer experimentally infected with a "moderate" number of *Parelaphostrongylus odocoilei*. 40X. Whipf's Polychrome.



Pulmonary pathology associated with the developing eggs and larvae of *P. odocoilei* has been described (Brunetti 1969) for Columbian black-tailed deer. Brunetti (1969) attributed the death of a yearling, female California mule deer to the presence of *P. odocoilei*. Reaction of lung tissue to these parasites was described as "granuloma like nodules ... produced by a proliferation of septal and interstitial cells" (Brunetti 1969). Lesions, identical to those described above, were found in experimentally infected mule deer in this study (Fig. 27). Similar reactions have been described for *P. macroni* by Nettles and Prestwood (1976), *P. tenuis* in white-tailed deer (Anderson 1963) and *Elaphostrongylus cervi* in reindeer (Bakken and Sparboe 1973) and red deer (Sutherland 1976).

The effect of these parasites on the pulmonary efficiency is unknown. Captive animals in the present study displayed no signs of respiratory distress; larval counts from wild deer (see Section V) were generally much lower than counts from experimental animals. Several died during the course of infection. While these losses were attributable to the effects of the parasite in the case of mule deer (Johnson pers. comm.), all animals showed gross signs and histological lesions similar to those of malignant catarrhal fever (MCF) (Wobeser *et al.* 1973). This is an area requiring considerable attention.

The failure to infect white-tailed deer with *P. odocoilei* and the establishment of a patent infection of this parasite in moose is perplexing. Establishment of a patent infection is indicative of minimum conditions required by the parasite for reproduction; however,

lesions found in the moose are evidence of a strong host response to the developing worm. Similar lesions were not evident in any other experimental animals. By inference, the absence of muscle lesions or the presence of LI's in the lungs or feces of white-tailed deer suggests the absence of the proper biochemical stimuli for, or the presence of an inhibitor to, *P. odocoilei* in this host. The presence of spined larvae in a high percentage of white-tailed deer in eastern Saskatchewan (Bindernagel and Anderson 1972; Shostak unpubl.) and southeastern British Columbia (Bindernagel 1973; Samuel unpubl.), in the absence of *P. tenuis* (Bindernagel and Anderson 1972; Bindernagel 1973; Wobeser pers. comm.) and the failure to establish *P. odocoilei* in white-tailed deer strongly suggest the presence of at least one undescribed species of lungworm or *E. cervi* in whitetails in these areas.

SECTION IV

FIELD STUDIES

A. Introduction

Interactions between a parasite and its intermediate host(s) can be summarized as follows: 1) identification of a suitable host; 2) entry into that host; 3) host response (if any) to the developing parasite; 4) larval longevity within the intermediate host, and 5) larval transfer to subsequent hosts. These aspects of host-parasite interactions are, however, poorly known for nematodes using terrestrial molluscs as intermediate hosts especially for the Elaphostrongylinae and *Parelaphostrongylus odocoilei*. The first and last interactions, the identification of natural intermediate hosts and larval transfer, require field investigations and are addressed here. The remainder are better suited to experimental investigations and are dealt with in Section V.

Nematodes of the superfamily Metastrongyloidea (as defined by Anderson 1978) require an obligate, molluscan intermediate host in the majority of cases that have been investigated, but there are exceptions (Dailey 1970; Georgi 1976). Although the need for a molluscan intermediate host was established nearly 50 years ago (Hobmaier and Hobmaier 1929, 1930), the natural intermediate hosts of the majority of these nematodes are unknown. The relative lack of host specificity at this level and the ease of experimentally infecting both terrestrial and aquatic gastropods (Davtjan 1945; Joyeaux and Gaud 1946; Panin and Rushkova 1964; Richards and Merritt 1967) has resulted in a lack of knowledge of natural intermediate hosts of these nematodes.

Within the Elaphostrongylinae, Panin (1964a) examined a large number of molluscs in an effort to determine the natural intermediate hosts of *Elaphostrongylus panlensis* (= *E. asiaticus panlensis*) on game farms in Kazakhstan SSR. He found 15 of the 20 species of gastropods in area were infected with *E. a. panlensis*. Lankester and Anderson found 7 of 12 species of terrestrial molluscs on Navy Island in southern Ontario were naturally infected with *Parelaphostrongylus* (= *Dorsal phostrongylus*) *tenuis*. These authors implicated the slug, *Deroceras laeve* and the snail *Zonitoides nitidus* as the most important hosts of *P. tenuis* in that area.

The study areas of Lankester and Anderson (1968) and Panin (1964a) were similar in that they were located where cervid populations were enclosed (Panin — game farm; Lankester and Anderson — small island). Cervid densities and the prevalence of elaphostrongylines were high. Such factors probably influenced prevalence and intensity of infection in the intermediate host populations. The examination of molluscs, from more usual deer habitat, for larvae of *P. tenuis* has yielded few larvae despite the examination of large numbers of molluscs (Kearney 1975; Gleich *et al.* 1977).

The natural intermediate hosts of the remaining species of *Parelaphostrongylus*, *P. odocoilei* and *P. andersoni* are unknown, although the life cycle of both species has been completed experimentally (Hobmaier and Hobmaier 1934a; Brunetti 1969; Prestwood 1972).

An investigation of the natural intermediate hosts of *P. odocoilei* in Jasper, Alberta, was undertaken from May to September 1976. The Jasper townsite in west central Alberta (Fig. 28) was chosen for several

reasons. First, *P. odocoilei* was positively identified from deer experimentally infected with larvae that originated from naturally infected mule deer in Jasper (see Sections II and III). Second, Jasper was identified as an enzootic area. Approximately 90% of the fecal samples collected from animals comprising the "townsite" mule deer herd were positive for larvae resembling those of elaphostrongyline nematodes (Samuel unpubl.). Finally, the area was accessible, thus facilitating the collection of terrestrial molluscs and fecal samples of mule deer.

The objectives of the study were to: 1) identify the terrestrial gastropods in the Jasper area; 2) determine the prevalence and intensity of *P. odocoilei* in those gastropods; 3) identify locations or "hot spots" of infection within the general area; 4) document changes in prevalence and intensity of *P. odocoilei* in infected molluscs, and 5) monitor larval output of this nematode in deer in the area during the collection period.

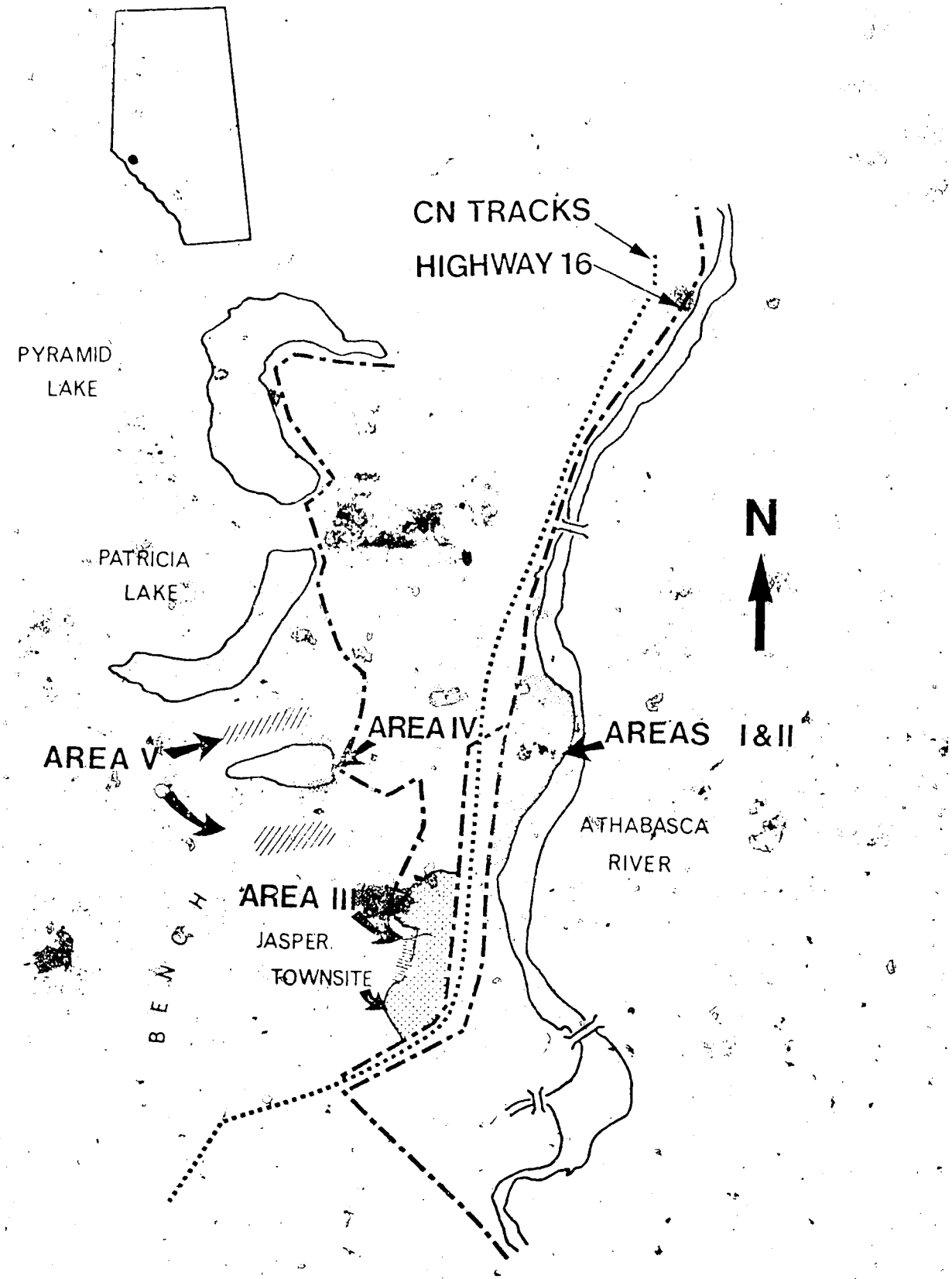
B. Materials and Methods

Study Area

Jasper, Alberta, is located 53°32'N by 118°36'W, approximately 400 km west of Edmonton, Alberta, in the eastern foothills of the Rocky Mountains. The townsite is situated in the center of Jasper National Park. Five primary collecting sites (Fig. 28), all located within a 9 Km radius of the townsite, were selected on the basis of major vegetation types.

Area I (Fig. 28) is an open, grassy area (Fig. 29) adjacent to the Highway 16 by-pass, east of the townsite. This area was disturbed

Figure 28. Schematic illustration of the Jasper townsite, showing the major collecting areas.


CN TRACKS
HIGHWAY 16
PYRAMID LAKE
PATRICIA LAKE
N
AREA V
AREA IV
AREAS I&II
ATHABASCA RIVER
AREA III
JASPER TOWNSITE
BENCH

by the construction of Highway 16. As a result of construction, a number of large rocks are available as sites for mollusc refuge. Native and introduced grasses are the predominant vegetation. There are a few juniper (*Juniperus* sp.) and bear berry (*Arct. taphylos uva-ursi*) are present as well as lodgepole pine seedlings (*Pinus contorta*).

Area II is a canopied area (Fig. 30) adjacent to Area I, bordered on east by the Athabasca River (Fig. 28). The predominant foliage is lodgepole pine, interspersed with aspen (*Populus tremuloides*). The predominant shrubs are juniper, bear berry, and buffalo berry (*Shepherdia canadensis*). This area is also considered a "disturbed" area, in the sense that use by tourists and illegal campers is high. Hence, there is a variety of material available for mollusc refuge sites as well as an abundance of logs (aspen and lodgepole pine) and leaf litter.

Area III is a transition area between valley vegetation and mountainous habitat, directly west of the townsite (Fig. 28). There is a sharp transition and a dramatic increase in elevation (Fig. 31). The predominant vegetation is grass and an occasional lodgepole pine. Buffalo berry, bear berry and juniper were common.

Area IV is situated northwest of the townsite (Fig. 28). It is a marshy area (Fig. 32). The predominant vegetation is sedge (*Carex* sp.).

Area V consists of two disjunct stands of aspen, surrounded by and interspersed with lodgepole pine (Fig. 33). This site is north and south of Area IV (Fig. 28). Bear berry, buffalo berry and juniper are the most common shrubs.

Figures 29-31. Primary collecting sites.

Fig. 29. Area I - open, grassy area adjacent to Highway 16.

Fig. 30. Area II - canopied area between Area I and the Athabasca River.

Fig. 31. Area III - transition area.


29


30

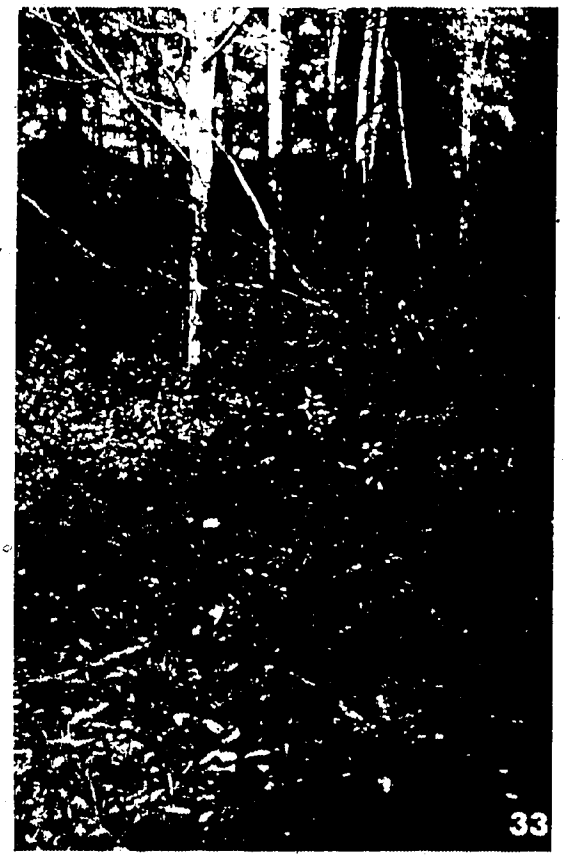

31

Figures 32 and 33. Primary collecting sites.

Fig. 32. Area IV - marsh zone.

Fig. 33. Area V - mixed aspen-conifers surrounding Area IV.


32


33

Molluscs were also collected outside the primary areas. A few (≈ 300) were collected at several locations along the bench (Fig. 28). Collections were made in a variety of habitat types throughout the collection period. These areas will be referred to as "Miscellaneous."

Collecting Procedures

Molluscs were collected on a weekly basis, with two exceptions, from May through August, 1976. In addition, molluscs were collected on April 23 and September 7 and 22. They were located by searching under rocks, logs, leaf litter and any other debris that might offer suitable refuge. Molluscs were placed in translucent plastic containers with a moist paper towel, and transported to the laboratory. Fifty to 100 molluscs, depending on size, were kept in each container.

Laboratory Procedures

All molluscs were sorted, by area of collection, into species groups prior to processing. Identifications were made with the aid of Burch (1962) and Pilsbry (1940-1948). Prior to examination the length of each mollusc was recorded. Slugs were allowed to crawl until fully extended and then measured to the nearest millimeter with a precision caliper. Measurement of snails was made on maximum shell diameter, with the exception of *Oxyloma retusa*, for which maximum shell length was recorded.

Molluscs were then minced with a fine scissors and placed in individual test tubes containing 5 to 10 ml of a standard artificial digestion fluid (0.7 ml concentrate HCl and 0.6 mg pepsin/100 ml distilled water). The test tubes were incubated at 37°C ± 1°C, for 1 to 3 hours, depending on the size of the mollusc. During incubation each tube was occasionally

shaken by hand to aid the digestion process. The tubes were centrifuged at 1500 RPM for approximately 5 minutes. Two ml of supernatant and the cellular plug were retained and examined for nematode larvae in a counting chamber with the aid of a Wild dissecting microscope at 25X.

Larvae found were classified as preinfective or infective (Gerichter 1948). Infective larvae were killed in hot glycerine alcohol and both groups of larvae were stored in that medium for examination.

Fecal Examination

In conjunction with the field collection of molluscs, an attempt was made to collect fresh fecal samples from deer utilizing the study area previously described. Routine examination of the study area was made from the highway after dawn and again prior to dusk for deer on the same days that mollusc collections were made. Once sighted, deer were followed on foot until they defecated, or darkness made continued observation impossible. When defecation was observed, the entire fecal sample was collected and placed in a plastic bag for subsequent examination. The following information was recorded for each sample collected: date, time, sex of the animal, age class (fawn, yearling or adult) and location.

Fecal material was returned to the laboratory and examined for first stage larvae of *P. odocoilei* via the Baermann technique (see Section III). Results are expressed as larvae/gram of feces wet weight or LPG.

C. Results

1. Collections

A summary of molluscs collected, including prevalence of infection with *P. odocoilei*, is given in Table V. A total of 8498 molluscs representing seven families and three suborders within the order Stylommatophora (Pulmonata) was examined. Seven species representing three families in the suborder Sigmaurethra were found to harbor larval *P. odocoilei*.

Deroceras laeve, a medium sized, active slug, had the highest prevalence of infection (4%), while the most frequently collected mollusc, the snail *Euconulus fulvus*, had a prevalence of 1.6%. Intensity of infection (Table VI) was similar in *D. laeve* and *E. fulvus*, while the remaining species harbored considerably fewer *P. odocoilei*.

The number of molluscs collected, species composition and percentage infection varied considerably between the five areas examined (Table VII). *Deroceras laeve* was collected in all study areas, while the succineid, *Oxyloma retusa*, was only found in Area IV, a marsh area, and in a similar "Miscellaneous" area. The following molluscs were collected in all locations with the exception of Area IV: *E. fulvus*, *Discus cronkhitei*, *Zonitoides arboreus*, *Zonitoides nitidus* and *Vitrina limpida*. *Discus shimeki* was primarily restricted to Areas I and II although a single specimen was found in Area V. The pupillids, primarily *Vertigo modesta*, but also *Columella edentula* and *Pupisoma* sp., were most commonly associated with the lodgepole pine portions of Area II. Additional specimens of these molluscs were collected in Areas I and V.

TABLE V. PREVALENCE OF *PARELAPHOSTRONGYLUS* [illegible] LARVAE IN GASTROPODS COLLECTED IN JASPER, ALBERTA (1976)

Species	Number Collected	Number Infected	Percentage Prevalence	Intensity*
Suborder Sigmaurethra				
Family Limacidae				
Deroceras laeve	1384	55	3.97	18 (1-295)
Family Zonitidae				
Euconulus fulvus	2298	36	1.57	17 (1-377)
Zonitoides arboreus	823	6	0.7	1.2 (1-2)
Zonitoides nitidus	263	4	1.5	1
Zonitoides sp.	5	0	0	-
Vitrina limpida	1062	10	0.9	1.7 (1-6)
Retinella electrina	12	0	0	-
Striatura ferrea	56	0	0	-
Family Endodontidae				
Discus cronkhitei	1320	9	0.7	2.4 (1-6)
Discus shimeki	171	2	1.7	1
Suborder Orthourethra				
Family Pupillidae				
Vertigo modesta	322	0	0	-
Vertigo ovata	4	0	0	-
Columella edentula	3	0	0	-
Pupisoma sp.	2	0	0	-
Suborder Heterurethra				
Family Succineidae				
Oxyloma retusa	793	0	0	0
TOTAL	8498	122	1.4	6.0

*Mean (Range)

TABLE VI. INTENSITY* OF INFECTION OF *PARELAPHOSTRONGYLUS ODOCOILEI* IN GASTROPODS FROM JASPER NATIONAL PARK (MAY to SEPTEMBER, 1976)

Species	$\bar{X}$	s**	Range
Deroceras laeve	18.1	50.9	1-295
Euconulus fulvus	17	66.3	1-337
Discus cronkhitei	2.4	1.9	1-6
Discus shimeki	1	0	1
Zonitoides arboreus	1.2	0.4	1-2
Zonitoides nitidus	1	0	1
Vitrina limpida	1.7	1.6	1-6

*Mean number of larvae/infected mollusc.

**Standard deviation.

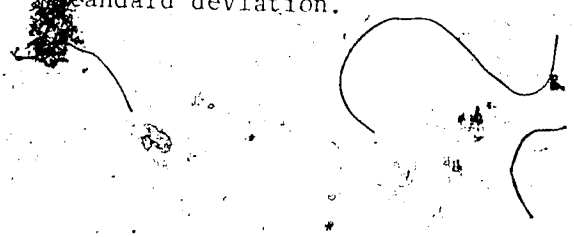


TABLE VII. PREVALENCE OF INFECTION OF LARVAL *PARELAPHOSTRONGYLUS ODOCOILEI* IN TERRESTRIAL GASTROPODS FROM THE MAJOR COLLECTING AREAS OF JASPER, ALBERTA (APRIL thru SEPTEMBER, 1976)

Gastropods	Areas of Collection														
	I			II			III			IV			V		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Deroceras laeve	1110	25	2.3	144	27	18.8	57	0	-	11	1	9	23	1	4.4
Euconulus fulvus	1280	14	1.1	469	18	3.8	81	1	1.2	0	-	-	437	2	0.5
Discus cronkhitei	432	5	1.2	79	0	-	1	0	-	0	-	-	742	4	0.5
Discus shimeki	147	2	1.4	23	0	-	0	-	-	0	-	-	1	0	-
Zonitoides arboreus	700	5	0.7	64	1	1.6	2	0	-	0	-	-	25	0	-
Zonitoides nitidus	182	4	2.2	26	0	-	1	0	-	0	-	-	38	0	-
Vitrina limpida	566	6	1.1	129	1	0.8	236	3	1.3	0	-	-	120	0	-
Vertigo modesta	91	0	-	179	0	-	0	-	-	0	-	-	42	0	-
Vertigo ovata	0	-	-	0	-	-	0	-	-	0	-	-	1	0	-
Pupisoma sp.	1	0	-	1	0	-	0	-	-	0	-	-	0	-	-
Columella edentula	0	-	-	3	0	-	0	-	-	0	-	-	0	-	-
Retinella electrina	7	0	-	0	-	-	0	-	-	0	-	-	5	0	-
Zonitoides sp.	5	0	-	0	-	-	0	-	-	0	-	-	0	-	-
Striatura ferrea	0	-	-	0	-	-	0	-	-	0	-	-	33	0	-
Oxyloma retusa	0	-	-	0	-	-	0	-	-	789	0	-	0	-	-
TOTALS	4521	61	1.4	1117	47	4.2	378	4	1.1	800	0	-	1465	7	0.5

a = Number of molluscs collected; b = number of molluscs infected; c = % prevalence of infection.

Pisidium laeve was most commonly encountered in the open portions of Area I, congregating under rocks in preference to logs or man-made habitats. *Pisidium fulvus*, a small, active snail, was most abundant in Areas I and II. *Pisidium laeve* and *P. fulvus* were replaced as the most abundant species by *Pisidium amnicum* in the aspen-dominated Area V. *Stagnicola jenneae* was only found in locations dominated by aspen (Area V and Miscellaneous).

Vitrina limpida, found in all areas except IV, is a late hatching, annual species. It overwinters as an egg, which hatches in early to mid-July and becomes extremely abundant. Adults survive the early frosts, deposit their eggs and die (Pilsbry 1948). Therefore, no *V. limpida* were collected until early July (Appendix II). During the latter portion of the summer this species was the most abundant of all molluscs. The fact that *V. limpida* was the predominant mollusc in Area III is an artifact of collection. Regular collections were not made in this region until August, ensuring the predominance of this species.

The highest prevalence of infection of molluscs was found in Areas I and II. Molluscs were not as readily available in Area II, the canopied area adjacent to the Athabasca River (Fig. 28). The overall prevalence of infection in Area II (Table VII) was influenced by the high prevalence of *P. laeve* and *E. fulvus* (18.8 and 3.8%, respectively).

Area I had the highest percentage of species infected (7 of 11). This is apparently good mollusc habitat, allowing a large build-up of a variety of molluscan species. There was no significant difference in prevalence of those species that harbored *P. odocoilei* in Area I.

The remaining areas had, by comparison, few infected molluscs. A single, infected *P. haweni* was collected in Area IV; however, none of the *P. parmacella* were infected. This is interesting in light of the rapid pentration of LI's of *P. odocoilei* into this species of snail under experimental conditions (unpubl. observ.) and reports that deer were frequently seen in the general vicinity of the marsh (Haney pers. comm.).

The aspen-conifer slopes surrounding the slough (Area V) were similarly depauperate of infected molluscs (Table VIII). The most common snail, *P. parmacella*, had a prevalence of approximately 0.5% as did *E. fulvus*. The remaining molluscs from [illegible] encountered too infrequently to be considered of any importance in the transmission of *P. odocoilei*.

The transition area (III) is difficult to equate with the other sites. Collections were initiated in mid-August. Although the overall prevalence of infection was 1.1% in this zone, it can be removed from consideration as an important area of infection. Three of four molluscs infected in this area were *V. limpida*. All infected *Vitrina* in this study harbored larvae indistinguishable from the LI's found in deer feces. It is unlikely that, if development was possible in this snail species, all of the *V. limpida* that were infected would harbor newly-penetrated LI's. Laboratory studies are needed to establish the ability of *P. odocoilei* to develop in *V. limpida*. In light of the preceding information, a single infected *E. fulvus* of 378 molluscs examined cannot delimit an important site of transmission.

A more detailed analysis comparing Areas I and II was done (Table VIII) since these areas accounted for 66% of all molluscs

TABLE VIII. COMPARISON OF PREVALENCE OF INFECTION OF MOLLUSCS IN AREA I AND II USING A 2X2 TEST OF INDEPENDENCE

Species	Area I			Area II		
	Collected	Infected	Prevalence (%)	Collected	Infected	Prevalence (%)
Family Limacidae						
Deroceras laeve	1110	25	2.3*	144	27	18.8*
Family Zonitidae						
Euconulus fulvus	1280	14	1.1*	469	18	3.8*
Zonitoides nitidus	147	2	1.4	26	0	0
Zonitoides arboreus	700	5	0.7 NS	64	1	1.6 NS
Vitrina limpida	566	6	1.1 NS	129	1	0.8 NS
Family Endodontidae						
Discus cronkhitei	432	5	1.2	79	0	0
Discus shimeki	147	2	1.4	23	0	0
TOTALS	4521	61	1.4*	1117	47	4.2*

*P < 0.001 2X2 Test of Independence (G-statistic)

collected and 88% of animals positive for *P. laeve*. The percent prevalence of infection in all molluses collected in Area II as well as *P. laeve* and *E. fulvus* was significantly higher ($P \leq .005$; $G_{adj} = 31.94$, 406.2 and 12.54, respectively) than those collected in Area I. There were no differences in prevalence ($P > 0.5$) for the other species common to both areas.

There was a distinct difference in the temporal patterns of the two species of molluses (*P. laeve* and *E. fulvus*) during the summer of 1976 (Fig. 34a). The slug was relatively abundant during May and June, while *E. fulvus* was most common in late August.

The initial prevalence of infection of *P. laeve* (25%) in April (Fig. 34b) is undoubtedly an overestimate due to a low sample size (n=8). Prevalence of infection of *P. laeve* remained below 5% until August. No slugs were infected with larval *Parabostrilus* in late July, while total prevalence in August and September was approximately 6%. Prevalence of infection of *E. fulvus* was erratic during the early collecting periods, reaching a peak of 3.5% in early July. Prevalence was 2.5% in August and September.

The mean length of *P. laeve* was plotted for each collection period (Fig. 35) in an attempt to assess the relationship between prevalence of infection and host age (size) in temporal collections. The size of slugs collected dropped from a mean of 15.9 mm (April to early July) to 12.3 mm in late July ($t_s = 4.112$; $t_{[\infty]}^{0.001} = 3.291$). It was not possible to collect similar data for *E. fulvus* which, due to small size (2 to 3.5 mm), made length a poor indicator of age differences.

Figure 34 a. The number of *Deroceras laeve* and *Euconulus fulvus* collected in Areas I and II from April to September 1976. Each point represents a two week interval and contains all animals collected during that time.

b. Prevalence of infection of *Parelaphostrongylus odocoilei* in *D. laeve* and *E. fulvus* from Areas I and II from April to September 1976.

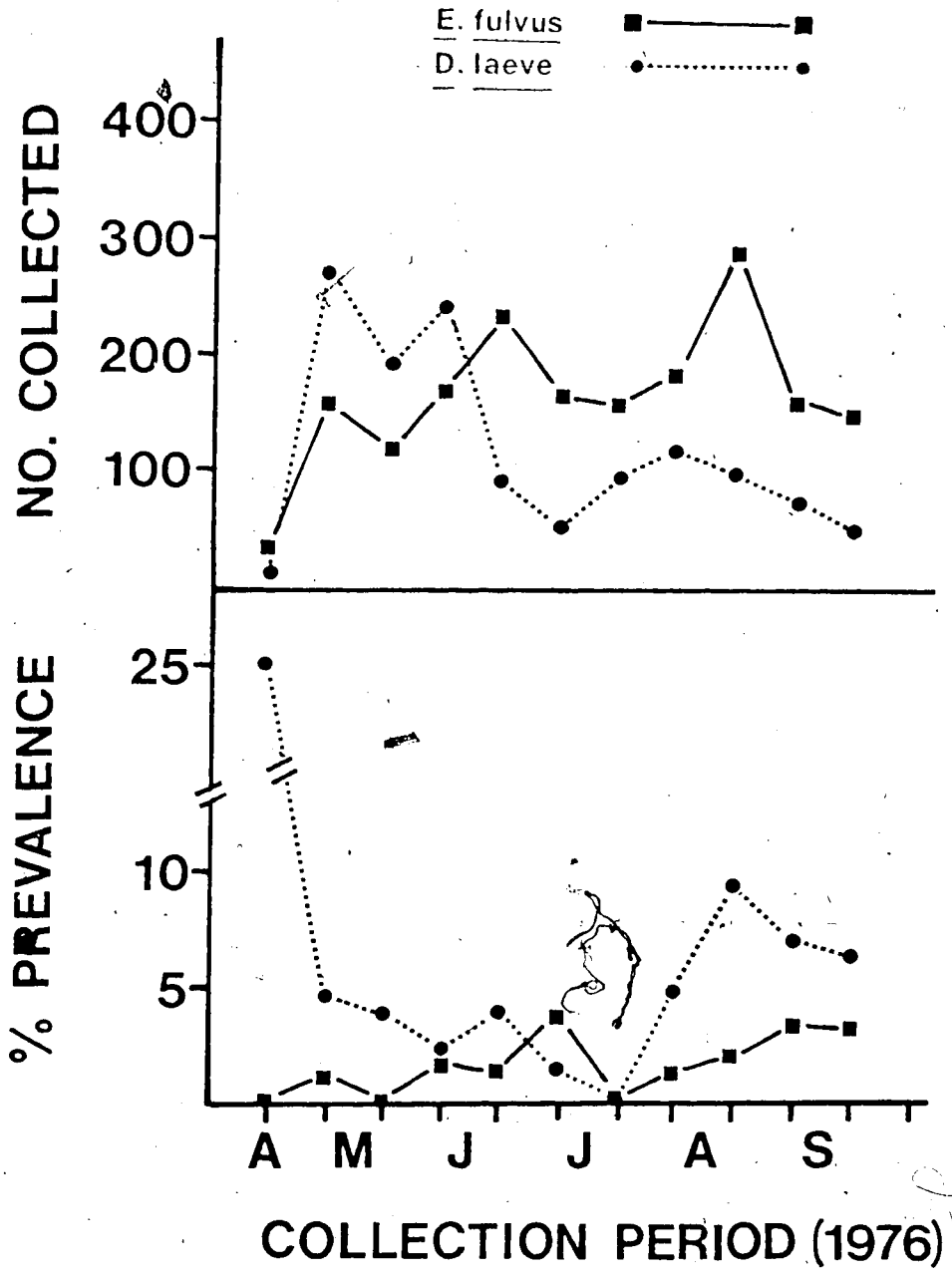
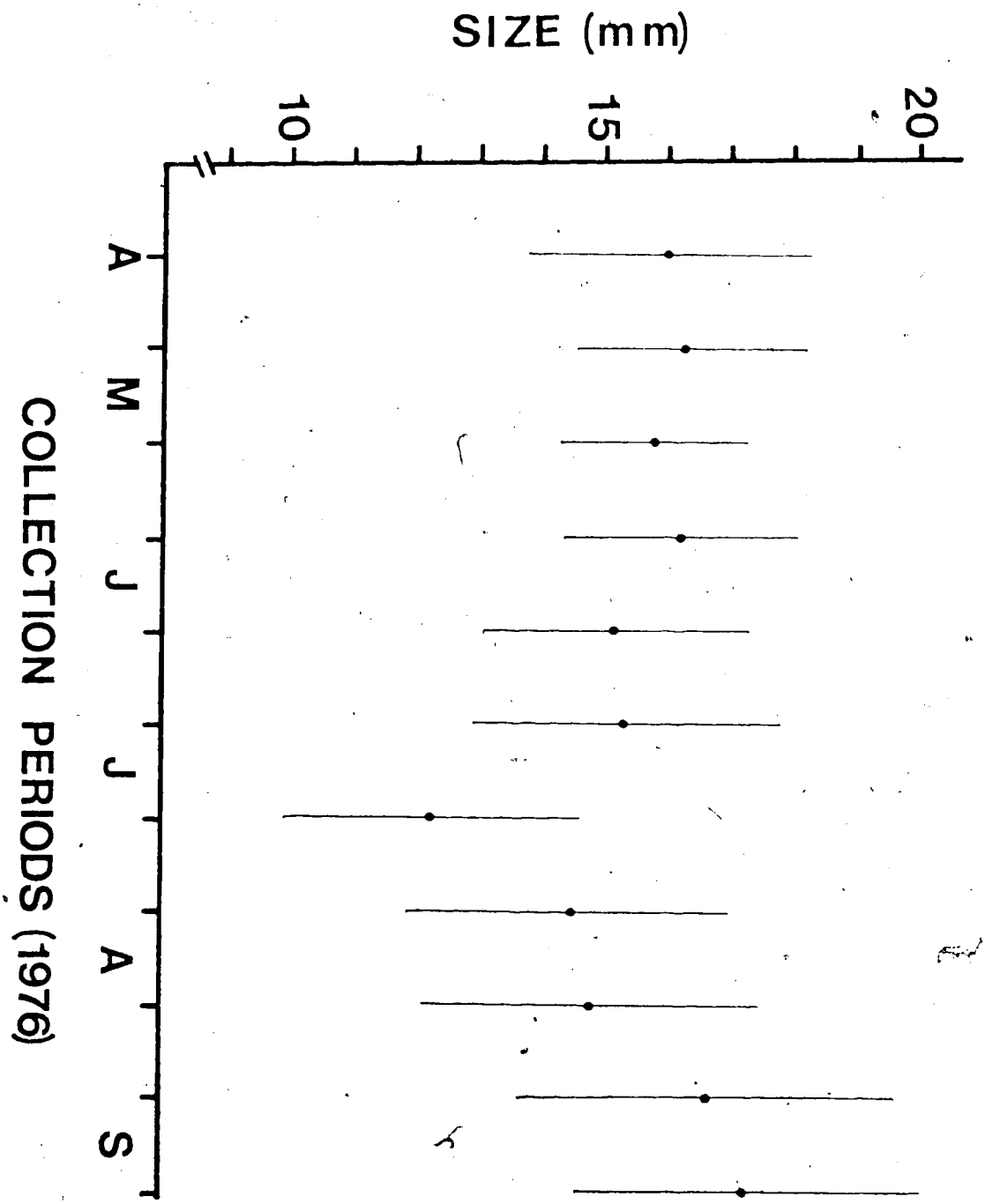

E. fulvus
D. laeve
NO. COLLECTED
400
300
200
100
% PREVALENCE
25
10
5
A
M
J
J
A
S
COLLECTION PERIOD (1976)

Figure 35. Mean length of *Pero[illegible]as laeve* collected in Jasper, Alberta, from April to September 1976. Each point represents a two week time interval and contains all animals collected during that time. The vertical bars represent one standard deviation.


SIZE (mm)
10
15
20
A
M
J
J
A
S
COLLECTION PERIODS (1976)

Slugs were divided into five arbitrary size classes (Fig. 36a). No attempt was made to distinguish between immature, juvenile and adult classes since preliminary observations indicated that a wide range of animals could be considered adult by reproductive criteria. Such determinations, made on the basis of size (Lankester and Anderson 1968), are meaningless. Therefore an arbitrary division of classes at 5 mm intervals was considered more realistic. The percent prevalence of infection is given in Figure 36b. No slugs less than 5 mm were infected, and prevalence increased with size.

Prevalence of preinfective and infective larvae (Gerichter 1948) in *D. laeve* and *E. fulvus* in Areas I and II showed distinct temporal trends (Fig. 37). Preinfective larvae were more frequently encountered early in the summer and during late August. A peak in the percent of infective larvae followed the preinfective peaks by approximately 1 month.

Fecal Collections

Forty-nine fecal pellet groups were collected in the general vicinity of the Jasper townsite between April and September, 1976. Forty-two (86%) were positive for larvae of *P. odocoilei* (Table IX). Two of the negative samples were collected from a single fawn on September 21 and 22. Two other deer were sampled on consecutive days. One, the mother of the fawn described above, passed 3 and 10 LPG, respectively. An adult buck was sampled on June 23 and 24. This animal passed 71 and 345 LPG, respectively. Fecal samples from the buck were collected in the same area at approximately the same time of day (7:30 hours).

Figure 36 a. Distribution of *Deroceras laeve* collected in Areas I and II, by size class.

b. Prevalence of infection of *D. laeve* with *Parelaphostrongylus odocoilei* according to the size class of the slug.

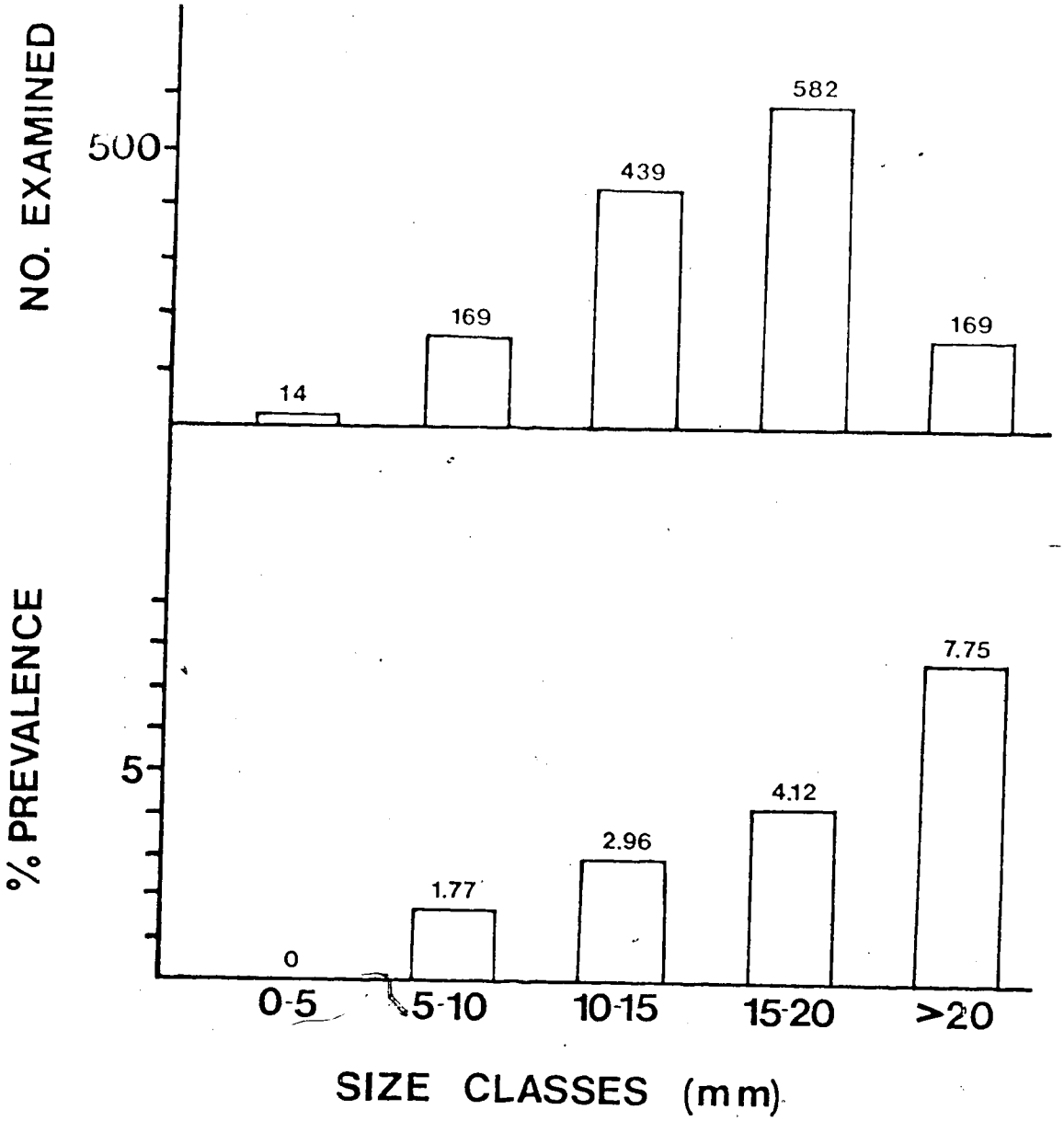

NO. EXAMINED
500
582
439
169
169
14
% PREVALENCE
5
7.75
4.12
2.96
1.77
0
0-5
5-10
10-15
15-20
>20
SIZE CLASSES (mm)

Figure 37. Prevalence of preinfective and infective larvae of *Parelaphostrongylus odocoilei* in *Deroceras laeve* from Areas I and II. Each point represents a two week time interval and contains all animals collected during that time.

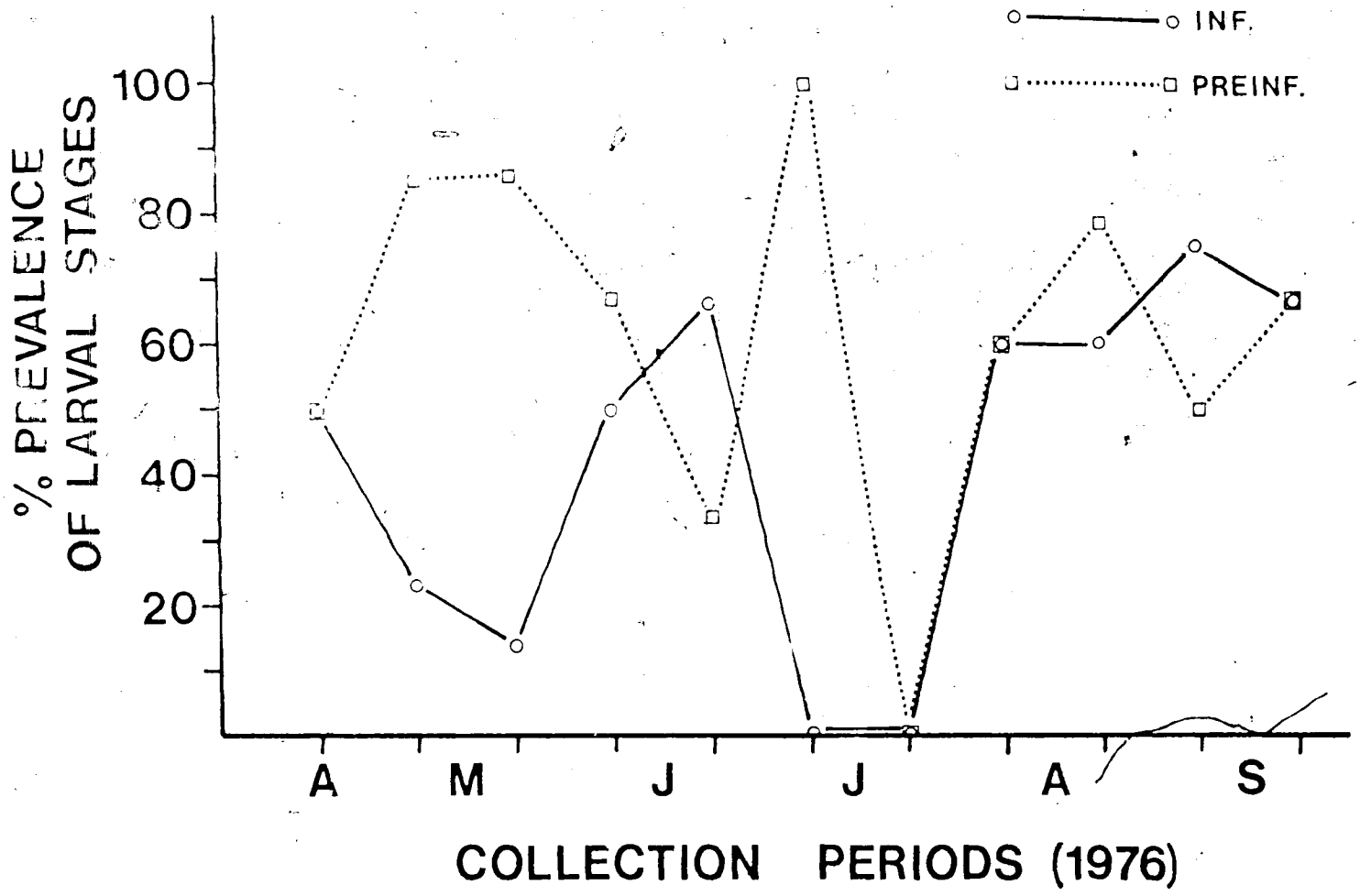

INF.
PREINF.
% PREVALENCE OF LARVAL STAGES
100
80
60
40
20
A
M
J
J
A
S
COLLECTION PERIODS (1976)

TABLE IX. SUMMARY OF LARVAL OUTPUT OF *PARELAPHOSTRONGYLUS ANDERSONI* FROM MULE DEER OF THE TOWNSITE HERD, JASPER, ALBERTA (APRIL to SEPTEMBER, 1976)

Month	Number of fecal groups examined	Number of fecal groups positive	$\bar{X}$ Intensity*	s**
April	6	6	101	63.5
May	7	7	190 (73)***	314.9
June	15	14	56	87.5
July	11	8	28	29.5
August	4	4	40	45.7
September	6	3	5	4.3

*Mean number of larvae per gram of feces (wet weight) per infected sample.

**Standard deviation.

***Reading ( ) is the mean intensity not including a single sample of 895 LPG and n = 6.

Larval output declined monthly, from April to September (Table IX). The May data were strongly influenced by a single yearling that passed 895 LPG. These data correspond to unpublished observations of fecal samples from the same area (Appendix III).

D. Discussion

A high percentage of mule deer constituting the Jasper townsite herd is infected with *P. odocoilei* (Samuel, unpubl.; this study). Eighty-six percent of fresh fecal samples collected during the present study were positive for spined larvae. The negatives were from fawns or from samples collected during periods of low larval output, possibly representing a seasonal reduction or cessation of larval production (Gevondyan 1958; and see Schad 1977 for a review of this phenomenon).

Due to the location of adult *P. odocoilei* in the muscles of the definitive host, it is difficult to assess worm burden in an individual deer. Comparison of larval output in experimentally infected mule deer (Section III) with field data suggests that deer in the townsite herd harbor relatively few worms. The highest larval output of samples collected in the field (895 LPG) corresponds most closely to the peak output of 720 LPG observed in MD 4, that received 14 L3's.

Several authors (Pillmore 1956; Uhazy *et al.* 1973) have attempted to establish criteria for determining the intensity of lungworm (= genus *Protostrongylus*) infections of bighorn sheep (*Ovis canadensis canadensis*). These authors correlated lung damage associated with varying worm burdens and larval output with some success. Results of experimental infections of deer with *P. odocoilei* (Section III) and

field data suggest that crude estimates of worm burden, on the basis of larval output, are possible for this species as well. These estimates must, however, be viewed with caution since larval output is known to vary as much as 300% in a 24 hour period in an experimentally infected animal (p. 43) and 500% in the same time period in a wild deer (p. 89). Additional factors, such as host age, immune status and age of the infection may also influence larval production to some degree.

The timing of larval output of the elaphostrongylines is poorly known. Temporal aspects of larval output may, however, be important in increasing the probability of larval contact with a suitable intermediate host. Peak larval output of *P. odocoilei* in the Jasper townsite herd occurred during the occupation of winter range (Table IX; Appendix III) when deer densities were relatively high. Low larval output, conversely, occurred in summer, when deer dispersed (Sullivan and Stelfox 1974). Similar situations have been noted by other workers (Uhazy *et al.* 1973; Gates 1975, and others) for bighorn sheep infected with *Protostrongylus* spp.

Saturation of an area containing suitable intermediate hosts with L1's would form a focus of infection, or hot spot. Subsequent dispersal of deer resulting in decreased deer densities would reduce the probability of infection through dilution of larvae-to-mollusc and infected mollusc-to-deer interactions. This is supported by field data from Areas IV, V and Miscellaneous, which form part of the summer range of the townsite herd (Sullivan and Stelfox 1974). These areas yielded few infected gastropods (Table VII). Mule deer are known to return on a yearly basis to a specific wintering area (Zalunardo 1965). This would enhance

the chance of an autumn exposure of the young of the year to molluscs infected the previous spring or summer.

The distribution and activity of first stage larvae of the elaphostrongyline nematodes is not well known. Evidence, primarily experimental, points to several mechanisms that would serve to increase the probability of larvae-mollusc contact. First stage larvae of these nematodes are resistant to freezing, yet do not tolerate drying (Mitskevich 1964; Lankester and Anderson 1968). First stage larvae of *P. tenuis* rapidly leave the fecal pellet when submerged in water (Lankester and Anderson 1968); therefore, larvae contained in feces deposited on snow would be able to survive in a frozen state until spring thaws could aid in their dispersal to subterranean habitats where the threat of desiccation would be reduced (Matekin *et al.* 1954; Mitskevich 1964). Larvae deposited during the summer would need to rely on sporadic rainfall for dispersal, increasing the risk of desiccation. The extent to which the dispersal of L1's actually occurs or is important for transmission, is unknown. The fact that large numbers of terrestrial molluscs, especially slugs, are subterraneous during the day, emerging to feed on surface vegetation at night (Newell 1966) makes this aspect of L1-mollusc contact an important area for future study.

Completion of the life cycle of *P. odocoilei* is dependent upon the accidental ingestion of an infected mollusc by grazing deer. Ingestion is considered accidental, as there are no reports in the literature of deer actively seeking molluscs as a food item. Mule deer are known to eagerly ingest emerging grasses during the spring before other vegetation is available (Cowan 1956; Einarsen 1956). Mule deer

in Jasper were observed to follow this pattern. Grazing continued to form an important part of the feeding regime of mule deer in the Jasper townsite for the duration of the observation period (pers. observ.). This proclivity toward grazing would aid in bringing deer into a zone of overlap (Holmes and Bethel 1972) with infected molluscs, increasing the probability of accidental ingestion.

A taxonomic assessment of the seven species of molluscs harboring larvae of *P. odocoilei* (Table V) shows that all belong to the suborder Sigmaurethra. Neither of the other suborders represented, Heterurethra (pupillids) and Orthourethra (*Oxyloma*), was infected. This may be the result of a lack of susceptibility of the snails (Wright 1971; Basch 1975) or ecological isolation (Panin and Rushkova 1964). The pupillids have been shown to be excellent hosts for a variety of ruminant lungworms of the subfamily Protostrongylinae (Matekin *et al.* 1954; Pillmore 1956) and while protostrongylids are able to use sigmaurethrans (Joyeaux and Gaud 1946; Gerichter 1951), elaphostrongylines have seldom been reported to use pupillids or vallionids (Heterurethra) as intermediate hosts (Panin 1964a). Attempts to experimentally infect pupillids with larvae of *Elaphostrongylus cervi.panticola* were unsuccessful (Panin and Rushkova 1964). Therefore, it appears the Heterurethra are, as a group, largely unsusceptible to infection by elaphostrongylines.

Ecological isolation is less likely as an explanation for the failure to find pupillids naturally infected with larval *P. odocoilei*. Mule deer and bighorn sheep are commonly seen together in Jasper National Park, although not in the study areas dealt with in this study. The

ubiquitous nature of these molluses in the present study as well as other investigations of this nature (Mitskevich 1960; Panin 1964a) argue strongly against ecologic isolation as a mechanism for the absence of infection in these molluses. However, this mechanism might be of some importance at the microhabitat level.

The succineids (Orthourethra, which includes *Oxyloma* of this study) are excellent hosts of *Elaphostrongylus cervi* in the USSR (Mitskevich 1964; Panin 1964a) and *P. tenuis* in North America (Lankester and Anderson 1968). These reports, however, refer to *Succinea* spp., which are less dependent upon moist conditions than *Oxyloma*. First stage larvae of *P. odocoilei* rapidly penetrated *O. retusa* under experimental conditions (pers. observ.). Development was not followed to completion, due to difficulty in maintaining this species in the laboratory, and therefore it is impossible to state whether *O. retusa* would be a suitable host for *P. odocoilei*.

Observations during this study suggest that an ecological rather than a physiological barrier to infection by *P. odocoilei* exists for *O. retusa*. It was found only in wet, marshy habitat (Area IV, Miscellaneous). Anderson (1963) demonstrated that larvae of *P. tenuis* had difficulty penetrating hosts in water, as did Looss (1905) for *Ancylostoma duodenale*, and that larvae would be quickly dispersed in an aquatic medium. Therefore, an amphibious mollusc such as *O. retusa* would have a reduced opportunity for contact with larval nematodes under conditions suitable for infection.

Aquatic molluscs (suborder Basommatophora) were not considered in the present study. Lankester and Anderson (1968) found no aquatic

molluscs infected with *P. tenuis* although the infection of terrestrial molluscs was common. Mowatt (1973) failed to find infective larvae of *P. tenuis* in a study of the nematode parasites of aquatic molluscs in Central Maine.

Deroceras laeve and *D. fulvus* must be considered the most important intermediate hosts of *P. odocoilei* in the vicinity of Jasper, Alberta. *Deroceras laeve* is a medium sized, active slug, holarctic in distribution (Pilsbry 1948) and considered a native North American species (Getz and Chichester 1971). This species combines all the attributes necessary to be an excellent intermediate host for a metastrongyloid nematode (Wallace and Rosen 1969). Ecological studies (Getz 1959) indicate that *D. laeve* occurs in a wide variety of habitat types, is catholic in its feeding habits (Getz 1959) and is able to survive subfreezing temperatures. *Deroceras laeve* shows a wide range of temperature tolerance, 14°C to 26°C, tolerating temperatures as high as 36°C (Getz 1959). A related species, *Agriolimax (= Deroceras) reticulatus*, has been observed feeding normally at 0.8°C (Mellanby 1961). The ability of *D. laeve* to withstand a wide range of climatic conditions, in addition to their diverse feeding habits, would place *D. laeve* in an excellent position to be accidentally ingested by foraging deer.

An overlap of the intermediate host into the feeding area of an herbivorous definitive host is essential in the completion of the life cycle of a parasite not based on a predator-prey relationship. There is no evidence to suggest a parasite induced alteration of molluscan behavior in this system. Although this type of interaction has been proven necessary for the successful completion of the life cycle of

some parasites (Holmes and Bethel 1972; Bethel and Holmes 1973), the normal activity patterns of molluscs and deer and the absence of rapid escape mechanisms in terrestrial molluscs may obviate the need for such a system.

Terrestrial molluscs are generally regarded as nocturnal (Hyman 1967), remaining quiescent during the day to avoid the direct rays of the sun and subsequent drying. Dainton (1954a) has shown experimentally that behavior of a variety of slug species was a response to diurnal temperature changes, and prolonged activity occurred when temperatures dropped below 21°C. Dainton (1954b) found that light had little effect on slug activity. This work was later confirmed using North American slugs (Karlin 1961). Subsequent investigations (Lewis 1969a) have shown that temperature is ineffective in timing slug activity and that locomotor activity was controlled endogenously (Lewis 1969b). Regardless of the mechanism controlling molluscan activity, the coincident timing of slug activity and deer feeding, in the absence of altered behavior of the intermediate host, must be considered important for the transmission of *P. odocoilei* and the other members of the Elaphostrongylinae.

Euconulus fulvus is a small, shelled mollusc with a holarctic distribution (Likhachev and Rammel'maier 1952; Burch 1962). It was abundant in all areas examined during the present study (Table V) with the exception of Area IV. The role of this snail in the transmission of *P. odocoilei* could prove important. Its small size, active nature and relative abundance gives it an increased probability of being accidentally ingested by grazing deer.

Prevalence of infection in *E. fulvus* was roughly one half that found in *P. laevis*; however, intensities of infection in these species were equal. *Euconulus fulvus* was considered a "suboptimal" host for *P. cornu pattiensis* on the basis of experimental infections (Panin and Rushkova 1964) although it had a high prevalence of infection in field studies (Panin 1964a). It is of some interest that *E. fulvus* was not reported from Navy Island, Southern Ontario (Lankester and Anderson 1968) as this species is common on mainland Ontario (Oughton 1948) as well as other regions of Canada (LaRocque 1953; Clark *et al.*, 1968).

The remaining snails that harbored larvae of *P. odocoilei* are discounted as playing a major role in the transmission of this parasite. *Discus cronkhitei* and *Z. arboreus*, although abundant, had low prevalences of infection. *Discus shimeki* and *Z. nitidus* had prevalences of infection similar to that of *E. fulvus*, but were encountered infrequently (Table V), and never harbored more than one larva per infected snail (Table VI). The final species, *Vitrina limpida*, harbored only preinfective larvae that had not completed any discernible development. This snail species may occur too late in the season to be effective in parasite transmission.

There is a distinct difference in the role of *Z. nitidus* as a primary host of *P. tenuis* on Navy Island (Lankester and Anderson 1968) and the secondary role of this mollusc as an intermediate host of *P. odocoilei*. This may reflect the preference of *Z. nitidus* for moist habitat (Lankester and Anderson 1968). *Zonitiodes nitidus* was encountered infrequently in Jasper Park and the drier areas of Navy Island, but was extremely abundant in the damp woodland areas of the Ontario site (Lankester and Anderson 1968).

Comparison of the study on Navy Island and the present study reveals some interesting similarities. Navy Island and the Jasper Townsite have several features in common. Both areas limit deer movement to some degree; Navy Island as a true island, and the present study area by acting as an island. Areas I and II of the present study, part of the winter range of the Jasper townsite mule deer herd, also included an undetermined number of animals that remained in the area on a year-round basis due to tourist activity (Sullivan and Stelfox 1974). These areas could be considered islands, in a loose sense, on the basis of constant deer utilization. Both areas have a high prevalence of *P. tenuis* and *P. odocoilei*, respectively. Other studies of molluscan intermediate hosts of *P. tenuis* failed to identify areas of concentrated deer usage and found few larvae of this nematode species, despite examining large numbers of molluscs (Kearney 1975; Gleich *et al.* 1977).

Reasons for the higher prevalence of infection of molluscs with *P. odocoilei* in Area II are not clear, but fall into two broad categories: deer usage and larval protection. All common species of molluscs were found in similar proportions in Areas I and II.

Personal observations of deer usage during the collecting season indicate that deer were likely to use Area II (canopied) more often than Area I (open). Deer were observed feeding in both locations and could be enticed to remain on or to enter Area I by tourists offering food. The majority of deer feeding in this area, however, would eventually retreat into the canopied portion to rest and/or continue feeding. Therefore, the deer I observed utilized the canopied area more frequently and as a consequence defecated in this area more often. This would,

theoretically, serve to increase the number of first stage larvae available to resident molluscs.

An additional factor affecting the prevalence of infection of molluscs is that the canopied area afforded protection from environmental factors for the first stage larvae. Several studies have demonstrated some resistance of metastrongyloid larvae to drying over short periods (Morgan 1929; Williams 1942; Rose 1957) and others (Seneviratana 1959b; Anderson 1962b) show extremely reduced survival under these conditions. Lankester and Anderson (1968) demonstrated a reduced level of infection of *P. tenuis* in the snail *Mesodon thyroidus* when molluscs were exposed to first stage larvae in either fecal material or inoculated into soil that had been allowed to dry, when compared to moist controls. Therefore, the canopy may aid in reducing the effects of evaporation, enhancing larval survival.

The canopy may also reduce the effects of ultraviolet radiation on exposed larvae, although Mitskevich (1964) stated that sunlight did not affect larvae of *E. cervi rangiferi*. Far and middle ultraviolet rays, however, have been demonstrated to have deleterious effects on the free-living larvae of *Trichostrongylus retortaeformis* by Gupta (1961), and artificial and natural ultraviolet radiation killed the free-living larvae of *Nematodirus spathiger*, another trichostrongyloid parasite (Senger 1964). The effects of u.v. radiation on larval nematodes certainly warrants further investigation.

The identification of a coniferous habitat as a "hot spot" of transmission of a parasite utilizing a molluscan intermediate host is somewhat anomalous. General works on molluscs (Hyman 1967) imply that

coniferous forests are poor mollusc habitat. Walker (1902) stated that "coniferous forests are usually quite barren of molluscan life." In a review of this subject (Clark *et al.* 1968), the authors stated that coniferous forests were "far from barren of molluscan life." This view is supported in part by the present work. All of the abundant species of molluscs were found in Area II, which is predominantly lodgepole pine. Although no accurate records of mollusc-site associations were made during the collections, it became evident that the majority of molluscs preferred non-coniferous sites in this area. Small areas containing aspen and aspen logs or man-made refuges yielded more molluscs than coniferous sites, which were more common. Therefore, a refinement of collecting procedures might reveal mollusc-plant or mollusc-refuge associations. These would represent "patches of transmission" within a site designated as a "hot spot."

Temporal collections of slugs (*D. laeve*) in this study were similar to those recorded on Navy Island (Lankester and Anderson 1968). Both studies indicate a single generation per year, with large, mature, overwintered animals lost from the population during June and July. These animals are subsequently replaced by small, immature individuals, as indicated in Figure 34, recruited into the population during late July and August. This loss of large, infected individuals may account in part for the zero prevalence of infection recorded in late July (Fig. 34b). The zero prevalence is an artifact of collection as infective larvae (L3) were found in both *D. laeve* and *E. fulvus* during the subsequent collection period (Fig. 34b; Appendix II). Since *P. odocoilei* requires a minimum of 22 days at 18°C to complete development

from penetration to the infective stage (Section V), it would have been impossible for first stage larvae to penetrate and develop to an infective form in the interval between collecting periods. The infected molluscs collected in early August must have been present in the population during July, but remained undetected. The loss of old infected animals and the recruitment of young, uninfected slugs would cause a dilution of the infected population making them rare (Lankester and Anderson 1968).

Size influenced the prevalence of infection of *D. laeve* with *P. odocoilei* (Fig. 36). Similar findings were recorded for this species of slug and *P. tenuis* by Lankester and Anderson (1968). This is attributable to factors associated with size and age (Wallace and Rosen 1969). Comparative susceptibility of various age classes of *D. laeve* has not been investigated experimentally.

Peak prevalence of infective larvae of *P. odocoilei* in *D. laeve* was reached in August-September in this study and June to July for *P. tenuis* on Navy Island (Lankester and Anderson 1968). Thus, infective larvae of these species, at these two locations, are most readily available to the cervid definitive host at different times. This suggests different strategies for transmission of these nematodes in these areas.

An increased occurrence of infective larvae in the late summer for *P. odocoilei* in the present study (Fig. 34a) coincides with observations (Samuel unpubl.) on the timing of infection of fawns in the townsite herd. Three fawns, examined on two occasions (29 Sept. and 2 Nov. 1974) were negative for first stage larvae at the initial sampling period, but positive at the second. Extrapolation of the prepatent period established

for experimental infections of *P. odocoilei* in mule deer (15 to 62 days) dictates that acquisition of the infection should have occurred between the last week in August and 15 September. This coincides with the peak prevalence of *P. odocoilei* in *P. haweeti*.

The early summer peak of infective larvae of *P. tenuis* on Navy Island suggests white-tailed deer are most likely to become infected as yearlings. This is substantiated to some degree by the findings of Anderson (1963). He found 41% of adult white-tails (> 1 year) infected with *P. tenuis*, while only 5.5% of fawns (< 1 year) harbored this parasite in Algonquin Park, Ontario. Additional observations on the temporal aspects of the infection of young animals are required to establish this concept with certainty.

The majority of molluscs collected during this study overwinter as adults, lay eggs in late spring or early summer and die. Lankester and Anderson (1968) gave circumstantial evidence that infective larvae (L3) survived the winter in the mollusc. The presence of infective larvae in the initial collection (23 April) coupled with the developmental data presented elsewhere, support this conclusion. Therefore, infected molluscs would be available to re-infect overwintered deer before they left the yarding area for summer range, as well as fawns entering the winter range for the first time the next fall.

EXPERIMENTAL LIFE CYCLE IN MOLLUSCS

A. Introduction

The ability of gastropod molluscs to remove foreign material has been recognized for some time (Tripp 1961; Brooks 1969). Humoral and cellular components of this defense system have been described.

Although humoral factors such as nonspecific agglutinins have been identified, their role in the maintenance of the internal steady-state of gastropods remains unclear (Tripp 1974). Cellular reactions are clearly regarded, at this point in time, as the more important means of defense against foreign material (Brooks 1969; Tripp 1970; Malek and Cheng 1974). Two basic types of cellular response in molluscs are recognized: phagocytosis and encapsulation (Tripp 1961).

These reactions as applied to parasitic helminths have been studied intensively during the past two decades (see Stauber 1961; Tripp 1961, 1974; Brooks 1969; Cheng and Rifkin 1970; Malek and Cheng 1974). The general pattern of an encapsulation response is followed by phagocytosis if the parasite is killed or becomes degenerate. The primary focus of these investigations has centered on trematodes of medical importance such as the schistosomes (Pan 1963, 1965) or molluscs of economic importance (Stauber 1961; Cheng 1966, 1967).

Studies of responses to nematodes utilizing molluscan intermediate hosts have not kept pace with those of their trematode counterparts. As Wright (1966) has stated: ". . . little or no work has been published on the response of molluscs to these parasites [nematodes]." Only recently have studies of the host-parasite relationships of mollusc-nematode

systems been undertaken and analyzed in a thorough fashion (Harris and Cheng, 1975a; Rachford 1976a; Sauerlander 1976).

Metastrongyloids are one of the major groups of nematodes that require a molluscan intermediate host. They are parasites of terrestrial and aquatic mammals, and although exceptions occur, terrestrial molluscs are generally required for the completion of the life cycle. The majority of studies that examined host-parasite relationships of these helminths utilized aquatic pulmonates (e.g. Courdurier *et al.* 1967; Richards and Merritt 1967; Harris and Cheng 1975a; Petter and Cassone 1975; Rachford 1976a) as a matter of convenience, and may not reflect a natural situation. There have been a few studies of host reaction involving terrestrial gastropods (Kassai 1958; Anderson 1962b; Zmoray *et al.* 1970; Svarc and Zmoray 1974; Saurelander 1976); however, here is only a single, incidental reference to the reactions of molluscs to elaphostrongyline nematodes (Anderson 1963).

The purpose of this portion of the investigation was to analyze the host-parasite interactions of *Parelaphostrongylus odocoilei* with several species of terrestrial gastropods, primarily *Triodopsis multilineata*. The specific objectives were as follows: 1) to determine the mode of parasite entry into the mollusc; 2) to determine the site of larval development; 3) to examine the host response to the developing larvae, and 4) to document larval growth of *P. odocoilei*.

B. Materials and Methods

Mode of Entry

Two species of snails, *Vitrina limpida* and *Zonitoides arboreus*, and two species of slugs, *Deroceras laeve* and *Deroceras reticulatum*, were used in these trials. *Vitrina* were collected in Jasper. Artificial digestion of over 100 individuals collected at the same time failed to reveal any metastrongyloid larvae. It was assumed, therefore, that *V. limpida* used in the experiments were also initially negative. Specimens of *Zonitoides* were collected near Elk Island National Park, Alberta. Elaphostrongylines are not known from the park, although feces of white-tailed deer have been examined for larvae (Bindernagel 1973) and deer, moose (Samuel *et al.* 1976) wapiti and bison (Samuel unpubl.) have been examined for lungworms. Several *Z. arboreus* were dissected and were negative for nematodes. All *Deroceras* were reared in the laboratory and were free from exposure to helminths prior to these trials.

Twenty-four specimens of each species were placed in a 12.4 cm finger bowl containing a disc of Whatman No. 1 filter paper to which approximately 30,000 L1 larvae had been added. The molluscs were placed in the finger bowls and permitted to move normally. If they began to climb the sides of the bowl they were gently replaced at the center of the filter paper disc. Sufficient water was added to keep the paper moist but not flooded.

Eight individuals of each species (five for *D. laeve*) were removed at intervals of 1/2, 1 and 1-1/2 hours. Molluscs were [redacted] placing them in boiling water. They were fixed in 10% neutral buffered formalin

and prepared for histological examination following standard procedures. Animals were subsequently sectioned at 7 µm and stained in Whipf's polychromatic stain (Vetterling and Thompson 1971).

Entry of first stage larvae into molluscs was scored as an index. From preliminary observations it was determined that, on the average, an individual larva was present in 8 consecutive histological sections of 7 µm. Therefore, all sections of larvae were counted in every seventh section and tabulated according to location, either foot or viscera. The total for each location was then divided by the number of sections counted, resulting in an index of entry based on the mean number of nematode sections per section of snail examined.

Larval Growth and Site of Development

Sixty *Triodopsis multilineata* were exposed to first stage larvae as described above for a period of 6 hours. Snails were maintained at 18°C ± 1° and fed a diet of lettuce and carrots. Two snails were digested 1, 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 20, 22, 24 and 26 days post-exposure. The de-shelled weight was recorded to the nearest 0.1 gm. The foot and viscera were separated and chopped into large pieces with a fine scissors, and digested separately. When digestion was complete the digestion fluid was examined and larvae counted. Preinfective larvae, by definition larvae that do not survive an artificial digestion procedure (Gerichter 1948), were fixed and stored in glycerine alcohol. Infective larvae were killed in boiling glycerine-alcohol and stored in that medium. Larvae were examined as whole mounts in pure glycerine. All measurements are in micrometers (µm) and were made with the aid of an ocular micrometer and measuring wheel.

Host Response

In conjunction with the previously described work on larval development, single *T. multilineata* were killed, at regular intervals, in boiling water, the foot and viscera were separated and fixed in 10% neutral buffered formalin. Snails were killed 3 hrs, 12 hrs, and 1, 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 20, 22, 24 and 26 days post-exposure. The foot was prepared for histological examination, sectioned and stained as previously described.

C. Results

Mode of Entry

Results of the entry of *P. odocoilei* into three of the species of molluscs are presented in Figure 38. *Deroceras reticulatum* is not included in this figure due to the low number of larvae found (Appendix IV). First stage larvae of *P. odocoilei* had entered all molluscs examined at 1/2 hour post-exposure (Fig. 38). In all cases the "index of entry" increased at 1 hour post-exposure and again at 1-1/2 hours PE. The overall index of entry was highest for *V. limpida* and lowest in *D. reticulatum*. The number and distribution of the larvae of *P. odocoilei* were distinct in each of the molluscs used in these trials.

Larvae of *P. odocoilei* were observed only in the foot of *D. laeve* (Fig. 38a). Larval penetration in *D. laeve* slowed between 1/2 and 1 hour PE but increased over seven times from 1 to 1-1/2 hours PE.

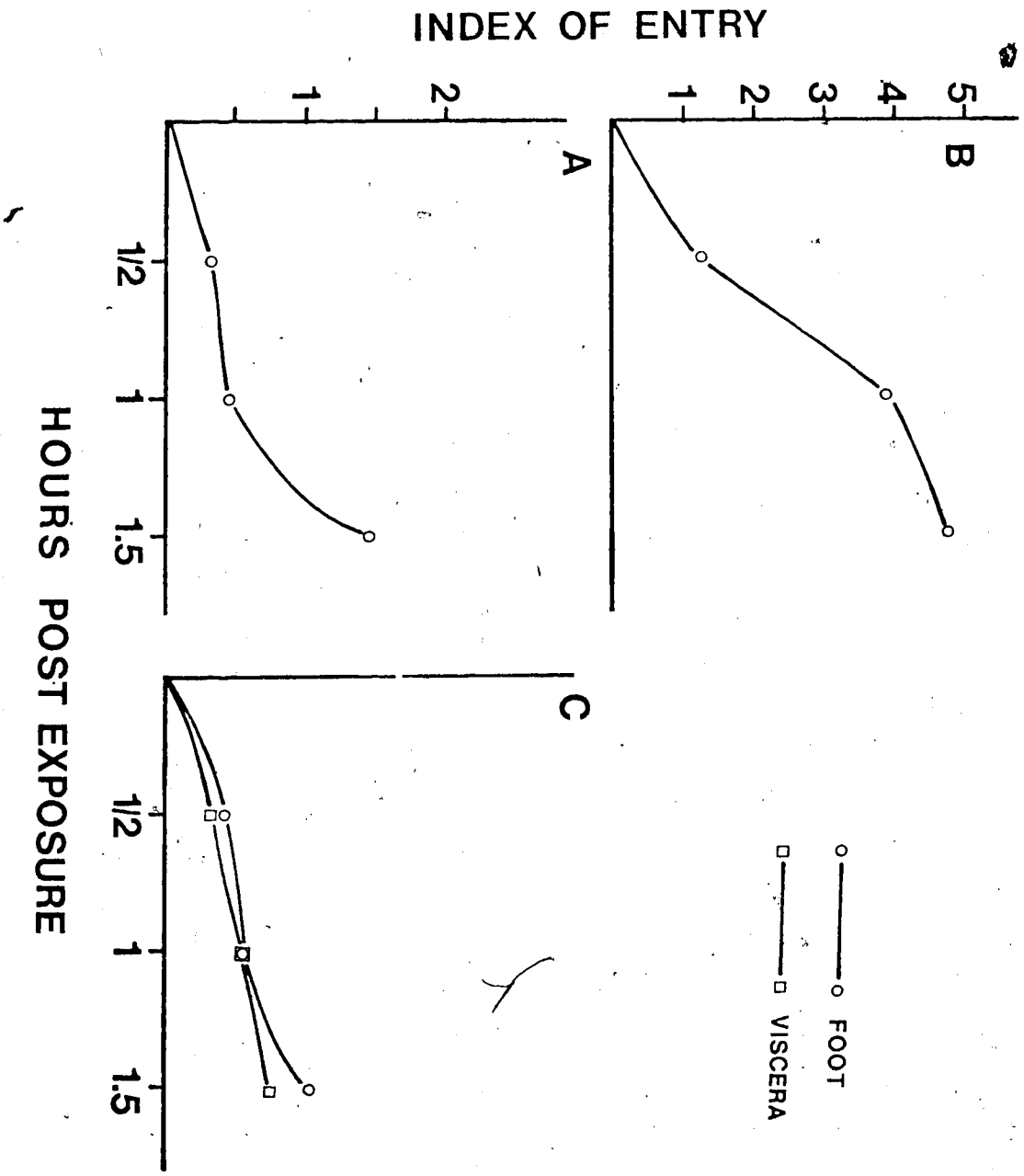
The vast majority of larvae of *P. odocoilei* were observed in the foot of *V. limpida* (Fig. 38b) with a small percentage (7%) in areas other than the foot. The largest increase in the entry index (2.86)

Figure 38. Index of larval entry of *Parelaphostrongylus odocoilei* into several species of terrestrial gastropods.

a. *Deroceras laeve*

b. *Vitrina limpida*

c. *Zonitoides arboreus*


INDEX OF ENTRY
A
B
C
1
2
3
4
5
1/2
1
1.5
HOURS POST EXPOSURE
FOOT
VISCERA

occurred for *V. limpida* between 1/2 and 1 hour PE and declined to 0.86 from 1 to 1-1/2 hours PE.

Zonitoides arboreus was the only species of mollusc that had a high index of entry for the viscera; i.e., via the alimentary canal. The index was similar at all time periods for the foot and viscera (Fig. 38c). Larval entry into the foot was highest (0.41 and 0.47) from 0 to 1/2 and 1 to 1-1/2 hours PE, respectively. The index was relatively stable for larvae appearing in the viscera; 0.34, 0.21, and 0.21 at 1/2, 1 and 1-1/2 hours PE.

Larval entry was poor in the slug, *Deroceras reticulatum*. The highest index value was 0.058 in the foot at 1-1/2 hours PE. This is only one-sixth of the next lowest recorded value, 0.35 for *D. laeve* at 1/2 hour PE. Larvae were observed in the viscera of a single *D. reticulatum* at 1 hour PE (see Appendix IV).

Larval Growth

Larval growth of *P. odocoilei* was monitored by measuring the following parameters: total length, maximum width, length of the esophagus and the distance of the excretory pore, nerve ring, genital primordium and anus from the anterior end of the worm. Ten larvae were measured from each of the time intervals previously described.

There was little evidence of change in any parameter except maximum width during the first six days of infection (Figs. 39, 40a-d). Regression analysis of total length during this period was not significantly different from zero (F_s=2.09; $F_{.05[1,3]}$ = 10.1). The first molt began on day 7 and was complete 10 days PE. Length increased gradually, after the first molt and showed a significant increase (Table X) at

Figure 39. Larval growth of *Parelaphostrongylus odocoilei* in experimentally infected *Triodopsis multilineata* ag 18°C. Length and width. Arrows indicate the first and second larval molts, respectively.

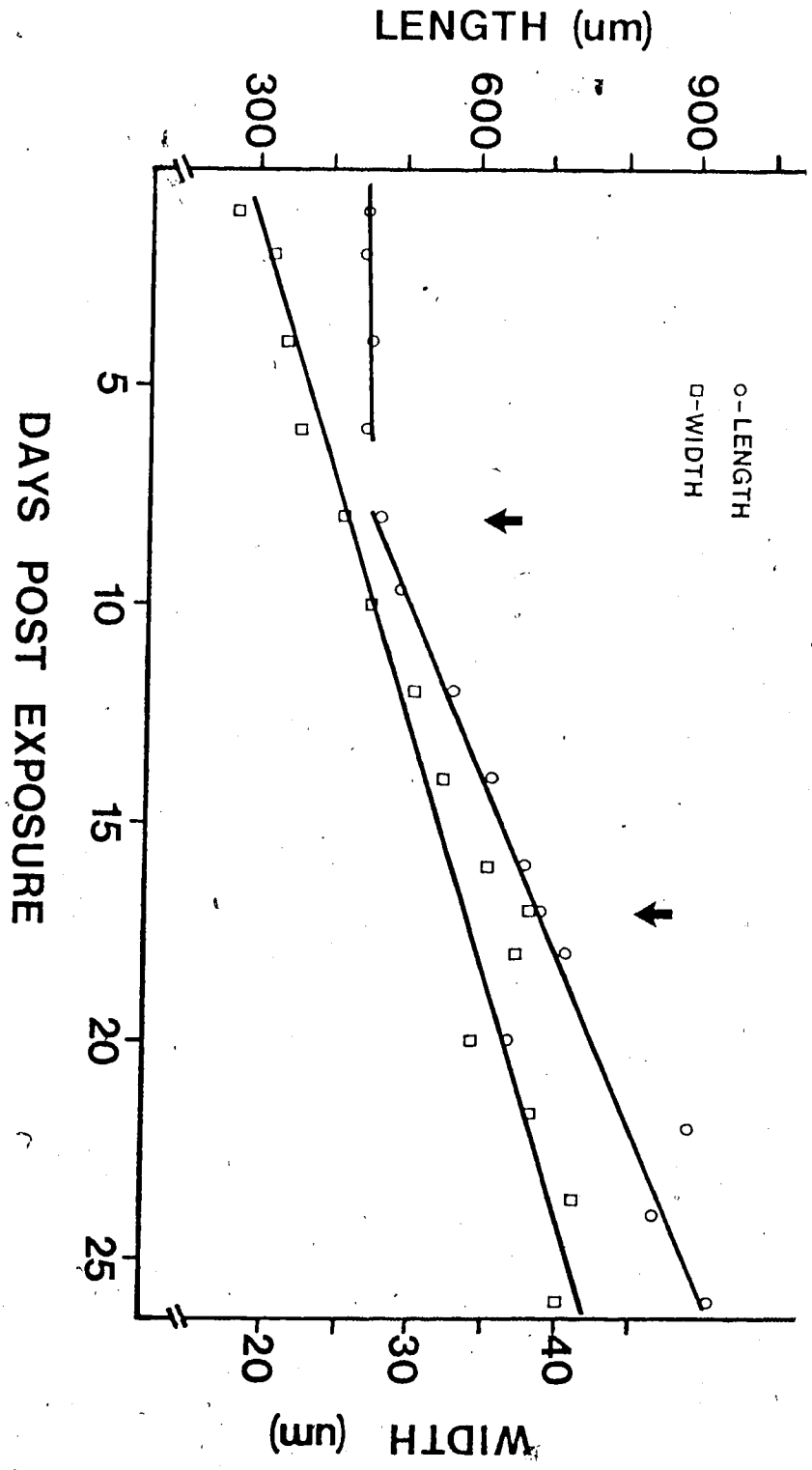

LENGTH (um)
300
600
900
DAYS POST EXPOSURE
5
10
15
20
25
WIDTH (um)
20
30
40
o-LENGTH
□-WIDTH

Figure 40. Larval growth of *Parelaphostrongylus odocoilei* in experimentally infected *Triodopsis multilineata* at 18°C. Arrows indicate the first and second larval molts, respectively. Additional parameters:

a. Distance of genital primordium from the anterior end.

b. Distance of the anus from the anterior end.

c. Distance of the excretory pore and nerve ring from the anterior end.

d. Length of the esophagus.

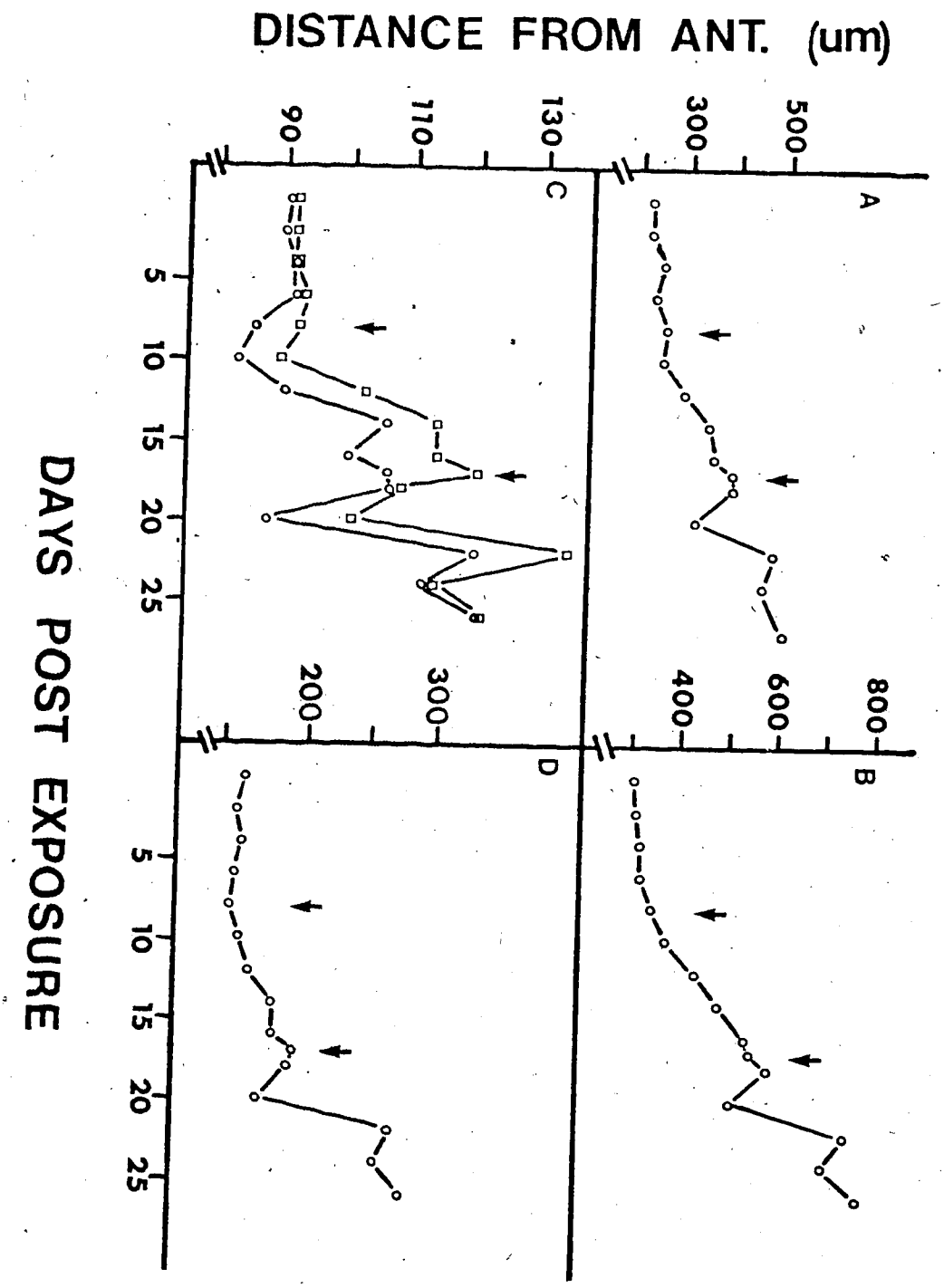

DISTANCE FROM ANT. (um)
A
B
C
D
300
500
400
600
800
90
110
130
200
300
5
10
15
20
25
DAYS POST EXPOSURE

TABLE X. *t*-VALUES FOR THE LENGTH OF *PARELAPHOSTRONGYLUS ODOCOILEI* LARVAE IN *TRIODOPSIS MULTILINEATA* (18°C) AT TWO DAY INTERVALS

Days	t_s	P<
4 vs 6	0.613	NS
6 vs 8	1.309	NS
vs 10	2.102	.05
10 vs 12	4.399	.001
12 vs 14	2.402	.05
14 vs 16	2.174	.05
16 vs 17	0.962	NS
16 vs 18	3.377	.01
18 vs 20	4.487	.001
20 vs 22	6.827	.001
22 vs 24	1.228	NS
24 vs 26	2.837	.05
22 vs 26	0.748	NS

$n = 10$

$t_{[.05]}^{18} = 2.101$

$t_{[.01]}^{18} = 2.898$

$t_{[.001]}^{18} = 3.965$

each two day interval from 8 to 22 days PE. Maximum width increased gradually throughout the examination period and did not display the initial plateau seen for maximum length (Fig. 39).

The length of the esophagus as well as the distance of the genital primordium and anus from the anterior end showed similar increases (Fig. 40). The distance of the excretory pore and nerve ring from the anterior end showed a slight decline on days 8 and 10, but increased rapidly through days 12 and 14 PE.

The second molt, to the third stage (L3), began 17 days PE and was complete by day 22. At day 20 there was a significant decline in all characters measured (Figs. 39, 40). A second group of ten larvae collected at this time was measured. Although measurements from this group were somewhat higher they showed a similar decline.

Infective larvae (Gerichter 1948) were found beginning 22 days PE; approximately 80% of the larvae collected were considered infective by day 26. These larvae increased in total length (Fig. 39), while width remained constant.

Host Response

Larvae of *P. odocoilei* penetrated the foot of *T. multilineata* by the first examination period, 3 hours PE (Fig. 41). Note the displacement of the epidermal cells and the absence of any visible cellular reaction to the invading larvae. There was no cellular reaction evident to the larvae of *P. odocoilei* at 12 hours PE (Fig. 42).

The host response was clearly evident 1 day PE (Fig. 43). A large number of amoebocytes surrounded larvae resulting in a focal response, 1 to 2 cell layers thick. At 2 days PE (Fig. 44), the number of

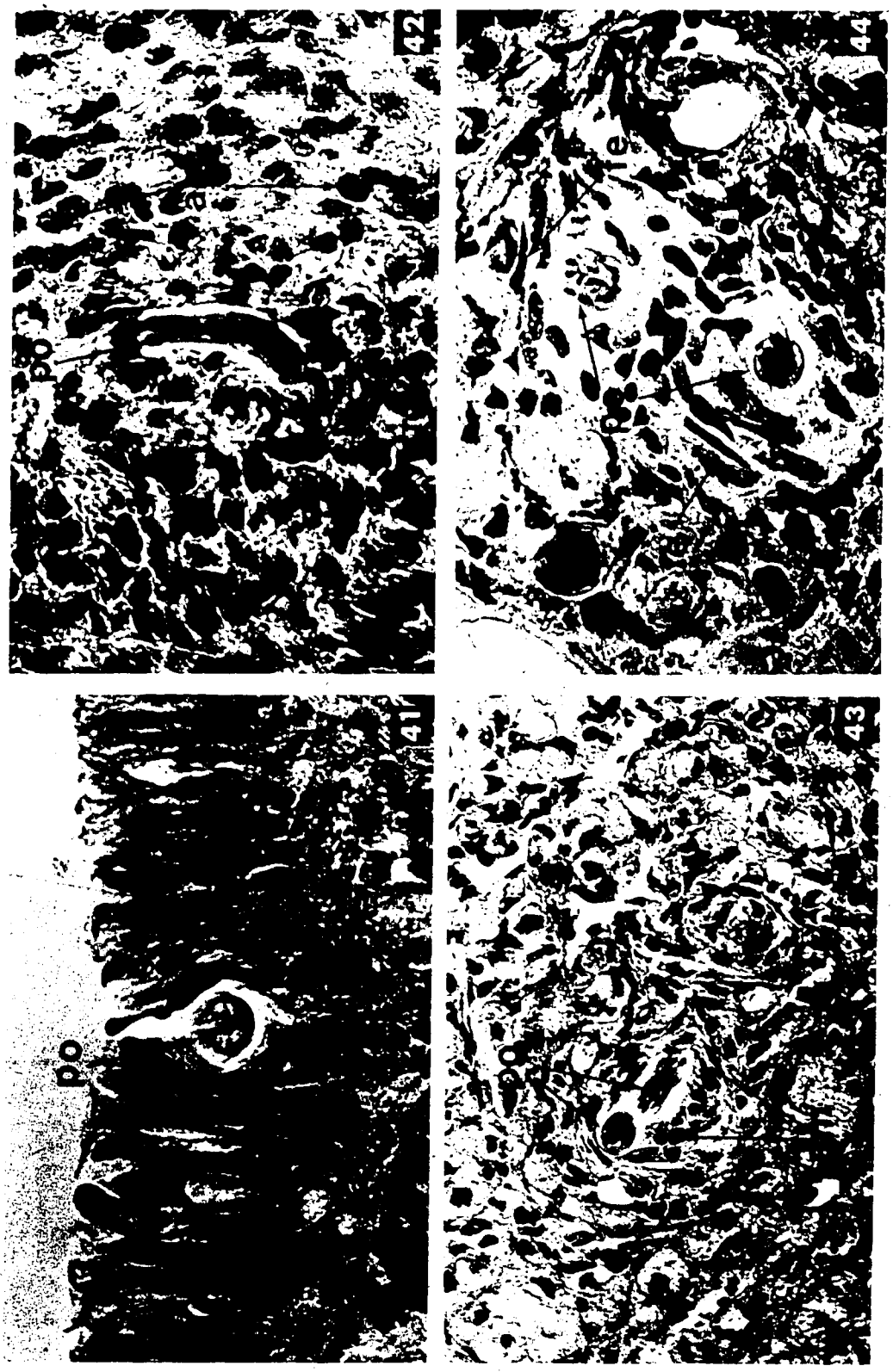
Figures 41-44. Ontogeny of the response of *Triodopsis multilineata* experimentally infected with *Parelaphostrongylus odocoilei*. (Foot region).

Fig. 41. 3 hours post-exposure. 250X. (ec - epidermal cells, po - *P. odocoilei*).

Fig. 42. 12 hours post-exposure. 400X. (am - amoebocytes, po - *P. odocoilei*).

Fig. 43. 24 hours post-exposure. 250X. (same as above).

Fig. 44. 2 days post-exposure. 400X. (fe - fibrotic elements, po - *P. odocoilei*).


42
Po
41
Po
44
Ie
43

amoebocytes surrounding larvae had increased substantially. The reaction had a solid appearance in histological section. Some of the amoebocytes at the periphery of the reaction were elongate, forming fibrous elements (Fig. 44). There was no sign of degeneration in the encapsulated larvae.

A large congregation of amoebocytes accumulated by 4 days PE (Fig. 45). There was no hyperplasia of myofibrous tissue, although this tissue may help establish boundaries and, hence, a focal point of reaction of an individual cyst. Amoebocytes at the periphery of the cyst continued to flatten, while there was no apparent change in the appearance of the cells at the center of the reaction. The influx of amoebocytes apparently stopped 6 to 8 days PE, as the majority of cysts were reduced in size from this point in time. The boundaries of the cyst were well defined (Fig. 46) and there was no evidence of a proliferative response. The larvae were normal in appearance.

At 8 days PE (Fig. 47) the cyst structure was similar to that observed at day 6. The first larval molt occurred and the gut of the nematode had begun to accumulate food granules. These granules stained strongly with Orange G, an acidophilic stain, which may be indicative of protein synthesis. There was little change in the overall cyst structure from 10 to 16 days PE (Figs. 48-51).

The second molt, to the third stage, was first observed 17 days PE. The cyst structure at 18 days PE had undergone little change (Fig. 52). The outer cyst wall was composed of 1 to 2 layers of flattened amoebocytes. The newly molted L3's of *P. odocoilei* contained a large number of food granules in the intestine at 20 days PE (Fig. 53). The cyst was still composed of a large number of amoebocytes in the central core

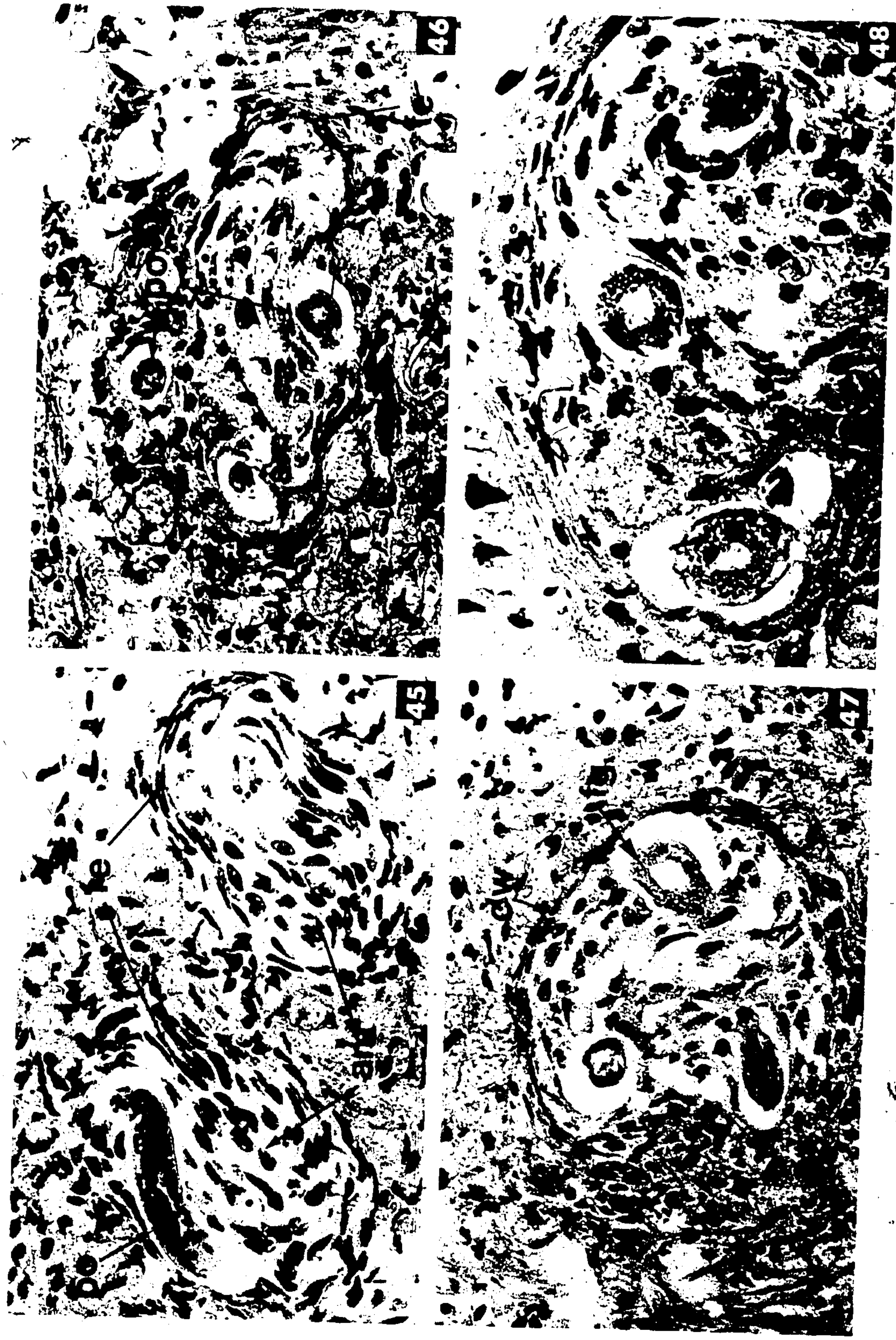
Figures 45-48. Ontogeny of the response of *Triodopsis multilineata* experimentally infected with *Parelaphostrongylus odocoilei*. (Foot region).

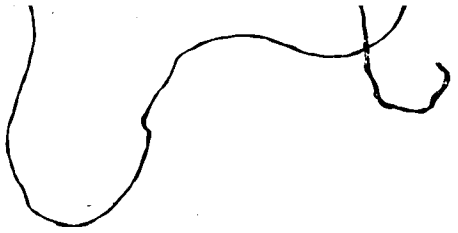
Fig. 45. 4 days post-exposure. 250X. (am - amoebocytes, fe - fibrotic elements).

Fig. 46. 6 days post-exposure. 250X. (am - amoebocytes, fe - fibrotic elements).

Fig. 47. 8 days post-exposure. 250X. (cw - cyst wall, fe - fibrotic elements).

Fig. 48. 10 days post-exposure. 400X. (Note the food granules in the intestine and cyst wall).


46
48
45
47



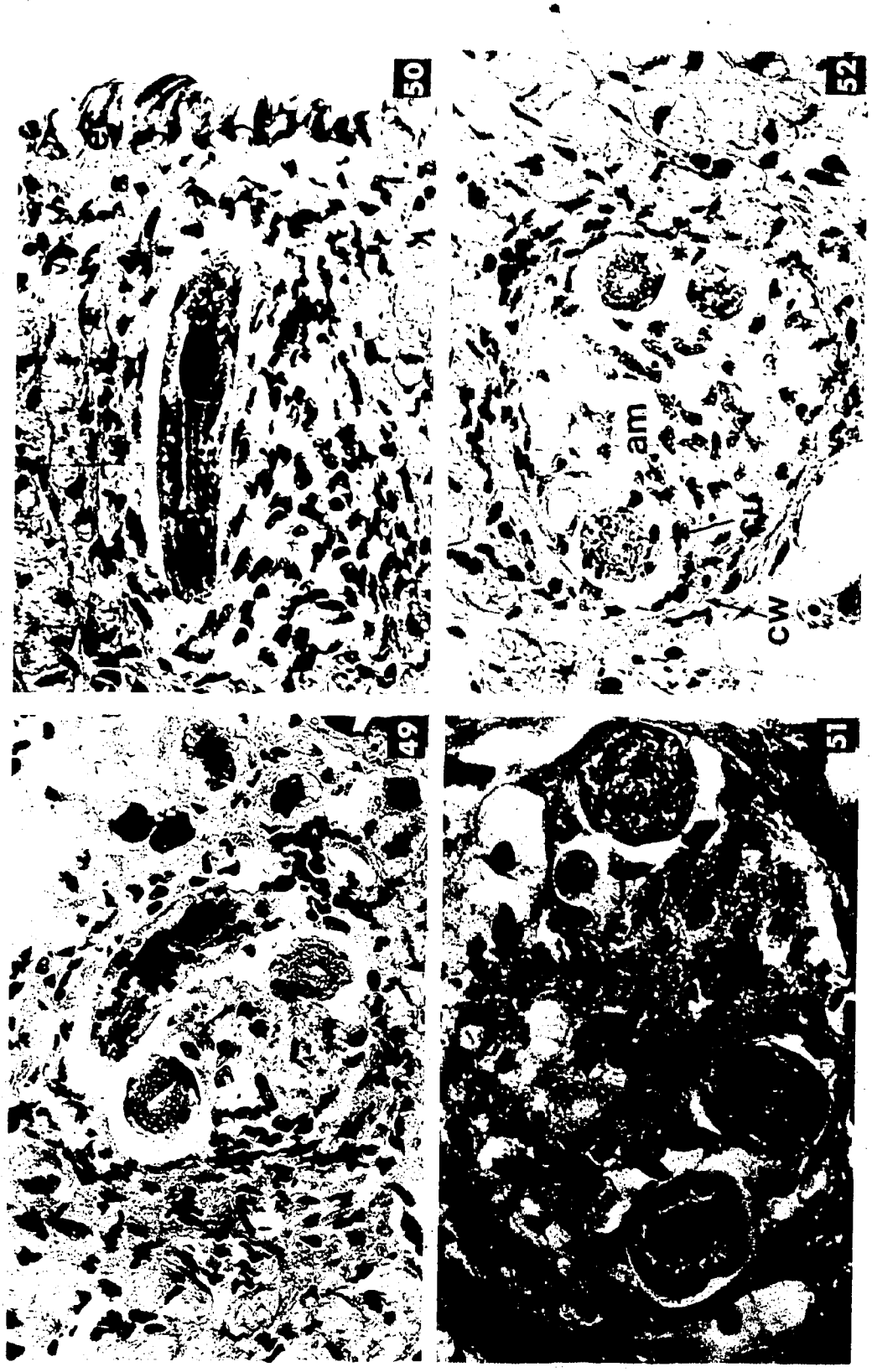
Figures 49-52. Ontogeny of the response of *Triodopsis multilineata* experimentally infected with *Parelaphostrongylus odocoilei*. (Foot region).

Fig. 49. 12 days post-exposure. 250X.

Fig. 50. 14 days post-exposure. 250X. Anterior end of larvae.

Fig. 51. 16 days post-exposure. 400X. (am - amoboecytes, cw - cyst wall).

Fig. 52. 17 days post-exposure. 250X. (cu - shed cuticle, cw - cyst wall, am - amoebocytes).


50
52
am
CW
49
51

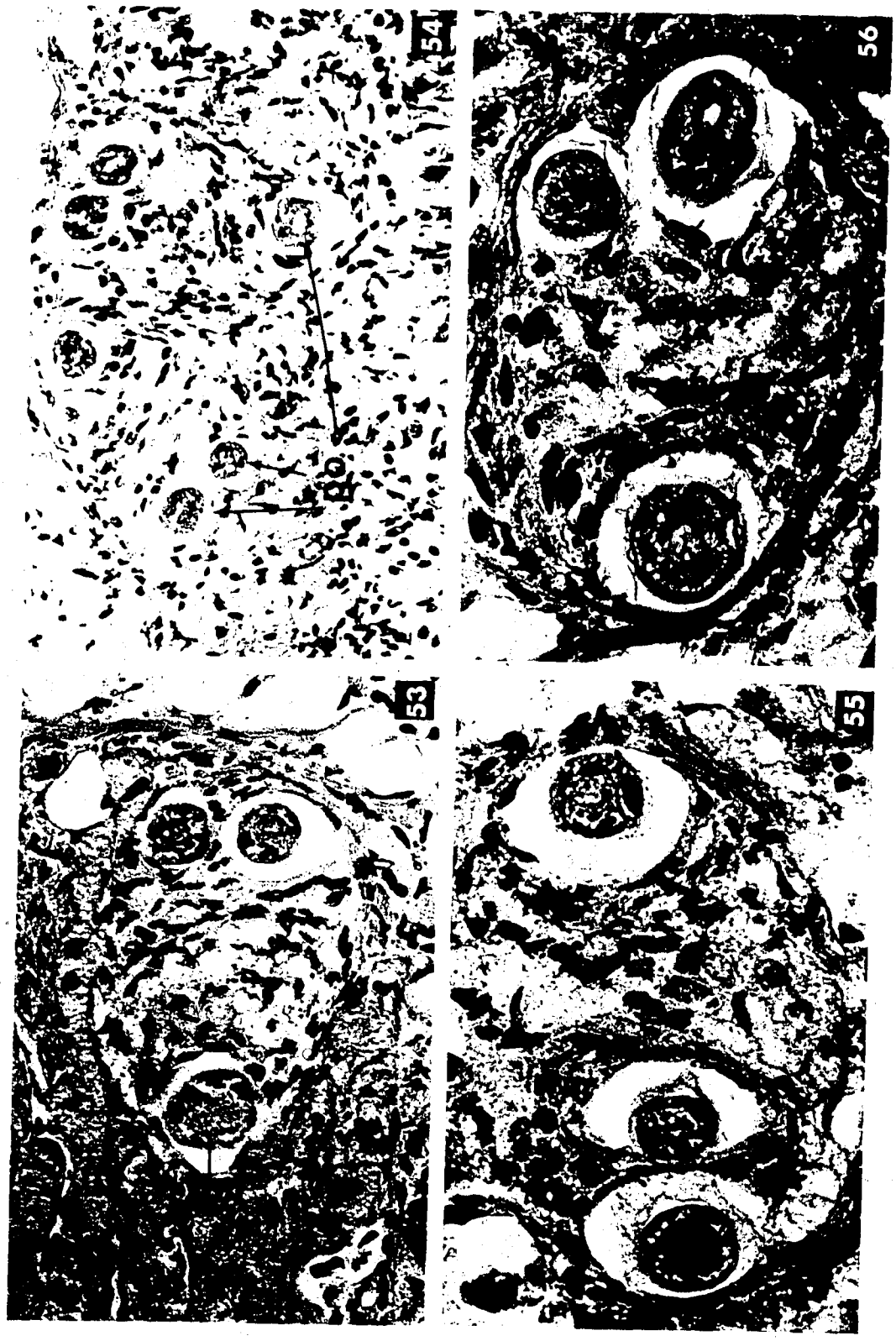
Figures 53-56. Ontogeny of the response of *Triodopsis multilineata* experimentally infected with *Parelaphostrongylus odocoilei*. (Foot region).

Fig. 53. 20 days post-exposure. 250X. (fg - food granule).

Fig. 54. 22 days post-exposure. 250X. (po - *P. odocoilei*). Note the presence of two separate cysts.

Fig. 55. 24 days post-exposure. 400X.

Fig. 56. 26 days post-exposure. 400X. Note the thin nature of the outer cyst wall.


54
56
53
55

(Figs. 54, 55) and a single or double layer of fibroblast-like cells at the periphery. Upon attaining the infective stage, larvae of *P. odocoilei* were practically devoid of the food granules that characterized the preinfective forms (Fig. 56).

Site of Larval Development

Larval *P. odocoilei*, at various stages of development, were recovered primarily from the foot region (Table XI) of *T. multilineata*. Analysis of snail size (non-shelled weight) versus the number of larvae found showed no relationship (r=0.22) and was not considered further.

For the purpose of analysis the snails were divided into three equal groups: early (1-8 days PE), middle (10-17 days PE) and late (18-26 days PE). Each group contained 10 snails and was roughly equivalent to the first, second and third developmental stages of *P. odocoilei* in *T. multilineata* at 18°C. Analysis of variance (Sokal and Rohlf 1969) identified a significant ($F_{[2,28]}$ $0.05 = 3.34$; $F_s = 3.67$) increase in the number of larvae found in the viscera through time. No significant increase was demonstrated for the number of larvae in the foot or the total number of larvae through time.

D. Discussion

The first-stage larvae of metastrongyloid nematodes have two options for gaining entry into the terrestrial molluscs that serve as intermediate host for the vast majority of these parasites. The first, direct penetration of the molluscan epithelium, represents an active mode of entry. The second, ingestion and subsequent penetration of the gut and a migration to the site of development, is a passive mode.

TABLE XI. DISTRIBUTION OF THE LARVAE OF *PARELAPHOSTRONGYLUS ODOCOILEI* BETWEEN THE FOOT AND VISCERA OF EXPERIMENTALLY INFECTED *TRIDOPSIS MULTILINEATA* (18°C) AT TWO DAY INTERVALS

(Days) Post-infection	Deshelled Weight of *T. multilineata* (grams)	No. Larvae		
		Foot	Viscera	Total
1	1.0	62	2	64
	1.96	40	1	41
2	1.56	275	1	276
	0.71	30	0	30
4	1.42	61	0	61
	0.97	128	0	128
6	1.53	102	0	102
	1.29	186	0	186
8	1.55	51	0	51
	1.30	94	0	94
10	1.10	97	2	99
	1.27	179	1	180
12	1.19	26	0	26
	1.45	459	1	460
14	1.34	174	6	180
	0.35	83	1	84
16	1.15	153	1	154
	1.15	122	7	129
17	1.37	316	3	319
	1.02	139	3	142
18	0.63	113	1	114
	1.10	125	7	132
20	1.16	279	0	279
	1.05	70	18	88
22	1.02	104	3	107
	1.70	124	1	125
24	0.97	112	2	114
	1.61	190	2	192
26	1.40	115	2	117
	1.28	141	18	159
TOTALS		4160	83	4243

Both means of entry have been reported for various species of this group, resulting in a minor controversy as to which mode, active or passive, is used by metastrongyloids in natural situations.

The confusion as to the primacy of active versus passive entry of larvae is primarily the result of the choice of molluscs used in the infection trials. Metastrongyloidea (sensu Anderson 1978) are parasites of terrestrial mammals, with the exception of the Pseudaliidae of marine mammals which apparently utilize fish as an intermediate host (Dailey 1970).

Field studies of the terrestrial metastrongyloids have overwhelmingly resulted in the identification of a terrestrial mollusc(s), generally of the order Stylommatophora, as the intermediate host of the nematode under investigation (Mitsekivich 1964; Panin 1964a; Alicata 1965; Lankester and Anderson 1968; Wallace and Rosen 1969; this study Section IV). Examination of aquatic molluscs for involvement in metastrongyloid life cycles has been negative (Panin 1964a; Lankester and Anderson 1968; Mowatt 1973) although aquatic molluscs are capable of harboring these larvae in experimental infections (Anderson 1963; Panin and Rushkova 1964; Cheng and Burton 1965). Results of Lim *et al.* (1965) are an exception. They found three species of aquatic gastropods (Order Basommatophora) naturally infected with *Angiostrongylus cantonensis*, the rat lungworm.

The majority of workers advocating a passive, peroral entry of these larvae into the intermediate host have used aquatic snails and exposed them to infection in an aqueous suspension of larvae (Courdurier *et al.* 1967; Richards and Merritt 1967; Drozdz *et al.* 1971;

Harris and Cheng 1975a; Petter and Cassone 1975; Rachford 1976a). Directional movements of larval nematodes are severely limited in an aquatic medium; penetration of a solid substrate under these conditions is virtually impossible (Looss 1905; Anderson 1962b). First stage larvae of *Aelurostrongylus pridhami* were unable to penetrate molluscs in an aquatic medium unless they used surface tension as a point to work against (Anderson 62b). These larvae would, therefore, have difficulty gaining entry into a mollusc in an aquatic medium unless they were ingested or accidentally swept into the lung (Anderson 1962b; Harris and Cheng 1975a).

Studies of this problem using terrestrial molluscs, on a solid substrate, have resulted in the conclusion that direct penetration of the foot is the primary, if not only mode of larval entry (Hobmaier and Hobmaier 1929, 1930, 1934a, 1934b; Hobmaier 1934, 1941; Kassai 1958; Svarc and Lest'tan 1969; Svarc and Zmoray 1974). It has been stated that larvae ingested by terrestrial molluscs are inviable (Hobmaier 1934; Hobmaier and Hobmaier 1934b; Anderson 1962b).

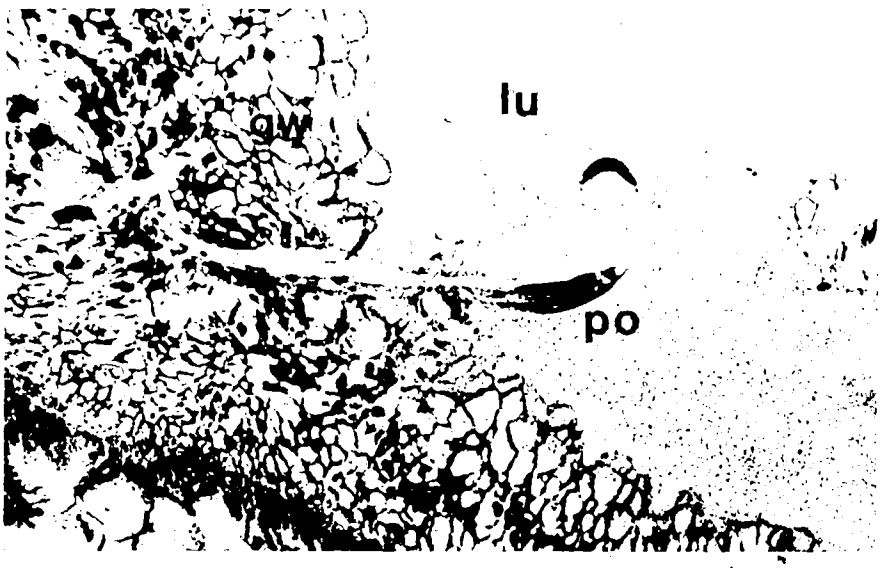
Results of the present study suggest that, although both modes of entry are possible, direct penetration of the ventral epithelium predominates for *P. odocoilei*. Ingestion of larvae occurred in three of the four species, but was only an important component for *Zonitoides arboreus*. There is no apparent explanation for this finding. Obviously, some component used in the experimental trials initiated a feeding response in *Z. arboreus*. A similar but reduced response was evident in the other snail, *V. limpida*, but with a single exception was absent in the slugs, *Deroceras* spp. Whether the larvae ingested by the molluscs

in this study were capable of completing development is unknown. Larvae of *P. odocoilei* were capable of penetrating the gut of *H. arborea* at escaping the digestive tract (Fig. 57). First-stage larvae of *A. cantonensis* were able to develop to the infective stage regardless of the mode of entry into the giant African land snail (Cheng and Alicata 1965).

The method of exposing molluscs to metastrongyloid larvae may, therefore, contribute to the primacy of one mode of entry over the other. The aforementioned differences in experimental design may also affect the site of larval development. The majority of authors favoring ingestion as the primary mode of entry (see above) also report larval development from a wide variety of sites in the mollusc. Those favoring direct penetration (see above) indicate that larval development is restricted to the region of the foot (however, see Hamilton 1969). Svarc and Zmoray (1974) concluded that larvae of *Muellerius tenuispiculatus* were nutritionally restricted to the gland-rich tissue of the mollusc foot. These authors also reported that larvae found outside this glandular region were retarded in their development. Larvae of *Parelaphostrongylus tenuis* found outside of the foot also failed to develop (Anderson 1963). This "site selection" hypothesis may account for reports of unequal rates of development of metastrongyloid larvae in individual molluscs (Hobmaier 1941; Gerichter 1948; Rachford 1976a).

In the present work the majority of larvae in experimental molluscs were found in the foot. From the first to second molt a small, but increased, number of larvae were found in tissue outside the foot region. There was a significant increase in the number of larvae

Figure 57. First-stage larvae of *Parelaphostrongylus odocoilei* penetrating the gut wall of *Zonitoides arboreus*. Experimental infection, 1 hour post-exposure. (Whipf's polychrome).


lu
gw
po

found in the visceral mass after the molt to the third stage which is indicative of larval activity and movement after becoming infective. Movement of L3's within a mollusc is not a new phenomenon and has been reported on several occasions (Senevitatna 1959a; Anderson 1962b; Harris and Cheng 1975a). Svarc and Zmoray (1974) flatly stated, however, that larvae of *M. tenuispiculatus* do not move once encapsulation has occurred.

Two components possibly involved in influencing the mode of entry and site of development of larval metastrongyloids that have received little attention are the systematic positions of the parasite and the molluscan intermediate host (Anderson 1962b). There is little concrete evidence in the present study concerning these aspects of the life cycle; however, trends are apparent in the literature and the present data that, I believe, warrant comment.

The striking observation is that the majority of studies reporting peroral entry and larval development in a wide variety of locations involve lungworms of carnivores and rodents (Angiostrongylidae and Filarioididae of Anderson 1978): *Filaroides martis* and *Aelurostrongylus pridhami* by Anderson (1962b); *Aelurostrongylus abstrusus* by Hamilton (1969), *Morerastrongylus andersoni* by Petter and Cassone (1975), *Angiostrongylus vatonensis* by Courdurier *et al.* (1967), Richards and Merritt (1967), Harris and Cheng (1975a) and Rachford (1976a), *Parastrongylus dujardini* by Drozdz *et al.* (1971). Workers reporting direct penetration and development in the foot have worked with ruminant lungworms (Protostrongylidae of Anderson 1978): *Synthetocaulus (=Muellerius) capillaris* and *Protostrongylus rufescens* by the Hobmaiers

(1929, 1930), *Parelaphostrongylus tenuis* by Anderson (1963), *Muellerius tenuispiculatus* by Svarc and Zmoray (1974) and several other protostrongylids (Kassai 1958). Differences in the biological valency of these organisms, as reflected by their systematic position, are far from proven (Anderson 1962b), but certainly warrant further examination under controlled conditions.

The role of the intermediate host is more difficult to define. Aquatic pulmonates (Order Basommatophora) are generally exposed to first stage larvae under different experimental conditions than their terrestrial counterparts, the Stylommatophora. Differences in the mode of entry and the site of development may be the result of experimental design rather than the systematic position of the molluscan host. Again, controlled studies would establish the relationship.

The ecological significance of the mode of entry of metastrongyloid larvae into a susceptible intermediate host is unclear. This relates primarily to the paucity of knowledge of the ecology and behavior of metastrongyloid larvae and terrestrial molluscs under field conditions. First stage larvae of *P. odocoilei* are capable of direct penetration of all species of mollusc tested experimentally. Therefore, without detailed knowledge of the location of the first stage larvae under field conditions and the events surrounding host-parasite contact, ingestion versus penetration is a moot point. Entry via ingestion is dependent upon either the larvae or the substrate the larvae rest on inducing a feeding response in the mollusc. Direct penetration only requires recognition of a susceptible mollusc by the larvae.

The ontogeny of the response of *T. multilineata* to the larvae of *P. odocoilei* is similar in many respects to the results of previous studies of this problem (Richards and Merritt 1967; Svarc and Zmoray 1974; Harris and Cheng 1975a; Rachford 1976a; Sauerlander 1976). Molluscs respond to foreign material that is too large to phagocytize by amoebocytic encapsulation (Tripp 1961). The response, as described by Harris and Cheng (1975a) is biphasic: 1) infiltration and aggregation of amoebocytes, and 2) the conversion of loosely arranged amoebocytes into a compact fibrotic capsule.

The first phase of the response was evident in *T. multilineata* 24 hours post-infection (Fig. 43). It is temperature dependent (Tripp 1970) and generally evident within 24 hours of initial exposure (Tripp 1961; Harris and Cheng 1975a; Sauerlander 1976). However, the response of *Lymnaea palustris* to *A. cantonensis* is apparently delayed for the first several days PE (Rachford 1976a).

The larvae move through the epidermal region of the foot and become quiescent either just below the ventral epithelium or at the proximal edge of the glandular tissue (Hobmaier 1934; Kassai 1958; Svarc and Zmoray 1974). Harris and Cheng (1975a) described amoebocytic "trails" as evidence of an active migration of larvae of *A. cantonensis* along the rectal ridge of *Biomphalaria glabrata*. Similar trails were not seen in *T. multilineata*, which is most likely the result of the direct nature of larval entry and the short distance involved.

The transformation of amoebocytes of *T. multilineata* into fibrous-like elements 4 days PE, corresponds to the infiltration of fibroblasts of previous workers (Tripp 1961, 1963). Harris (1975) demonstrated

conclusively, using transmission electron microscopy, that the fibrous capsule formed in nematode infections in *B. glabrata* was the result of a large number of cytoplasmic extensions formed from granular leukocytes (= amoebocyte) already present, and not the result of a later influx of formed, fibroblast cells supporting the observations of Pan (1965) at the light microscope level.

The result of the reaction is that the nematode larvae are sequestered in a fibrous capsule. The larvae retain a normal appearance and development and are infective to the definitive host, when such trials are made. There do not appear to be any major differences in reaction of aquatic (Basommatophora) and terrestrial (Stylommatophora) gastropods to the larvae of metastrongyloid nematodes. Thus, species of either group can be used in further research of this phenomenon.

The molluscan response to foreign material, first described by Tripp (1961) and later defined more precisely by Pan (1963, 1965), is a non-specific, non-proliferative, cellular reaction, or more simply, a Type 1 reaction (Pan 1963). The Type 1 reaction may or may not result in the destruction of the encapsulated organism. Larval trematodes (mother sporocysts) were destroyed and resorbed (Pan 1963, 1965). There was no histologic evidence in the present study to indicate that larval metastrongyloids are destroyed by this form of host response. Drozdz *et al.* (1971) reported tissue reactions in aquatic and terrestrial molluscs to *Parastrongylus dujardini* that ranged from no observable reaction to the complete destruction and resorption of the parasite. Rachford (1976a) also reported the destruction of a few larvae of *A. cantonensis* by *Lymnaea palustris*. Neither author provided histological evidence to substantiate these observations.

Histochemical studies (Harris and Cheng 1975b) of *A. cantonensis* encapsulated by *B. glabrata* revealed the presence of acid phosphatase, nonspecific esterase, alkaline phosphatase and β-glucuronidase activity localized in the cells forming the capsule. These authors hypothesized that the larvae may be resistant to these enzymes, or more probably, that the enzymes are not released into the lumen of the capsule, hence the larvae are not destroyed. There is no explanation for this failure to destroy the encapsulated material.

The capsule changes only slightly from the formation of the fibrotic elements. Additional layers of fibrous material may be laid down; however, from approximately 8 days PE the capsule decreases in size, sooner than previously reported (Harris and Cheng 1975a). Some destruction of amoebocytes occurs (Sauerlander 1976), eventually resulting in a cyst consisting of one to two layers of fibrous cells. In a few instances (Kassai 1958) only a slight tissue reaction was reported. His results were from a two month old infection and undoubtedly represented the end result of a normal encapsulation reaction as presently described.

The encapsulation process is dependent solely upon activity of amoebocytes (Pan 1965; Harris and Cheng 1975a; Rachford 1976a). Myofibrous encapsulation has been reported (Cheng and Rifkin 1970) in the giant African land snail, infected with *A. cantonensis*. Muscle fibers are passively involved in the encapsulation of *P. odocoilei* in *T. multilineata*. As amoebocytes collect around a larva, the myofibers may become displaced and form a border; however, there is no evidence of hyperplasia in these cells or that they engage actively in the

encapsulation process (Harris and Cheng 1975a; Sauerlander 1976).

The effect of this encapsulation process on the developing nematode or its benefit to the mollusc remain unclear. Several authors (Harris and Cheng 1975a; Harris 1975) have stressed the dynamic aspects of this process and that it represents more than a mechanism for the isolation of foreign material. The fact remains that polystyrene spheres and pollen grains elicit an identical response (Tripp 1961) and neither is damaged as a result of being encapsulated. A certain percentage of degenerate mother sporocysts, encapsulated in an analogous fashion (Pan 1963, 1965), are destroyed. Therefore, irregardless of the dynamic aspects of the process, the end result of this response to the larvae of metastrongyloid nematodes is the isolation of tissue recognized as foreign with no apparent injury to the parasite or benefit to the mollusc. Additional studies, accurately delimiting the nature of this interaction, are required.

Larval development and growth of *P. odocoilei* in *T. multilineata* are similar to that of other metastrongyloids (Mackerras and Sandars 1955; Seneveristna 1959b; Drozdz *et al.* 1971; Svarc and Zmoray 1973; Rachford 1976b) and larval nematodes in general (Scott 1929; Stoll 1940; Sommerville 1960; Fisher 1970). Little increase in total length is evident during the initial phase of infection (Mackerras and Sandars 1955; Bhaibulaya 1975). This phase is also characterized by the accumulation of a large number of refractile granules, presumably lipid reserves, in the gut (Gerichter 1948; Seneveristma 1959a; Svarc and Zmoray 1973; Bhaibulaya 1975). The decline in all characters measured at day 20 PE is an enigma. Bhaibulaya (1975) reported a

decrease in overall length of *Angiostrongylus makerrasae* in *Helicarion* sp. at 2 days PE, however, and initial decrease in length, accompanied by an increase in width is generally expected. At day 20 the larvae of *P. odocoilei* had molted, but had not yet reached the infective stage. Aestivation of the mollusc is known to retard larval development (Lankester and Anderson 1968); however, this does not merely result in slightly stunted worms, but suspends development completely. According to Kassai (1958) this decline is best viewed as host induced variability.

Gerichter (1948) proposed that larval development of the metastrongyloids within the mollusc be viewed as four distinct phases rather than the three commonly recognized. The first three stages of Gerichter encompass the common stages, separated by two intervening molts (Chitwood and Chitwood 1951; Hyman 1951). The fourth stage, according to Gerichter, includes the period from the second molt (L3) to the point of development at which the larva was infective to the definitive host or under laboratory conditions survived an artificial digestion procedure. Although the 'maturation period' has not been described for all species of metastrongyloids examined (Seneviratna 1959a) the concept of an obligate period of maturation of the L3's of some species of this group of nematodes may be ecologically important and must be identified for each species. The maturation period for *P. odocoilei* is approximately 5 days, the second molt begins 17 days PE and infective larvae were not found until 22 days PE.

SECTION VI

EVOLUTION AND ZOOGEOGRAPHY

A. Evolution

Introduction

The use of parasites as a tool for zoogeographic analysis was initiated by von Ihering (1891) as a minor component of a general attempt to demonstrate similarities of the freshwater fauna and flora of South America, Australia and New Zealand. He later expanded this to a comparison of the helminth fauna of the vertebrates of South America and other parts of the world (von Ihering 1902).

The use of parasites to establish zoogeographical connections of host organisms, later termed the "von Ihering Method" (Metcalf 1929), was independently pursued by a number of investigators at the turn of the 20th century (see Harrison 1928 and Metcalf 1929 for reviews of the early literature). More recently, Manter (1955) analyzed the zoogeographic relationships of the trematodes of marine fishes: Bullock (1970), the Eoacanthocephala of fishes; and Stromberg and Crites (1974) the Camallanidae (Spiruroidea:Nematoda) of the world. Manter (1967) briefly reviewed aspects of geographical distribution of parasites and outlined rules of analysis which were proposed more formally by Stromberg and Crites (1974). They are:

1. Parasites with generalized host requirements are the most likely to disperse.

2. Evolution gradually results in the specialization of parasites and host specificity increases as the relationship gets older.

3. The greatest variety of parasites is likely to occur where the host has lived the longest.

Concomitant to the zoogeographical analysis of host-parasite relationships is the concept of co-evolution of host and parasite. This concept has had a long history in the parasitological literature (Metcalf 1929, 1940; Dogiel *et al.* 1964; Cameron 1964). Cameron (1964) stated, "parasites have obviously evolved coincidentally with their hosts" and that "parasite phylogeny and classification can only be interpreted in terms of host phylogeny and classification." Phylogenetic relationships have been demonstrated for several parasites and their hosts: opalinid ciliates of frogs (Metcalf 1923, 1929, 1940); botflies of [illegible]s and perissodactyls (Rubtsov 1939); and *Setaria* (*s.l.*) sp[illegible]tes (Yeh 1959).

Recently [illegible] of host and parasite, particularly nematodes, has come under attack (Chabaud, 1957; Inglis 1965). Inglis (1965) stated, "evolution of most groups of nematodes has tended to occur in groups of hosts with similar ecological requirements." This has been supported by the work of Durette-Desset (1971) for the trichostrongyles of rodents and by Quentin (1971) for helminths of rodents. These authors have demonstrated that helminth radiation and evolution is tied to availability of new niches (i.e., host diversification), but not strictly to host phylogeny.

A more moderate approach (Osche 1963) is that co-evolution may be demonstrated, provided the proper group of hosts and parasites are chosen for examination. Each example of potential co-evolution must be analyzed on its own merits (Ashlock 1974).

The use of endoparasitic organisms as indicators of host phylogeny and zoogeographic relationships is recognized as a potentially valuable

tool for parasitologists and non-parasitologists (Hennig 1966; Ashlock 1974). The purpose of this work is to analyze the phylogenetic history of the Elaphostrongylinae by the methods of Hennig (1966) and use these data in assessing current concepts of parasite evolution. The phylogenetic relationships and causal zoogeography of the molluscan intermediate and cervid definitive hosts will be examined, in so far as they relate to evolution of the parasite species.

Phylogenetic Systematics

Phylogenetic systematics, as far as I am aware, has not been applied to the study of endoparasitic helminths. Therefore, a brief review of the methods used in the following analysis of the Elaphostrongylinae is presented.

Phylogenetic systematics, as outlined by Hennig (1966), is a method for analysis of kinship on the basis of recency of common ancestry through recognition of apomorphic (derived) character states. Speciation is viewed as a dichotomous branching event in which the stem (or ancestral) taxon diverges into two or more "sister groups" distinct from the ancestral taxon; i.e., they share a common stem ancestor. Thus phylogenetic analysis attempts to relate past history by the analysis of present relationships (Kavanaugh 1972). Sister groups are accorded the same formal rank in the hierarchy.

Hennig (1966) described three methods for analyzing these relationships, but only the holomorphological method can be applied. Holomorphological analysis uses methods similar to other types of morphological investigations. Hennig stresses the importance of comparing all types of information (biochemical, ecological,

physiological, etc.) at each corresponding stage (semaphoront) in the life history of the organisms under consideration. Degree of kinship can only be established on the basis of shared derived characters, termed synapomorphies by Hennig. Ancestral characters (plesiomorphies) are not considered indicators of common ancestry, as they may have continued unchanged in widely divergent lines through numerous speciation events. Thus, shared ancestral characters (symplesiomorphies) are of only limited value in determining sister group relationships. Apomorphic character states that delimit a monophyletic taxon are termed autapomorphies.

It is imperative to be able to interpret the direction of evolution in a series of homologous character states (Hennig 1966) or morphocline (Maslin 1952). Although Hennig and Maslin set forth detailed criteria for establishing the direction of evolution (plesiomorphic -apomorphic) a more simplified scheme proposed by Ross (1974) is used in the following analysis, as outlined below:

1. Fossil sequences — for groups with extensive fossil history; the character state appearing first in the fossil record is probably ancestral.

2. Comparison with related groups (*Ex*-group comparisons) — if one of the two character states occurs in related groups, it is probably ancestral.

3. In group comparisons — characters appearing in a group and not comparable to other groups. The state associated with the most plesiomorphic member is considered plesiomorphic (Ball 1975).

4. Group trends — characters appearing to follow the same evolutionary development in independent lineages.

The first method, paleontological, would be of little use, as the fossil record of endoparasitic helminths is limited to the presence of

eggs in coprolites. The remaining methods (*ex*-group, in-group and group trends) should provide means for analyzing direction of evolution within a group of character states. The preceding summary of phylogenetic systematics is, by necessity, brief. Interested readers are directed to the original English version, *Phylogenetic Systematics* (Hennig 1966) or to any of the following reviews: Maslin (1952), Griffiths (1972), Kavanaugh (1972) and Giffin (1974).

Phylogeny of the Elaphostrongylinae

The Elaphostrongylinae are parasitic in members of the mammalian family Cervidae, although these worms have been reported sporadically from other ungulates, sharing range with infected deer (Kennedy *et al.* 1952; Alden *et al.* 1975; Mayhew *et al.* 1976). I believe that the elaphostrongylines constitute a monophyletic group. A rigorous evaluation of their relationship to the remaining protostrongylids is impossible at this time due to the unsatisfactory subfamilial groupings presently proposed by Russian helminthologists (Boev 1975; Kontramavichus *et al.* 1976).

The character states used in the following analysis are outlined in Table XII. Each character is assigned a letter, lower case for a plesiomorphic state and a capital letter for an apomorphic state. An outline and explanation of the decision for the determination of plesiomorphic versus apomorphic states is presented below:

A. Crura of gubernaculum — the gubernaculum of the vast majority of lungworms (Protostrongylidae) is complex, consisting of at least a corpus and crura. Therefore the presence of crura is plesiomorphic and the loss or reduction of these structures is considered apomorphic. (*Ex*-group).

TABLE XII. CHARACTER STATES FOR THE PHYLOGENETIC ANALYSIS OF THE SPECIES OF THE ELAPHOSTRONGYLINAE

Characters	States			
	Plesiomorphic		Apomorphic	
Gubernacular crura	well developed	(a)	reduced	(A_1)
			absent	(A_2)
Gubernacular corpus	bifurcate	(b)	solid	(B)
Spicule tip	solid	(c)	bifid	(C)
Foramen of spicule	absent	(d)	present	(D)
Dorsal ray	bulb-like	(e)	not bulb-like	(E)
Branches of dorsal ray	located ventrally	(f)	terminal	(F)
Location of dorsal ray	ventral	(g)	dorsal	(G)
Location of adult worm	lung		Central nervous tissue and connective tissue	(H)
			CNS only	(H_1)
			Muscle connective tissue	(H_2)

B. Corpus of the gubernaculum — the corpus of the majority of lungworms is divided distally. This is considered the plesiomorphic condition. The subsequent fusion of this structure is considered apomorphic. (*Ex*-group).

C. Spicules — separation of the lamina from the shaft of the spicule is common in the Protostrongylidae and is considered plesiomorphic. The bifid nature of the spicular shaft of *P. andersoni* is unique, and therefore autapomorphic (see Maslin 1952).

D. The presence of a foramen in the spicules of *P. tenuis* is unique, and therefore autapomorphic.

E. Dorsal ray — the dorsal ray in all protostrongylids (except *Muellerius*) and in most other metastrongylids is a small bulb-like structure. A bulb-like dorsal ray is plesiomorphic. (*Ex*-group).

F. Branches of the dorsal ray — the Metastrongyloidea commonly possess small, ventral papillae on the dorsal ray. This is considered plesiomorphic. A terminal placement of these projections is apomorphic. (*Ex*-group).

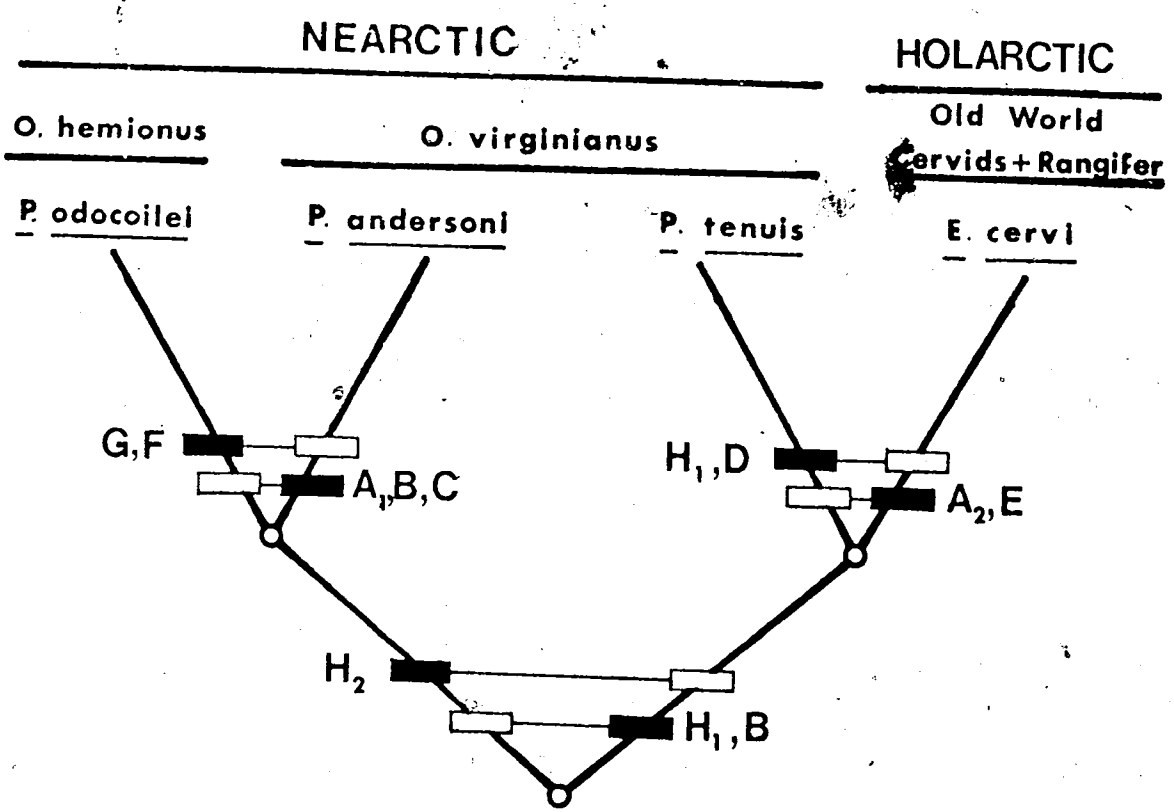
G. Location of dorsal ray — the dorsal ray is terminal in metastrongyloids. The dorsal location of this ray in *P. odocoilei* is unique, and therefore autapomorphic.

H. Site of localization — I consider the Elaphostrongylinae to be an aberrant group of lungworms (see Discussion). Specialization of location is considered apomorphic. Complete development in the CNS (*P. tenuis*) is considered apomorphic to a continued migration to muscle and/or connective tissue outside the CNS (*E. cervi*). (Group trends).

Analysis. The phylogenetic scheme proposed for the Elaphostrongylinae is outlined in Figure 58. The open boxes represent plesiomorphic states in a given character, while closed boxes represent apomorphic states. The letters correspond to the apomorphic states outlined in Table XII.

This analysis outlines two primary lines of development, one parasitizing a muscular-connective tissue habitat (*P. odocoilei* and *P. andersoni*) and the other adopting a sojourn in the CNS (*P. tenuis*

Figure 58. Phylogenetic analysis of the species of the subfamily Elaphostrongylinae. Abbreviations of character states as in Table XII. Open boxes represent plesiomorphic character states and closed boxes represent apomorphic states.


NEARCTIC
HOLARCTIC
O. hemionus
O. virginianus
Old World
Cervids + Rangifer
P. odocoilei
P. andersoni
P. tenuis
E. cervi
G,F
A1,B,C
H1,D
A2,E
H2
H1,B

and *E. cervi*). From the available evidence it is impossible to determine which of these habitats should be considered primitive. On the basis of *ex*-group comparisons, CNS migrations have evolved several times in the Metastrongyloidea (see Anderson 1968) and should be considered plesiomorphic. At the present time, however, such a determination would be premature. The life cycle of many of the metastrongyloids includes a phase in the lymphatic system (Bhaibulaya 1975). The migration route of the Elaphostrongylinae has not been established although Anderson and Strelive (1967) hypothesized a migration via the circulatory system for *P. tenuis* in white-tailed deer.

Within the muscular-connective tissue lineage *P. odocoilei* and *P. andersoni* are allopatric, occurring in mule deer (*O. hemionus* spp.) and white-tailed deer (*O. virginianus* spp.), respectively. Holomorphological analysis identified several autapomorphic character-states isolating *P. odocoilei* from its sister species; e.g., bifurcate gubernacular corpus, branches of dorsal ray terminal and the dorsal location of the dorsal ray. *P. andersoni* possesses three autapomorphies: reduced crura, solid gubernacular corpus and bifurcate spicular shaft, separating it from *P. odocoilei*.

E. cervi was isolated from *P. tenuis* in the CNS lineage by the loss of the gubernacular crura and unusual shape of the dorsal ray. *P. tenuis* forms a monophyletic unit by the presence of the spicular foramen and the restriction of the life cycle to the CNS.

Review of the Phylogeny of the Suborder Strongylina

The suborder Strongylina Pearse 1936 is wholly parasitic, consisting of forms parasitizing a variety of invertebrates as well as all

classes of vertebrates. Components of the suborder are grouped into the following superfamilies: Strongyloidea Weinland 1858, eustomatous parasites of the alimentary canal with a monoxenous life cycle; Trichostrongyloidea Cram 1927, meiostomatous parasites of the intestinal tract of all classes of vertebrates, life cycle monoxenous; Metastrongyloidea Leiper 1927, meiostomatous, extraintestinal parasites, life cycle heteroxenous with an annelid or molluscan intermediate host, parasites of mammals. The strongyles, while considered a monophyletic group (Inglis 1965), display an extensive range of definitive hosts, location within the host and type of life cycle.

The trichostrongyloids and metastrongyloids are generally considered to have arisen independently from a primitive strongyloid stem group (Dougherty 1949, 1951a; Inglis 1965) although the possibility of the two being sister groups (*sensu* Hennig 1966) is not completely discounted (Inglis 1965). Proposals by Russian workers for the phylogeny of and within the Metastrongyloidea (see Skrjabin *et al.* 1952) are considered unacceptable and the outline of Dougherty (1949 and 1951b) is followed here.

Dougherty (1949, 1951b), following extensive study of the metastrongyloids, proposed a phylogeny of the group based on host phylogeny and evolution. He viewed the filarioids and skrjabingylids evolving with carnivores, the pseudaliids with cetaceans and the protostrongylids (including the elaphostrongylines) as early parasites of ungulates and secondarily of lagomorphs. Dougherty (1951b) included *Dictyocaulus* with the metastrongyloids; however, this genus is more commonly regarded as an aberrant trichostrongylid (Skrjabin

et al. 1952; Chitwood 1969). Thus, at the level of family or subfamily, the Metastrongyloidea follow the general concept of co-evolution of host and parasite as suggested by Cameron (1964).

The subfamily Elaphostrongylinae is considered an aberrant branch of the lungworm family Protostrongylidae. Establishment of phylogenetic relationships within the family is beyond the scope of this investigation. In any event this type of analysis would be dependent upon a critical re-examination and phylogenetic assessment of all genera constituting the family as presently defined by Boev (1975) and Kontramavichus *et al.* (1976). Regardless of these limitations, several facts attest to the specialized nature of the Elaphostrongylinae.

Morphologically, lack of a telamon (ventral cloacal supporting structure) in the males is interpreted as primitive. The Protostrongylinae as well as the majority of other genera and species in the family display some, if not an elaborate, development of this characteristic structure. The dorsal ray of the protostrongylids, with the exception of the Muellerinae, has the form of a small, compact knob adorned with sessile or pedunculate papillae. The dorsal ray, although bulb-like in *Parelaphostrongylus*, possesses projections larger than papillae. In *E. cervi* the bulb-like nature of the dorsal ray has apparently been lost and only the projections remain. The gubernaculum of the elaphostrongylines, although simple in comparison to the protostrongyline gubernaculum, is reminiscent of that structure in a number of species in the Varestrongylinae. It would be possible to perceive an ancestral condition of the elaphostrongyline gubernacula similar to *Varestrongylus (=Leptostrongylus) alpenae* or *Varestrongylus (=Capreocaulus) capreoli*.

Although no female characters were used in the preceding analysis, the absence of a provagina from the Elaphostrongylinae is considered plesiomorphic. This is based on the absence of a provagina in the majority of the metastrongyloids, while within the Protostrongylidae the presence of this structure is erratic.

The elaphostrongylines possess one and possibly two apomorphic characters that isolate them from the other protostrongylids. These are the extra-pulmonary site of adult maturation and the asymmetrical condition of the buccal capsule. Extra-pulmonary habitats have been exploited by several distantly related genera and species within the Metastrongyloidea (e.g. *Angiostrongylus cantonensis*, *Gurltia*; see Anderson 1968 for a review of this subject) and must be considered apomorphic. The nature of the buccal capsule, symmetrical versus asymmetrical, is too poorly known within the group to determine if the asymmetrical condition is unique to the elaphostrongylines.

The subfamily Elaphostrongylinae is considered a specialized branch of the Protostrongylidae that radiated into a new habitat. The ancestral stock that gave rise to the subfamily was unquestionably a group of primitive lungworms, possibly similar in some respects to the genus *Varestrongylus*.

The foregoing phylogenetic analysis calls into question the validity of the genus *Parelaphostrongylus*. A strict application of the principles of phylogenetic systematics for ranking (Hennig 1966) would dictate that all species in Figure 58 be contained in a single, monophyletic genus — *Elaphostrongylus* Cameron 1931. However, due to the speculative nature of the analysis, the genera *Elaphostrongylus*

Cameron 1931, and *Parelaphostrongylus* Boev and Schul'ts 1950, are retained in the interest of taxonomic stability. Additional information and further analysis are required to determine the validity of *Parelaphostrongylus* in a phylogenetic system.

B. Zoogeography

Introduction

The evolutionary fate of a parasite is directly linked to that of the host (Cameron 1964) regardless of the nature of the relationship, be it ecological or phylogenetic. The current distribution of any parasitic helminth must reflect, at least in part, the current distribution of the intermediate host (if required) as well as the definitive host. After defining the area of distribution of the Elaphostrongylinae it will be necessary to attempt an explanation of that distribution in terms of causal zoogeography (Illies 1974).

Several authors (von Ihering 1902; Metcalf 1929, 1940; Ma er 1955, 1967; Stromberg *et al.* 1974) have successfully used this method to explain the distributions of the higher taxa of parasites and their definitive hosts. More recently, Brooks (1977) analyzed the co-evolution and causal zoogeography of plagiorchiid trematode genera and their anuran definitive hosts.

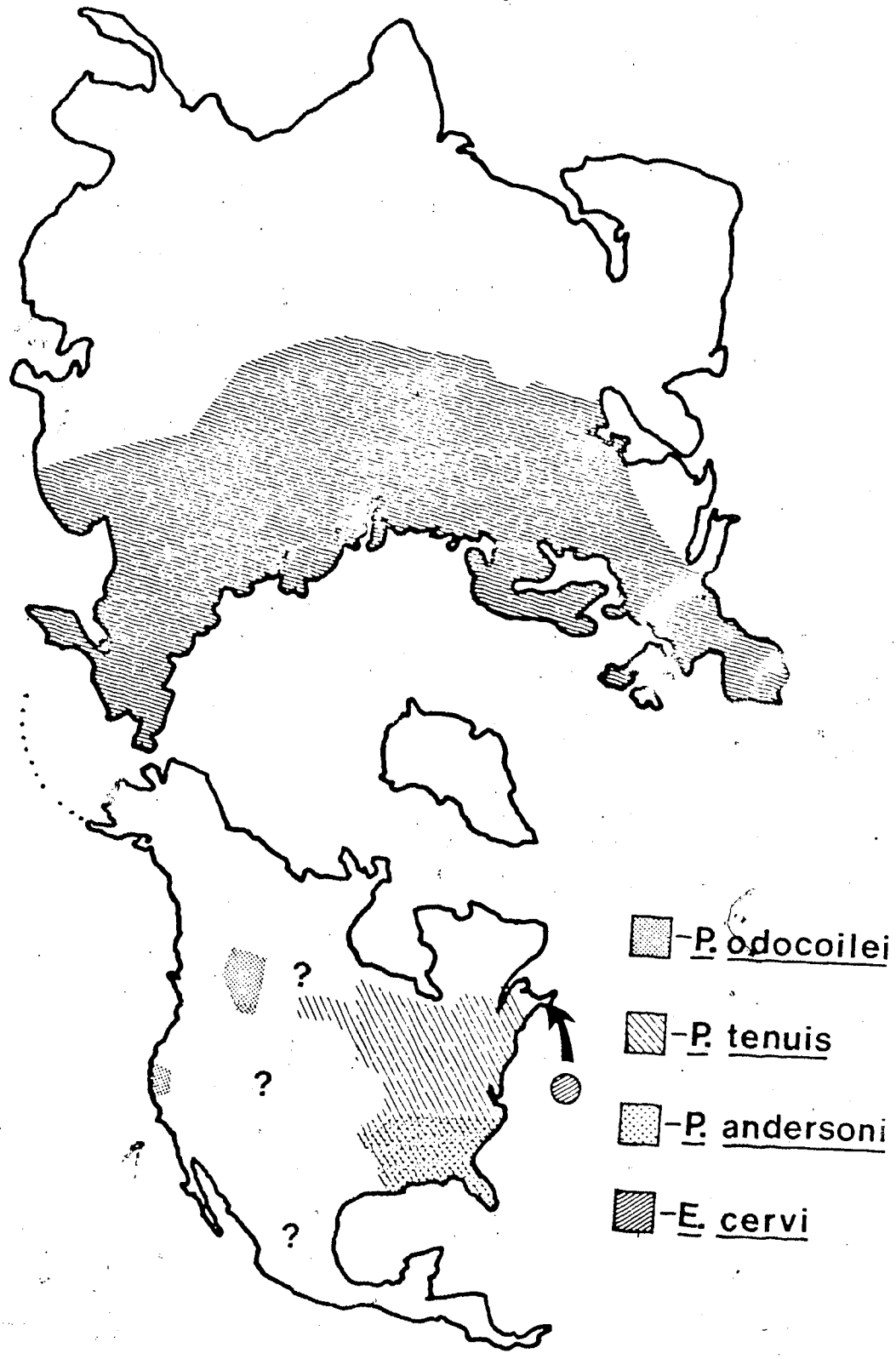
Analysis of the host-parasite relationships within the Elaphostrongylinae consists of three primary areas of investigation: 1) host specificity at the intermediate and definitive host level; 2) current distribution of the Elaphostrongylinae, susceptible gastropod intermediate hosts and cervid definitive hosts, and 3) causal zoogeography of the Cervidae.

An attempt to ascertain the zoogeographical history of a group of organisms as poorly known as the Elaphostrongylinae may appear to be hopeless. The author, however, must concur with previous workers (D.R. Whitehead 1972; Ross 1974; Ball 1975) in that it is the obligation of individuals involved in systematic research to attempt to explain, causally, the distribution of the organisms under investigation. Progress in this difficult field can only be made by analysis of previous efforts. Future workers, with additional information and/or analytical techniques may support or disprove the original effort. The result, regardless of the outcome, will be an increased understanding of the distribution of living organisms.

Elaphostrongylinae

A generalized distribution of the Elaphostrongylinae is presented in Figure 59. *Elaphostrongylus cervi* subspp. have been reported from a wide variety of locations in Europe and Asia (see Kontramavichus *et al.* 1976 for a general review). Cameron (1931) originally described *E. cervi* from *Cervus elaphus* in Scotland. Since that time this species has been reported from the Iberian peninsula (Lopez-Neyra 1947), Scandinavia (Roneus and Nordkvidt 1962 and Halvorsen *et al.* 1976), Austria (Kutzer and Prosl 1975), Czechoslovakia (Barus and Blazek 1973) in Europe as well as over much of the Soviet Union (Pryadko and Boev 1971). Recently, Lankester *et al.* (1976) reported a spined protostrongylid larvae from caribo (*Rangifer tarandus caribou*) in Ontario and Manitoba, identical to those reported for the Elaphostrongylinae, and *E. cervi* has been reported from naturally infected caribou in Newfoundland establishing the presence of this parasite in North America

Figure 59. World-wide distribution of the Elaphostrongylinae.


?
?
?
P. odocoilei
P. tenuis
P. andersoni
E. cervi

(Lankester 1976). Thus *P. cervi* has a holarctic distribution. *P. cervi* has also been found in wapiti and red deer introduced to New Zealand (Mason *et al.* 1976 and Mason and McAllum 1976).

Parelaphostrongylus is at present restricted to North America (Fig. 59) (Anderson 1972). The meningeal worm, *P. tenuis*, normally a parasite of white-tailed deer, has been reported from a wide variety of locations in the southeastern United States (Prestwood and Smith 1969), Maine (Gilbert 1973), Michigan (DeGuisti 1955), Ontario (Anderson 1956), Manitoba (Lankester 1974) and reliably as far west as western Manitoba (Bindernagel and Anderson 1972). Bindernagel and Anderson (1972) and Bindernagel (1973) reported larvae "indistinguishable from those of *P. tenuis*" from fecal samples of white-tailed deer in eastern Saskatchewan and eastern British Columbia. There has been no confirmation of the specific or generic identity of these worms; however, there is evidence (Bindernagel pers. comm.; Wobeser pers. comm.) that *P. tenuis* is not involved. It is possible that one of several undescribed species of either elaphostrongylines or one of the genera of protostrongylid lungworms with spined, first stage larvae is involved.

Parelaphostrongylus andersoni was recently described from the dorsal muscles of white-tailed deer in southeastern United States (Prestwood 1972). The distribution of *P. andersoni* has been documented within this region (Prestwood *et al.* 1974); however, there have been no reports of this species outside the southeastern U.S. *P. andersoni* and *P. tenuis* are sympatric over a large portion of the range of the former (Prestwood and Smith 1969; Prestwood *et al.* 1974). *P. andersoni* is more commonly found in deer inhabiting southern floodplain, southern

mixed and oak-hickory-pine vegetation zones (Prestwood *et al.* 1974). These authors also reported two instances of concurrent infections of deer by *P. tenuis* and *P. andersoni* in North Carolina.

The range of *P. odocoilei* is not as well documented as the other members of the genus. Previous reports restricted the distribution of *P. odocoilei* to mule deer and black tail deer of the California Coast Range (Hobmaier and Hobmaier 1934a) and the north central Sierra Range in California (Brunetti 1969). The present study indicates this species is widespread in west central Alberta (Fig. 60). Identification of *P. odocoilei* from Jasper, Alberta, has been discussed elsewhere in this work. The remaining locations on Figure 60 are based solely upon the presence of the characteristic spined larvae in the feces of deer, without the corroboration of recovery of adult worms.

Molluscan Intermediate Hosts

The elaphostrongylines show little specificity at the level of the intermediate host (Mitskevitch 1964; Panin 1964a; Panin and Rushkova 1964; Lankester and Anderson 1968; this study, Section III). Within the pulmonate order Stylommatophora, two of the four suborders (Heterurethra and Sigmaurethra) contain a number of species capable of harboring elaphostrongylines, while only the [illegible] urethra (Pupillidae, Vallionidae) have proven refractory (Panin and Rushkova 1964); the Cochliocopidae (=Cionellidae), however, are susceptible. Several species of aquatic pulmonates (Bassomatophora) have been experimentally infected as well (Anderson 1963; Panin and Rushkova 1964).

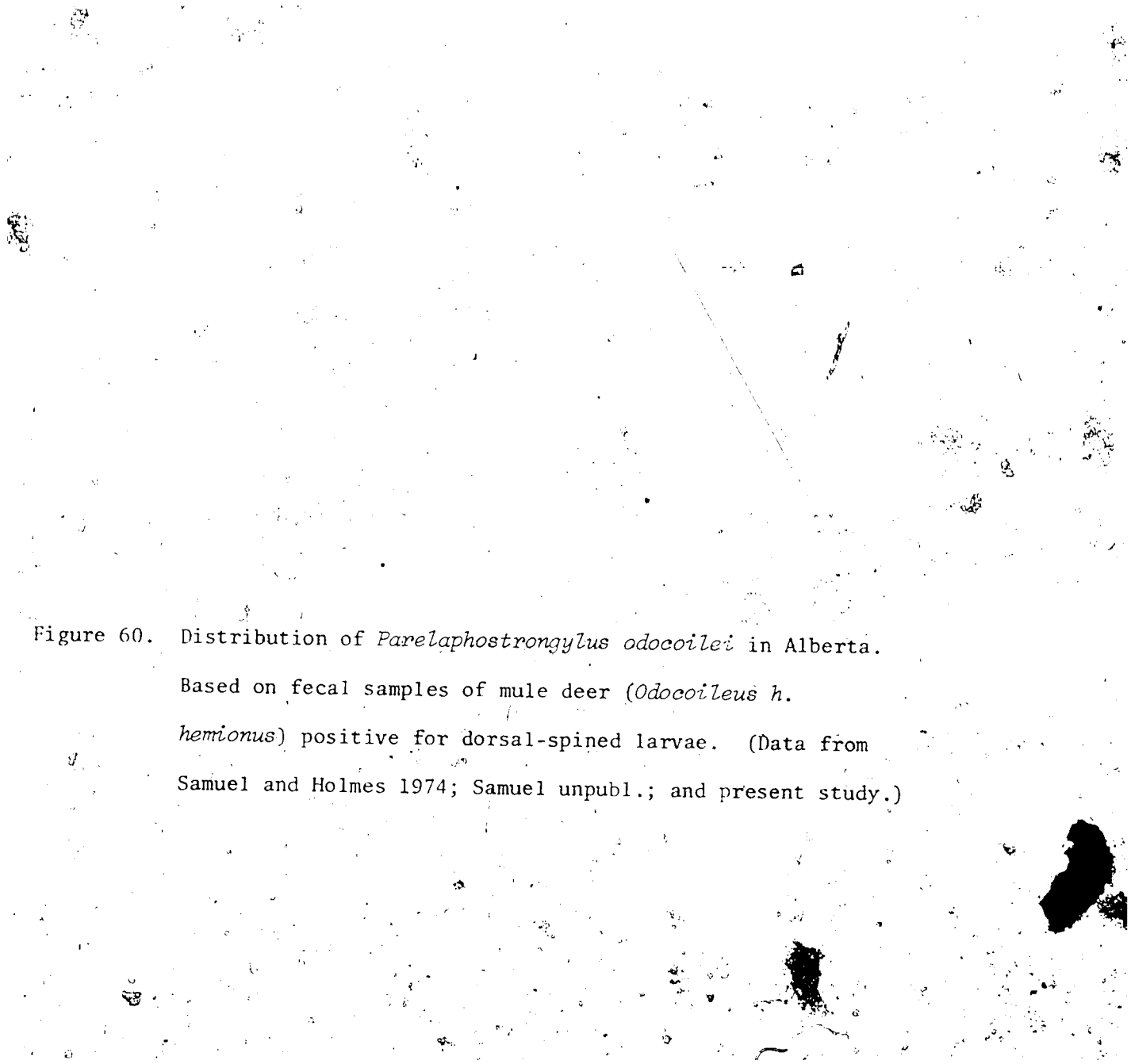
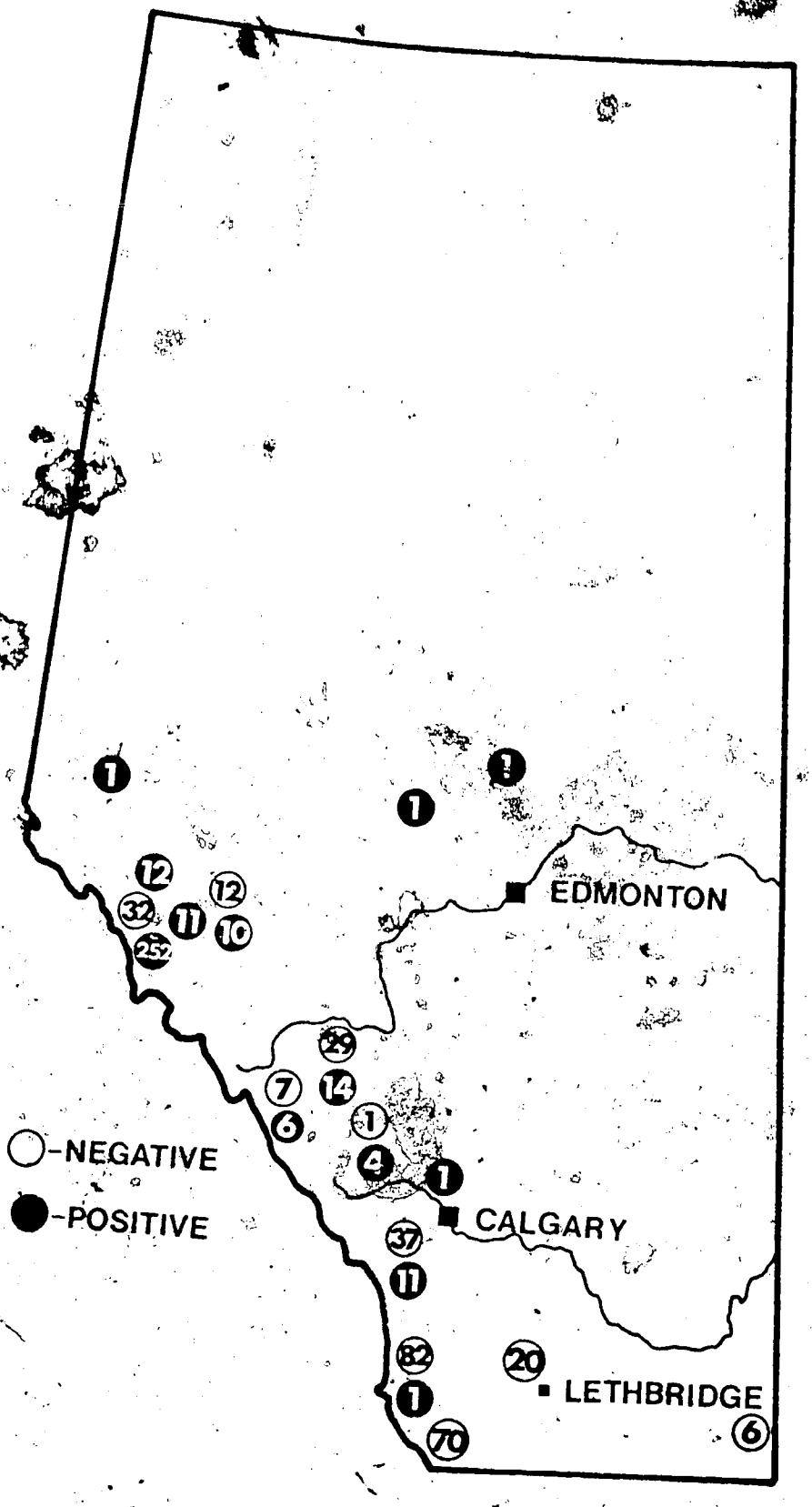


Figure 60. Distribution of *Parelaphostrongylus odocoilei* in Alberta. Based on fecal samples of mule deer (*Odocoileus h. hemionus*) positive for dorsal-spined larvae. (Data from Samuel and Holmes 1974; Samuel unpubl.; and present study.)


EDMONTON
CALGARY
LETHBRIDGE
○-NEGATIVE
●-POSITIVE

Six genera of terrestrial molluscs, recognized as intermediate hosts (Table XIII), are holarctic in distribution. The following species of these genera are widely distributed and locally abundant: *Deroceras laeve*, *Euconulus fulvus* and *Zonitoides nitidus*. The remaining genera have related species in Eurasia and North America that are also excellent hosts for these nematodes. Molluscs capable of acting as intermediate hosts of these nematodes are common species, and therefore available in practically all habitats deer are likely to use.

Historical relationships of the holarctic malacofauna have been studied in some detail. Waldén (1963) postulated that holarctic molluscs were of Palearctic origin and the Bering Land Bridge played an important role in determining their current distribution. This fauna, including the majority of the genera in Table XIII, may be relics from the Cretaceous or Late Tertiary (Waldén 1963). Russian workers (Likhachev and Rammel'maier 1952) present evidence for molluscan movements both east and west on the Bering Land Bridge; however, they feel this fauna is younger than suggested by Waldén. This is based on the assumption of the Russian workers that the Stylommatophora originated in the Tertiary, while Morton (1955) placed the origin of the suborder in the Carboniferous. The presence of extant molluscan genera in the Paleocene (Waldén 1963; Likhachev and Rammel'maier 1952) argues for an earlier establishment of this oup than the Tertiary.

A comparison of the Tertiary mollusc fauna of North America and Eurasia is difficult. Pre-Pleistocene records for the Nearctic are rare (Taylor 1960). Waldén (1963) listed five extant genera of

TABLE XIII. PRE-PLEISTOCENE RECORDS OF EXTANT MOLLUSCAN TAXA (STYLOMMATOPHORA) THAT ARE KNOWN INTERMEDIATE HOSTS OF ELAPHOSTRONGYLINE NEMATODES

Taxon	Time	Source
Discus cr[illegible]chitei	Upper Cretaceous	Leonard, 1950
Zonitoi	Oligocene	Waldén, 1963
Succi	Paleocene	Waldén, 1963
Vitri	Oligocene	Waldén, 1963
Deroceras	Pliocene	Waldén, 1963
Euconulus	Paleocene	Waldén, 1963

terrestrial molluscs present in North America prior to the Pleistocene (Table XIII). Taylor (1960) listed *Deroceras*, as well as three additional extant genera, from mid-Pliocene deposits in the mid-western United States.

The Eurasian malacofauna has received considerably more attention. Differentiation of the European-Siberian fauna began during the Tertiary and all contemporary mollusc genera were present in Europe by the end of the Pliocene (Likhachev and Rammel'maier 1952). Therefore, a relatively modern terrestrial gastropod fauna was present when modern cervid groups were becoming firmly established during the Late Miocene and Early Pliocene (Flerov 1952) as suggested by Russian helminthologists (Pryadko and Boev 1971).

Speciation rates of molluscs are considerably lower than those of their mammalian counterparts (Taylor 1960; Wilson *et al.* 1975). The continuity of the terrestrial mollusc fauna during the Late Tertiary and Pleistocene would have given the elaphostrongylines a long association with and relatively little selection pressure from their molluscan intermediate hosts. This hypothesis is supported by the concept that metastrongyloid larvae acquired an intermediate host as a mechanism for escaping desiccation and are, in effect, ecto- rather than endoparasites of molluscs (Hobmaier 1934). Thus, the causal zoogeography of these nematodes must be explained at the level of the definitive host.

Cervidae

The cervid genus *Odocoileus* (*s.s.*) is currently widespread in North America (see Taylor 1956 and G.K. Whitehead 1972) and northern

South America (G.K. Whitehead 1972). The white-tailed deer (*O. virginianus*) is composed of approximately 38 subspecies (G.K. Whitehead 1972) that range as far north as the Northwest Territories (Scotter 1974) and as far south as Brazil (Brokx 1972a). This species is found in all areas of North America with the exception of the mountainous and sonoran regions of the southwestern U.S., including California. The mule deer (*O. hemionus*) includes 14 subspecies (G.K. Whitehead 1972) that are primarily distributed in the western parts of the U.S., Canada and Mexico (Kraemer 1973). The Columbian black-tailed deer (*O. h. columbianus*) and Sitka deer (*O. h. sitkensis*) have been described as "a species in the making" (Taylor 1956) and are located west of the Rocky Mountains, in California, Washington, Oregon, and southern British Columbia, and in northern British Columbia, Alaska and Sitka Island, respectively (Cowan 1956; G.K. Whitehead 1972).

The evolutionary history of *Parelaphostrongylus* is closely linked to the history of *Odocoileus*. The prehistoric, evolutionary and dispersal patterns of *O. virginianus* and *O. hemionus* are, unfortunately, unknown (Martin and Webb 1974).

Odocoileus is an old taxon, extending into the Pliocene with little change (Scott 1913). Deer, indistinguishable from extant *O. virginianus*, have been reported from the Blancan of Florida, although additional confirmation of the age of these strata is required (Webb 1974). Stock (1930) reported deer "apparently" related to mule deer (*O. h. hemionus*) from the Rancho La Brea tar pits of California.

Records of a variety of North American fossil cervids are abundant (e.g. Frick 1937); however, to my knowledge, there have been no attempts to assess their relationship either to extant or other fossil forms. The most consistent attempt at classifying and determining the phylogenetic relationships of cervids on the basis of paleontological as well as neontological data is by Flerov (1950, 1952). He did not, however, discuss zoogeographical relationships to any extent. The causal zoogeography of cervids remains unclear and virtually unstudied, with the exception of superficial treatment in more general works (Scott 1913; Simpson 1945, 1947; Darlington 1957).

Brokx (1972a) proposed a possible evolutionary history and the relationships of South American cervids and, in addition, the relationships of *O. hemionus* and *O. virginianus*. The following account of these relationships is based primarily on his work.

North American cervids are felt to have evolved from an ancestral stock, similar in some respects to the primitive Pampas deer (*Ozotoceros*) of South America. This ancestral group inhabited Central America and Mexico and subsequently split into two main lines during the Late Miocene to Early Pliocene. One line gave rise to the more primitive South American genera (or subgenera): *Ozotoceros*, *Blastocerus*, *Hippocamelus*, *Mazama* and *Pudu*, while the second line gave rise to the northern genera *Odocoileus* and *Rangifer*.

Movement of the ancestral South American forms across the Panamanian isthmus could have occurred earlier than previously considered possible (e.g. Simpson 1950, 1969), as recent evidence indicates this connection was in existence throughout the Pliocene

(Savage 1974) and that faunal exchange occurred prior to the Pleistocene (Hershkovitz 1966, 1972). The presence of *O. virginianus* in northern South America is regarded as a secondary invasion of a more primitive whitetail (Hershkovitz 1966), after establishment and diversification of the original South American stock into well defined, specialized forms.

Based on this (Brokx 1972a), the establishment of *Odocoileus* as a widespread genus in North America most probably occurred during the Late Pliocene. The timing of subsequent events is particularly difficult to establish. Mule deer are considered to be specialized descendants of a more primitive white-tailed ancestor (Brokx 1972a). Speciation in this case was probably the result of the isolation of eastern and western populations by grasslands in central North America (Blair 1958). This isolation, possibly as early as the Nebraskan glaciation, gave rise to the mountain-adapted mule deer (*O. hemionus*) and the woodland dwelling whitetail (*O. virginianus*). Isolation of these stem forms into various geographical and ecological areas then gave rise to the subspecific taxa currently recognized for each group.

The lack of further differentiation of these stem forms into additional species or genera may be attributed to the lack of distinct habitats in North America comparable to those of South America (Brokx 1972a) or the limited ability of *Odocoileus* to invade new habitats (i.e. the far North) and compete with northern-adapted species.

There has been a great deal of debate as to the origin of the genus *Rangifer*. Many authors (Lydekker 1898; Simpson 1947; Kurtén 1968) have argued for an Eurasian origin. Other workers (Flèrov 1952,

Brokx 1972a, Giffin 1974) have presented convincing data for a Nearctic origin for reindeer. The large number of characteristics shared by *Rangifer* and other New World cervids (Lydekker 1898; Flerov 1952; Brokx 1972a,b; Giffin 1974) leaves little doubt as to the Nearctic affinities of the genus and its subsequent dispersal during the Pleistocene as a cold-adapted, tundra specialist.

What are now considered Eurasian forms: *Cervus*, *Elaphurus* and *Capreolus*, originated in Eurasia (Simpson 1947; Flerov 1950, 1952; Brokx 1972a, Giffin 1974) and *Alces* is also generally considered to have Eurasian affinities (Flerov 1952; Kurtén 1968; Giffin 1974). The New World deer: *Odocoileus*, *Rangifer* and endemic South American genera, originated in the western hemisphere (Flerov 1952; Brokx 1972a; Giffin 1974). A classification of these animals is given in Table XIV. The events leading to the present distribution of these genera, however, remain uncertain. There are two theories of the causal distribution of modern cervids. They are briefly outlined below.

The first theory postulates the autochthonous development of the New World cervids from ancestral forms (Blastomerycinae, Leptomerycinae) present during the Oligocene and Miocene. This view has been supported in modified forms by a variety of workers (Matthew 1908; Scott 1913; Simpson 1947; Flerov 1950, 1952).

The second hypothesis is that cervids extant in the North American Miocene became extinct, leaving no descendants. Modern North American Cervidae are, therefore, descended from a more recent, antlered, Asian form of protocapreoline type (Brokx 1972a,b) which arrived via the Bering Land Bridge during the Early Pliocene (Lydekker 1898; Pilgrim

TABLE XIV. CLASSIFICATION OF THE GENERA OF CERVIDS DISCUSSED IN THE TEXT (AFTER G.K. WHITEHEAD, 1972)

Family Cervidae

Subfamily Cervinae

Dama Frisch, 1775

Axis H. Smith, 1827

Cervus Linneaus, 1758

Alces Gray, 1821

Subfamily Odocoileinae

Odocoileus Rafinesque, 1832

Capreolus Gray, 1821

Rangifer H. Smith, 1827

Blastocerus Wagner, 1844

Ozotoceros Ameghino, 1891

Hippocamelus Leuckhart, 1816

Mazama Rafinesque, 1817

Pudu Gray, 1852

1941; Brokx 1972a). Scott (1913) and Simpson (1947), although apparently supporting an independent origin of New World cervids did not completely dismiss the possibility of a later Eurasian immigrant acting as an ancestor to the Odocoileinae.

C. Host Specificity

Host specificity of the elaphostrongylines within the Cervidae is low, in agreement with Inglis' (1965) contention that ecologically similar organisms are more likely to share related helminths than species that are ecologically divergent, but with a close phylogenetic kinship. A list of hosts that each of the four species of elaphostrongylines has been reported from is presented in Table XV. The most intriguing lapsus is the inability of *P. odocoilei* to establish a patent infection in the white-tailed deer (see Section III).

Odocoileus virginianus and *O. hemionus* are closely related forms and are, at least presently, sympatric in areas of western North America (Kraemer 1973). The inability of *P. odocoilei* to establish a patent infection in *O. virginianus* must represent a distinct physiological or immunological barrier to the parasite. Detailed life history studies of *P. odocoilei* in a variety of cervid hosts are necessary to resolve the stage and manner in which *P. odocoilei* is sequestered. Moose (*Alces alces*) and black tailed deer, while able to harbor experimental infections, do not appear to be primary hosts of this parasite (see Section III).

The meningeal worm, *P. tenuis*, is difficult to assess in terms of specificity. Anderson (1968, 1972) has shown *P. tenuis* to be

TABLE XV. DEFINITIVE HOSTS OF ELAPHOSTRONGYLINE NEMATODES

Parasite	Host	Source
Elaphostrongylus cervi cervi	*Cervus elaphus scoticus*	Cameron, 1931
	C. e. hippolaphus	Kutzer and Prosl, 1975
	Capreolus capreolus	Kutzer and Prosl, 1975
	Rangifer tarandus	Lankester, 1976
E. c. panticola	*Cervus elaphus*	Kontramavichus *et al.*, 1976
	C. e. sibericus	Kontramavichus *et al.*, 1976
	C. e. brauneri	Kontramavichus *et al.*, 1976
	C. e. maral	Kontramavichus *et al.*, 1976
	C. nippon	Kontramavichus *et al.*, 1976
	Alces alces	Kontramavichus *et al.*, 1976
E. c. rangiferi	*Rangifer tarandus*	Mitskevitch, 1960
Parelaphostrongylus tenuis	*Odocoileus virginianus*	Anderson, 1963
	* *O. hemionus*	Anderson *et al.*, 1966
	C. e. canadensis	Woolf *et al.*, 1977
	* *Alces alces americanus*	Anderson, 1964
	* *Rangifer tarandus*	Anderson, 1971
	* *Dama dama*	Kistner *et al.*, 1977
	* domestic sheep and goats	Alden *et al.*, 1975
	* guinea pigs	Anderson and Strelive, 1966
P. odocoilei	*Odocoileus h. hemionus*	Present study
	O. h. columbianus	Present study
	O. h. californicus	Brunetti, 1969
	Alces alces andersoni	Present study
P. andersoni	*Odocoileus virginianus*	Prestwood, 1972

*denotes a non-patent infection

lethal to a wide variety of North American cervids. Patent infections of *P. tenuis* are only recorded in the white-tailed deer, the normal host (Anderson 1963) and wapiti (*Cervus elaphus canadensis*) by Anderson *et al.* (1966), Karns (1966), and Woolf *et al.* (1977).

From an academic viewpoint, the question of host specificity remains unanswered. If the migrating larvae of *P. tenuis* did not kill the host prior to the onset of patency, would the life cycle be completed? The conclusion of this line of reasoning is that, under suboptimal conditions, *P. tenuis* is capable of completing the life cycle in a number of cervid species, but destroys the habitat due to the delicate nature of the CNS used as a larval migration route. The meningeal worm, therefore, cannot be considered more host-specific than *P. odocoilei* which does not (presumably) undergo a CNS migration. Host specificity of *P. andersoni* is unknown, although it may become patent in black-tailed deer (Nettles *et al.* 1977).

Host specificity of *Elaphostrongylus cervi* is also difficult to assess. Pryadko and Boev (1971) were unable to identify characters differentiating the three species of *Elaphostrongylus* and ranked them as subspecies of *E. cervi* based upon host species. No cross transmission studies have been done to determine whether these represent a series of cryptic species or a widely distributed helminth of low host specificity.

D. Discussion

Dougherty (1949, 1951b) proposed an hypothesis for the evolution of the Metastrongyloidea (*s.l.*) on the basis of the co-evolution of a

subfamily (or subfamilies) of nematode with an order of mammalian host. Inglis (1965), although generally opposed to the concept of co-evolution on the basis of phylogenetic relationships, felt that Dougherty's scheme was justified. Co-evolution appears to have occurred in the Elaphostrongylinae at the species level as well. An analysis of speciation events, based on parasite phylogeny, host evolution and climatic conditions supports this contention.

The Elaphostrongylinae are of Nearctic origin, and originally parasites of New World cervids (Odocoileinae). Reasons for this view are presented later. The initial speciation event divided the ancestral worm population into two basic lineages: one, a muscular form with no neurotropic phase (*P. andersoni* - *P. odocoilei*) and the other, a CNS inhabiting lineage (*P. tenuis* - *E. cervi*), more similar to *P. tenuis* in appearance, but with a wider degree of host tolerance. Determination of the plesiomorphic condition, i.e., muscle versus CNS, is not clear and of little concern at this point. The impetus for this initial radiation is unknown and may simply represent exploitation of a new niche by either or both lines.

The subsequent isolation of eastern and western populations of the ancestral *Odocoileus* gave rise to the more specialized *O. hemionus* in the mountain and foothill regions of North America and the more generalized *O. virginianus* in the East (Brokx 1972a). This separation also gave rise to allopatric conditions necessary for the speciation of *P. odocoilei* in the mule deer and *P. andersoni* in whitetails. The absence of neurotropic forms in mule deer can only be explained by a low tolerance of the proto-*hemionus* population to the CNS inhabiting forms.

This hypothesis could be tested by experimental infections of *O. hemionus* with *E. cervi*, the prediction being that mule deer would succumb to the infection or at least show signs of CNS disturbance, therefore decreasing fitness under natural conditions. White-tail deer apparently show no ill effects of infection with *E. cervi* (Lankester pers. comm.). Isolation of host populations could have occurred as early as the Kansan glaciation. Dry conditions and the formation of extensive grasslands in central North America would have formed a barrier to exchange between these populations (Blair 1958).

Events leading to the division of the original neurotropic form into the specialist (*P. tenuis*) and generalist (*E. cervi*) lineages are not clear. *P. tenuis* evolved in close association with the white-tailed deer. During the mid-Pleistocene (Aftonian?) a morphologically distinct, cold-adapted, tundra cervid, *Rangifer*, emerged in the Nearctic (Simpson 1947; Flerov 1950). An allopatric shift of this population into areas inaccessible to *Odocoilous* could have triggered a rapid specialization of *P. tenuis*, while *Rangifer* and wapiti (*Cervus*) and moose (*Alces*), the latter two recently introduced from Eurasia (Simpson 1947; Flerov 1952; Darlington 1957; Flerov 1967), shared the generalist, *E. cervi*. Range descriptions of these cervids (Murie 1951; Guthrie 1966; G.K. Whitehead 1972; Kelsall and Telfer 1974; LeResche *et al.* 1974) indicate a greater overlap in the range of *Rangifer*, with *Cervus* and *Alces*, than any of them with white-tailed deer.

Although *O. virginianus* may be able to harbor patent infections of *E. cervi* experimentally (Lankester pers. comm.), competition from

the specialist *P. tenuis* could have resulted in competitive exclusion (Holmes 1973) and prevented a re-introduction of *E. cervi* into whitetail populations. Late Pleistocene emigration of *Rangifer* established its present Holarctic distribution (Flerov 1952; Darlington 1957; Brokx 1972a) and introduced *E. cervi* to Eurasia. Eurasian practices of reindeer farming (Scandinavia and USSR) and deer farming (*Cervus* - USSR) undoubtedly increased the distribution and enhanced transmission, resulting in epizootics of elaphostrongylosis (e.g. Boev *et al.*, Pryadko *et al.* 1963).

On the basis of the phylogenetic and zoogeographic evidence presented above, there can be little question as to the Nearctic origin of the Elaphostrongylinae and the subsequent introduction of *E. cervi* into Eurasia accompanying the emigration of *Rangifer* from North America to the Palearctic. This is in direct opposition to the Eurasian origin proposed for the group by Russian helminthologists (Pryadko and Boev 1971); however, they failed to supply any supporting evidence.

A re-examination of Manter's (1967) rules for helminth zoogeography with reference to the elaphostrongylines is warranted. These nematodes are more diverse in North America. All four species and both genera are found in the Nearctic, while a single genus and species, *E. cervi*, is Holarctic. Invoking Occam's Razor, the most parsimonious interpretation is to assume a Nearctic origin and a single trans-Beringian movement rather than an Eurasian origin and two migrations.

Manter (1967) suggested that evolution results in specialization and increased host specificity. *Elaphostrongylus cervi* is the least

host-specific of the elaphostrongylines, with morphologically identical forms infecting five cervid genera (Pryadko and Boev 1971; Lankester pers. comm.). Had this association been one of long standing, speciation would have been expected to occur.

Host specificity within *Parelaphostrongylus* (see above), although not rigid, is more restricted than in *Elaphostrongylus*. Intergeneric infections do occur in this genus (e.g. *P. odocoilei* in *Alces* and *P. tenuis* in [illegible]). The resulting infections, however, demonstrate a reduced [illegible]al output. The concept of specificity predicts Manter's initial rule, that parasites with the most generalized requirements are most likely to disperse. *E. cervi*, the most general of the four species, has the widest distribution of all the Elaphostrongylinae. The wide distribution of *P. tenuis*, a specialist, is related to the adaptability of the host and possibly the concept of "germ warfare" (Barbehenn 1969) used by a host against competing species. White-tailed deer are able to occupy territory where they are competitively inferior by causing the death of the superior competitor through infection with *P. tenuis*. This has been documented in a field situation for white-tailed deer and moose in Maine (Gilbert 1973, 1974; Saunders 1973).

Co-evolution of host and parasite does occur (Cameron 1964) with reference to the elaphostrongyline-cervid system. Specificity of the subfamily for their cervid hosts has prevented the spread of these parasites to ecologically similar groups (e.g. bovids and lagomorphs) which do share other protostrongylids (Kontramavichus *et al.* 1976).

Although there is evidence for host specificity within the group (see above) reduced specificity (Inglis 1965) at the generic level has undoubtedly played a large part in the current distribution this group currently enjoys. This is particularly applicable to the catholic choice of intermediate hosts.

The apparent paucity of species in the Elaphostrongylinae may reflect one of the following: 1) an unsuccessful group — the extra-pulmonary habitat may be difficult to exploit initially and does not promote rapid speciation; 2) the species described to date are a remnant of a previously diverse fauna that was severely reduced by extinction of a number of large cervids at the end of the Pleistocene; 3) difficulty in locating these worms has resulted in many species being overlooked during necropsy, or 4) our knowledge of the helminth fauna of many cervids is inadequate and surveys of these genera (e.g. *Mazama*, *Pudu*, *Hippocamelus*, *Ozotoceros*, *Blastocerus*, *Rusa*, *Axis*, etc.) will reveal a number of additional genera and species. The general lack of host specificity of these worms within the Cervidae argues against the second option. The third and fourth options are certainly worth consideration. Brokx (1972a) reported unidentified lungworm larvae from *O. virginianus gymnotis* in Venezuela. These larvae were not described and could, therefore, belong to any of the protostrongylid genera. The first option, that the elaphostrongylines are simply an unsuccessful group from the standpoint of species diversity, is supported by the apparent inability of *E. cervi* to speciate in the cervids of the Palearctic.

Analysis of the phylogenetic relationships of this group of parasites does little to illuminate the relationships within the Cervidae. While speciation within *Parelaphostrongylus* agrees with the model proposed for *Odocoileus* by Brokx (1972a), the relationships within *Odocoileus* remain unclear, as do the general relationships of Old and New World cervids. Time relationships for these organisms are speculative at best. The low host specificity of *P. cervi* indicates an ecological association with northern cervids as proposed by Inglis (1965) rather than a phylogenetic relationship as proposed by Cameron (1964). More precise estimates of the time of origin of the cervid genera as well as a critical examination of their phylogenetic affinities are required before these relationships can be firmly substantiated.

SECTION VII

CONCLUDING REMARKS

In this study, I have attempted to test several hypotheses regarding elaphostrongyline nematodes and to document aspects of the life cycle of *Parelaphostrongylus odocoilei*. The results are a positive addition to our knowledge of the life cycle and systematics of the Elaphostrongylinae.

The results can be divided into two broad categories. The first group are those that are not unique to *P. odocoilei* or, in some instances, to the genus *Parelaphostrongylus*. Testing the following hypotheses: the primary mode of entry of first-stage larvae into a molluscan intermediate host is direct penetration, the site of larval development is the foot region of the mollusc and the reaction of terrestrial gastropods to nematode larvae is different than that of aquatic gastropods, has resulted in findings of broad significance. Entry of Ll's of *P. odocoilei* into terrestrial molluscs is primarily by direct penetration and evidence is presented to suggest that fundamental differences in biological valency of various lungworm taxa and/or experimental design may be responsible for the controversy surrounding this phenomenon. The cellular reaction of *T. multilineata* to these nematodes is biphasic (Harris and Cheng 1975a) and is identical to what has been described for aquatic pulmonates.

Results of field studies on snails in Jasper, Alberta, prove that local foci of infection occur for *P. odocoilei*. Additional work is needed to confirm the presence of these infection foci for other elaphostrongylines. A focus is delimited, at least in part, by deer

and snail abundance. Fundamental studies of the bionomics and ecology of first-stage larvae and terrestrial molluscs are required.

A phylogenetic analysis of the species of the Elaphostrongylinae has resulted in the identification of two lineages: a muscle-connective tissue line (*P. odocoilei* and *P. andersoni*) and a CNS line (*P. tenuis* and *E. cervi*). The concept of co-evolution of host and parasite has recently come under attack. Results of the present study indicate that a close phylogenetic relationship exists between *Parelaphostrongylus* and the cervid genus *Odocoileus*. The generalist, *E. cervi*, appears, however, to have an ecological relationship with cervids of the far north. The molluscan intermediate hosts of this parasite apparently have had little effect on the distribution of the Elaphostrongylinae.

The second group of hypotheses to be tested and phenomena studied are unique to *P. odocoilei*. The discovery that the alternate nematode required by the hypothesis of Samuel and Holmes (1974) was *P. odocoilei*, and not a new species, resulted in a redescription and the establishment of a neotype specimen for *P. odocoilei*. These actions will act as a stabilizing influence on the nomenclature of the group.

Documentation of the life cycle and host specificity of *P. odocoilei* resulted in the unique opportunity to study a parasite of big game animals in a variety of closely related hosts. I was, therefore, able to confirm a primary tenet of Parasitology, i.e. that parasites in the preferred host have a higher reproductive potential and a shorter prepatent period (Croll 1973). These studies

also resulted in the hypothesis that the duration of the prepatent period is inversely related to the size of the infective inoculum. Additional experimental evidence is required to support this concept.

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APPENDIX I. History of the cervids used in experimental infections (Section III) with *Parelaphostrongylus odocoilei*.

Species and Identification number	Sex	Locale	Age at infection
Mule Deer 1	♂	Cochrane, Alberta	Not available (Fawn)
" 2	♀	Brooks, Alberta	Not available (Fawn)
" 3	♂	Brooks, Alberta	3-1/2 months
" 4	♀	Claresholm, Alberta	2 weeks
" 5	♀	Cranbrook, B.C.	2 months
" 6	♂	Cardston, Alberta	1-1/2 months
" 7	♀	Cranbrook, B.C.	3 days
" 8	♀	Cranbrook, B.C.	1-1/2 months
Black-tail Deer 2	♀	Simon Fraser University	6 months
" 7	♀	Vancouver Island, B.C.	2 months
" 23D	♂	Simon Fraser University	6 months
" 15	♀	Vancouver Island, B.C.	2 weeks
" 18	♂	Vancouver Island, B.C.	2 weeks
" 22	♀	Vancouver Island, B.C.	2 weeks
White-tailed Deer 5	♂	Brooks, Alberta	3-1/2 months
" 14	♂	Vilna, Alberta	1-1/2 months
" 15	♀	Cranbrook, B.C.	2 months
" 17	♂	Stettler, Alberta	2 weeks
" 18	♂	High River, Alberta	1 month
Moose 1	♀	Slave Lake, Alberta	5-1/2 months
" 9	♂	Sundre, Alberta	2 weeks

APPENDIX II. Prevalence of *Parelaphostrongylus odocoilei* in terrestrial molluscs of Jasper, Alberta, by date of collection.

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
23 April	*Deroceras laeve*	8	2	25
	Euconulus fulvus	20	0	--
	Discus cronkhitei	4	0	--
		32	2	6.25
4-7 May	*D. laeve*	100	12	12
	E. fulvus	64	2	3.1
	D. cronkhitei	46	0	--
	Zonitoides arboreus	60	0	--
	Oxyloma retusa	100	0	--
		370	14	3.8
12-14 May	*D. laeve*	174	0	--
	E. fulvus	82	0	--
	D. cronkhitei	29	0	--
	Discus shimeki	12	0	--
	Z. arboreus	31	1	3.2
	O. retusa	117	0	--
	Retinella electrina	5	0	--
	Vertigo modesta	2	0	--
		452	1	0.2
25-27 May	*D. laeve*	186	7	3.8
	E. fulvus	118	0	--
	D. cronkhitei	37	0	--
	D. shimeki	10	0	--
	Z. arboreus	51	1	2
	O. retusa	92	0	--
	V. modesta	2	0	0
		496	8	1.6

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
1-3 June	*D. laeve*	169	6	3.6
	E. fulvus	112	3	2.7
	D. cronkhitei	57	0	--
	D. shimeki	11	0	--
	Z. arboreus	47	0	--
	Zonitoides nitidus	13	1	7.7
	O. retusa	56	0	--
	R. electrina	2	0	--
	Striatura ferrea	6	0	--
		473	10	2.1
9-11 June	*D. laeve*	68	0	--
	E. fulvus	63	0	--
	D. cronkhitei	33	0	--
	D. shimeki	2	0	--
	Z. arboreus	18	1	5.6
	Z. nitidus	1	0	--
	O. retusa	51	0	--
	R. electrina	3	0	--
	V. modesta	3	0	--
	S. ferrea	3	0	--
		244	1	0.4
15-17 June	*D. laeve*	55	0	--
	E. fulvus	149	1	0.7
	D. cronkhitei	51	1	2
	D. shimeki	12	0	--
	Z. arboreus	73	0	--
	Z. nitidus	36	1	2.8
	O. retusa	51	0	--
	V. modesta	6	0	--
	S. ferrea	4	0	--
		437	3	0.7

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
22-24 June	*D. laeve*	44	3	6.8
	E. fulvus	160	2	1.3
	D. cronkhitei	178	0	--
	D. shimeki	19	0	--
	Z. arboreus	71	1	1.4
	Z. nitidus	15	0	--
	O. retusa	37	0	--
	R. electrina	2	0	--
	V. modesta	6	0	--
	S. ferrea	5	0	--
		537	6	1.1
28 June-1 July	*D. laeve*	21	1	4.8
	E. fulvus	121	3	2.5
	D. cronkhitei	75	1	1.3
	D. shimeki	16	1	6.3
	Z. arboreus	40	1	5
	Z. nitidus	11	1	9.1
	V. modesta	18	0	--
	Vertigo ovata	1	0	--
	Vitrina limpida	12	1	8.3
		315	9	2.9
12-14 July	*D. laeve*	41	1	2.4
	E. fulvus	153	4	2.6
	D. cronkhitei	114	1	0.9
	D. shimeki	13	1	7.7
	Z. arboreus	62	0	--
	Z. nitidus	23	0	--
	O. retusa	44	0	--
	S. ferrea	1	0	--
	V. limpida	114	0	--
	V. modesta	34	0	--
		602	7	1.2

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
19-21 July	*D. laeve*	91	1	1.1
	E. fulvus	77	0	--
	D. cronkhitei	151	0	--
	D. shiv	6	0	--
	Z. arboreus	103	0	--
	Z. nitidus	44	0	--
	O. retusa	53	0	--
	V. modesta	13	0	--
	V. ovata	3	0	--
	S. ferrea	9	0	--
	V. limpida	47	0	--
		597	1	0.2
27-28 July	*D. laeve*	36	0	--
	E. fulvus	140	0	--
	D. cronkhitei	109	1	0.9
	D. shimeki	25	0	--
	Z. arboreus	72	1	1.3
	Z. nitidus	32	0	--
	O. retusa	40	0	--
	V. modesta	53	0	--
	S. ferrea	1	0	--
	V. limpida	104	0	--
		612	2	0.3
3-5 August	*D. laeve*	75	4	5.3
	E. fulvus	131	0	--
	D. cronkhitei	41	1	2.4
	D. shimeki	5	0	--
	Z. arboreus	46	0	--
	Z. nitidus	16	0	--
	O. retusa	40	0	--
	V. modesta	40	0	--
	S. ferrea	4	0	--
	V. limpida	64	2	3.1
		462	7	1.5

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
9-10 August	*D. laeve*	40	1	2.5
	E. fulvus	132	4	3
	D. cronkhitei	104	2	1.9
	D. shimeki	14	0	--
	Z. arboreus	41	0	--
	Z. nitidus	37	1	2.7
	O. retusa	40	0	--
	V. modesta	71	0	--
	V. limpida	94	2	2.1
	Columella edentula	2	0	--
	Zonitoides sp.	5	0	--
	Pupisoma sp.	1	0	--
		581	10	1.7
17-18 August	*D. laeve*	81	5	6.2
	E. fulvus	197	5	2.5
	D. cronkhitei	75	0	--
	D. shimeki	6	0	--
	Z. arboreus	45	0	--
	Z. nitidus	16	0	--
	O. retusa	5	0	--
	V. modesta	38	0	--
	Pupisoma sp.	1	0	--
	V. limpida	144	3	2.1
		638	13	2.0
24-25 August	*D. laeve*	49	4	8.2
	E. fulvus	207	2	1
	D. cronkhitei	84	0	--
	D. shimeki	13	0	--
	Z. arboreus	31	0	--
	Z. nitidus	10	0	--
	O. retusa	37	0	--
	V. modesta	22	0	--
	S. ferrea	4	0	--
	V. limpida	89	1	1.1
	C. edentula	1	0	--
		547	7	1.3

Date of collection (1976)	Species	Number collected	Number infected	Percentage infected
7-8 September	*D. laeve*	75	4	5.3
	E. fulvus	222	5	2.3
	D. cronkhitei	71	2	2.8
	D. shimeki	6	0	--
	Z. arboreus	21	0	--
	Z. nitidus	6	0	--
	V. modesta	7	0	--
	V. limpida	169	0	--
		577	11	1.9
21-22 September	*D. laeve*	71	3	4.2
	E. fulvus	150	4	2.7
	D. cronkhitei	61	0	--
	D. shimeki	1	0	--
	Z. arboreus	11	0	--
	Z. nitidus	3	0	--
	V. modesta	7	0	--
	V. limpida	222	1	0.5
		526	8	1.5

APPENDIX III. Numbers of larvae with spined tail, recovered from fresh fecal samples of mule deer in Jasper, Alberta (22 April to 30 November 1974).

Sample Number	Date of Collection	Sex of Animal	Larvae/Gram
1	22-24 April 1974	female	31
2	"	male	36
3	"	male	228
4	"	female	61
5	"	male	10
6	"	female	60
7	"	female	226
8	"	female	324
9	"	female	255
10	"	male	223
			$\bar{X}$ = 145
11	5 May 1974	female	10
12	"	female	93
13	"	male	6
14	"	female	32
15	"	female	105
16	"	male	27
17	"	female	46
18	"	female	17
19	"	female	3
20	"	male	8
21	"	female	140
22	"	female	42
23	"	male	42
24	"	male	33
25	"	male	265
26	"	male	7
27	"	female	119
28	"	female	57
29	"	female	11
30	"	male	144
			$\bar{X}$ = 60

Sample Number	Date of Collection	Sex of Animal	Larvae/Gram
31	28-31 May 1974	male	2
32	"	male	3
33	"	male	12
34	"	male	33
35	"	male	39
36	"	female	10
37	"	male	7
38	"	male	8
39	"	male	0
40	"	female	73
41	"	male	52
42	"	female	5
43	"	male	0
44	"	male	49
45	"	male	14
46	"	female	68
47	"	male	18
48	"	male	16
49	"	male	0.2
50	"	female	31
			$\bar{X}$ = 22
51	11-14 June 1974	male	17
52	"	male	13
53	"	male	1
54	"	female	129
55	"	female	17
56	"	male	25
57	"	female	6
58	"	female	109
59	"	female	1
60	"	male	1
61	"	female	135
62	"	male	52
			$\bar{X}$ = 42

Sample Number	Date of Collection	Sex of Animal	Larvae/Gram
63	24-28 June 1974	male	19
64	"	male	52
65	"	female	0.2
66	"	male	39
67	"	female	29
68	"	female	94
69	"	female	0.2
70	"	female	4
71	"	male	24
72	"	male	+ (no count)
73	"	female	60
74	"	male	37
75	"	male	22
			$\bar{X}$ = 32
76	8-12 July 1974	female	142
77	"	female	0
78	"	male	5
79	"	male	1
80	"	male	0.3
81	"	female	177
82	"	male	2
83	"	male	26
84	"	female	77
85	"	female	33
86	"	male	11
87	"	female	0
88	"	male	0
			$\bar{X}$ = 36
89	5-9 August 1974	female	28
90	"	male	1
91	"	male	74
92	"	male	0
93	"	male	1
94	"	male	9
95	"	female	136
96	"	female	1
97	"	female	6
			$\bar{X}$ = 28

Sample Number	Date of Collection	Sex of Animal	Larvae/Gram
98	25-28 August 1974	female	2
99	"	female	19
100	"	male	0
101	"	male	5
102	"	male	5
103	"	male	6
104	"	female	8
			$\bar{X} = 6$
105	29 September 1974	male	100
106	"	fawn	0
107	"	male	55
108	"	male	25
109	"	male	167
110	"	male	69
111	"	fawn	0
112	"	fawn	0
113	"	female	4
114	"	male	149
			$\bar{X} = 57$
115	2 November 1974	female	5
116	"	female	189
117	"	female	47
118	"	male	47
119	"	male	19
120	"	male	372
121	"	female	26
122	"	fawn	20
123	"	fawn	273
124	"	fawn	9
125	"	female	4
126	"	fawn	0
127	"	male	140
128	"	female	2
129	"	male	42
			$\bar{X} = 80$

Sample Number	Date of Collection	Sex of Animal	Larvae/Gram
130	15 November 1974	male	113
131	"	male	270
			$\overline{X}$ = 192
132	30 November 1974	?	14
133	"	?	5
134	"	?	0
135	"	?	27
136	"	?	70
137	"	female	46
138	"	female	5
139	"	female	42
140	"	male	40
			$\overline{X}$ = 28

APPENDIX IV. Entry of first-stage larvae of *Parelaphostrongylus odocoilei* into various species of terrestrial molluscs. Raw data.

Deroceras laeve

Animal #	# Sections Examined	# Worm Sections in Foot	# Worm Sections in Viscera
		1/2 hour Post-exposure	
1	32	8	0
2	25	9	0
3	27	13	0
4	29	9	0
5	36	13	0
		1 hour Post-exposure	
1	34	17	0
2	27	9	0
3	33	16	0
4	28	13	0
5	35	20	0
		1-1/2 hours Post-exposure	
1	28	22	0
2	43	82	0
3	36	89	0
4	24	25	0
5	35	27	0

Deroceras reticulatum

Animal #	# Sections Examined	# Worm Sections in Foot	# Worm Sections in Viscera
		1/2 hour Post-exposure	
1	22	0	0
2	25	0	0
3	22	3	0
4	25	0	0
5	32	0	0
6	25	1	0
7	22	0	0
		1 hour Post-exposure	
1	20	0	6
2	19	3	0
3	21	1	0
4	16	3	0
5	24	1	0
6	27	0	0
7	19	0	0
8	22	0	0
		1-1/2 hour Post-exposure	
1	20	1	0
2	23	4	0
3	19	0	0
4	22	2	0
5	22	1	0
6	21	0	0
7	27	1	

Vitrina limpida

Animal #	# Sections Examined	# Worm Sections in Foot	# Worm Sections in Viscera
		1/2 hour Post-exposure	
1	52	89	6
2	34	35	0
3	50	109	0
4	39	63	0
5	36	61	0
6	60	50	0
7	49	29	0
8	58	31	0
		1 hour Post-exposure	
1	51	369	5
2	44	161	1
3	33	113	10
4	34	136	9
5	36	72	5
6	59	186	14
7	43	159	7
8	66	238	2
		1-1/2 hours Post-exposure	
1	35	185	1
2	36	122	0
3	67	439	4
4	28	135	0
5	40	195	3
6	30	154	14
7	56	168	2

Deroceras reticulatum

Animal #	# Sections Examined	# Worm Sections in Foot	# Worm Sections in Viscera
		1/2 hour Post-exposure	
1	22	0	0
2	25	0	0
3	22	3	0
4	25	0	0
5	32	0	0
6	25	1	0
7	22	0	0
		1 hour Post-exposure	
1	20	0	6
2	19	3	0
3	21	1	0
4	16	3	0
5	24	1	0
6	27	0	0
7	19	0	0
8	22	0	0
		1-1/2 hour Post-exposure	
1	20	1	0
2	23	4	0
3	19	0	0
4	22	2	0
5	22	1	0
6	21	0	0
7	27	1	

Vitrina limpida

Animal #	# Sections Examined	# Worm Sections in Foot	# Worm Sections in Viscera
		1/2 hour Post-exposure	
1	52	89	6
2	34	35	0
3	50	109	0
4	39	63	0
5	36	61	0
6	60	50	0
7	49	29	0
8	58	31	0
		1 hour Post-exposure	
1	51	369	5
2	44	161	1
3	33	113	10
4	34	136	9
5	36	72	5
6	59	186	14
7	43	159	7
8	66	238	2
		1-1/2 hours Post-exposure	
1	35	185	1
2	36	122	0
3	67	439	4
4	28	135	0
5	40	195	3
6	30	154	14
7	56	168	2