

THE BIOLOGY OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS*, A LUNG
NEMATODE OF MUSKOXEN IN THE CANADIAN ARCTIC: FIELD AND
LABORATORY STUDIES

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Philosophy
in the Department of Veterinary Microbiology
University of Saskatchewan
Saskatoon

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December 1999

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ABSTRACT

A new genus and species of protostrongylid lungworm *Umingmakstrongylus pallikuukensis* was described in muskoxen (*Oviobos moschatus*) from the west-central Canadian Arctic mainland in 1995. This parasite was considered unique among the Protostrongylidae. A series of integrated experiments were designed to investigate important aspects of the biology of this parasite in the definitive and intermediate hosts and in the arctic environment.

It was confirmed that the *U. pallikuukensis* requires gastropod intermediate hosts to develop from first-stage larvae (L1) to third-stage larvae (L3). The life cycle of *U. pallikuukensis* was completed in 3 captive muskoxen; 2 were infected with L3 which were digested from slugs (*Deroceras reticulatum*), the third with L3 that emerged from slugs (*D. reticulatum* and *D. laeve*). In 2 animals the prepatent period was 91 and 95 days, larval production peaked at 13-14 mo post-infection (PI), and the patent periods extended to the time of euthanasia, 14 and 26 mo PI. The third muskox was euthanized at patency. No clinical signs of respiratory compromise were apparent in any of the 3 experimentally infected muskoxen. At post mortem on day 97 PI, parasitic cysts were difficult to find and some parasites were not enclosed in cysts, but by months 14 and 26 PI all parasites were within clearly defined cysts. Cyst size was positively correlated with the number of adult parasites contained. Lung pathology appeared to be localized to the cysts, which had a thick fibrous capsule surrounding adult nematodes, larvae and eggs. These lesions appear to be unique among the protostrongylids. Bronchioles penetrated the cyst capsule providing a direct route for larvae to leave the lungs. Third-stage larvae of *U. pallikuukensis* were given to domestic sheep, but adult parasites did not establish.

Medical imaging techniques were used to describe the lesions caused by *U. pallikuukensis*. Parasite-associated lesions were detected with standard radiography by 178 and 191 days PI in 2 muskoxen. Lesions increased in size, then appeared to stabilize or decrease in size. Post mortem radiography was useful for locating cysts in a lightly infected animal and post mortem computed tomography was useful for locating, quantifying and characterizing cysts. These medical imaging techniques could be useful for characterizing lung nematodes in wild animals (*e.g.* muskoxen, bighorn or Dall's sheep) at post mortem.

The phenomenon of emergence of L3 of *U. pallikuukensis* from gastropod intermediate hosts was examined. This phenomenon has been reported for some protostrongylids, but the patterns and significance have not been investigated in detail.

Third-stage larvae of *U. pallikuukensis* emerged from live specimens of *D. laeve*, *D. reticulatum*, and *Catinella* sp. in the laboratory, and from live or dead *D. laeve* in field experiments on the tundra. In the laboratory, emergence occurred over a wide range of infection intensities and patterns of emergence were independent of infection intensity. In field experiments, L3 were found on vegetation by 10 wk PI, and were recovered from the vegetation of some experiments after over-wintering. Emerged L3 survived in tap water and distilled water for 13 mo at 0-4 C. Emergence of L3 may increase the temporal and spatial availability of L3 in the environment.

The morphologic and morphometric aspects of larval development of *U. pallikuukensis* in *D. laeve* at 23.4 C were investigated and compared to other protostrongylids. The majority of the larval growth of *U. pallikuukensis* occurs immediately following the second molt. The features most useful for defining larval stages are the separation of the cuticular sheaths, tail structure and viability following digestion.

The rates of larval development for *U. pallikuukensis* in *D. laeve* and *D. reticulatum* at various temperatures in the laboratory were investigated. In *D. laeve* the temperature threshold was 8.5 C, in *D. reticulatum* it was 9.5 C and the degree-days required to develop to L3 in both gastropod species was 167. These developmental parameters were compared among several species of the Protostrongylidae and it is hypothesized that they may be influenced both by the environment and by features of the parasites and intermediate hosts, including phylogeny.

Understanding the development of *U. pallikuukensis* in *D. laeve* in the Arctic is critical for understanding its epidemiology. Over a period of 2 years a series of experiments was established in the tundra of the central Arctic mainland near Kugluktuk, Nunavut, Canada. The locally abundant slug, *D. laeve*, was experimentally infected with L1 of *U. pallikuukensis* and the slugs were placed in enclosures in the tundra every 1-2 wk. Microhabitat temperatures were measured both inside and outside enclosures. Rates of larval development were determined by weekly or bi-weekly examination of the slugs and larvae from a subset of enclosures. Some slugs were left in enclosures over the winter. In 1997, L3 were recovered from slugs infected by 17 July at 4-6 wk post-infection. In 1998, slugs infected by 25 June contained L3 by the end of July. Slugs containing larvae were recovered from the enclosures after over-wintering, and larvae which probably entered the winter as second-stage larvae had resumed development to L3. The greatest mean number of L3 in slugs occurred in August, after which time the number of L3/slug decreased and L3 were found on the vegetation. Rates of larval development in the Arctic corresponded with those predicted using surface microhabitat

temperatures and laboratory derived threshold temperatures (8.5 C) and thermal constants (167 degree-days). This enclosure system has far-reaching applications for investigation of other protostrongylids, in other geographic regions or under different or changing climatic conditions (including global warming).

The distribution of *U. pallikuukensis* is potentially linked to the distribution of suitable intermediate hosts. The terrestrial and freshwater gastropod faunas were surveyed on the Arctic mainland near Kugluktuk and on Victoria Island. The ability of several of the species recovered to support development of *U. pallikuukensis* from L1 to L3 was then determined. On the mainland 6 terrestrial and 4 freshwater species were found. On Victoria Island 1 terrestrial (*D. laeve*) and 3 freshwater species were found. The terrestrial species; *D. laeve*, *Catinella* sp. *C. alticola*, and *Euconulus fulvus*, and the freshwater species; *A. hypnorum* and possibly *Physa jennessi jennessi*, supported development of *U. pallikuukensis* from L1 to L3. *Deroceras laeve*, because of its high mobility, widespread distribution, and the high proportion of larvae which establish and develop to L3 in a short time, may be the most important intermediate host. It is possible that *U. pallikuukensis* could establish on Victoria Island if introduced. In addition, it is likely that suitable intermediate hosts are present on mainland east of Kugluktuk, and that the climatic conditions in this region (central and eastern mainland) would support larval development.

It is apparent that *U. pallikuukensis* may utilize several strategies to successfully maintain itself in the muskox population, including: localized pulmonary pathology; high levels of L1 production; extended patency; use of a variety of terrestrial and freshwater intermediate hosts; rapid development in the arctic environment; overwinter survival; and larval emergence. This research has greatly increased our understanding of several aspects of the biology of not only *U. pallikuukensis*, but other protostrongylids and arctic parasites in general.

ACKNOWLEDGMENTS

My thesis work has spanned several disciplines and several degrees in latitude and would never have been possible without the extensive support of numerous individuals and organizations. I would like to extend my deepest gratitude to all those who have aided in the numerous aspects of this project and have provided me with great moral support.

In Kugluktuk from the Department of Resources, Wildlife, and Economic Development, (DRWED) Kitkmeot Region I thank John Stevenson and Sandy Buchan for welcoming me into their office and providing vital logistical and financial support; Colin Adjun and Josh Hunter for transporting me safely to and from field camps and assisting with field research; Damian Panayi and Monica Kapakatoak for valuable assistance in the laboratory and field; Jacob Keanik, Grant Corey, Joe Ashevak and Remi Krikort and Andy McMullen for facilitating the collection of muskox samples from hunters; Damian for retrieving numerous references and odds and ends and giving me a reason to smile during hectic times, and Alasdair Veitch and Brett Elkin for their interest and moral support. Thank-you to the many Work Experience students from the Kugluktuk High School, and especially to Christabelle Westwood, Shannon Ekhiohina, and Theresa Westwood for doing excellent jobs both in the laboratory and field. The Kugluktuk Hunters' and Trappers' Association provided logistical support, and numerous hunters in the Northwest Territories and Nunavut put the extra effort in to collect and transport muskox samples back to town for analysis. Mindy Willet, Harold Mulder, Rita Kutz, Gwyn Kutz, Brent Wagner, Lois Wooding, Dave and Mary Elder, Eric Doig, and Joyce and Mrs. Nishi all provided valuable assistance in the field studies.

My work in Saskatoon represents the fruit of many peoples' labours. Sue Tedesco, Jan Adamczewski, Ewald Lammerding, and Peter Flood provided valuable advice and insight into muskox husbandry and handling. From the Center for Animal Parasitology Terry Steeves and Tracy Goldade provided gastropods and advice on gastropod maintenance and care. The WCVM Animal Care Unit (Margo Buckley, Kyle Constantinoff, Doug Walde, Marlow Thue, Monique Burmester, Sandy Parsons, and Katherine Hljeski) took excellent care of the research animals and were a guaranteed source of smiles and treats.

Dissection of cyst of *U. pallikuukensis* is a very long and tedious affair, to which a select group of very special people can attest. Thank-you for your hours of patience and dedication: Noel Moens, Angela Schneider, Paul Gagnon, Rick Espie, Nicole Paquette and Brent Wagner. Thank-you to Manuel Chirino-Trejo for

bacteriological examination of the cysts, and Gary Wobeser for assistance in interpreting the histology.

Richard Back, Nadine Morris, and Sharon Martin exhibited great patience and expertise in radiographing my research animals; Charles Farrow and John Pharr assisted with interpretation of radiographic films. Doug Wenzel of the Computed Tomography Unit at the Royal University Hospital provided excellent technical assistance, Kendra Fisher aided with the interpretation of films and J. Loewy facilitated the scans.

Juliane Deubner has aided me extensively through the years in preparation of figures and slides, usually at the last minute; for her patience and expertise I am truly indebted. A special thank-you to Darren Harder and Chris Gaschler for very timely and generous assistance. Kathy Caspell has provided excellent technical assistance in preparation of some very frustrating photographs and Ian Shirley has generously provided computer assistance.

I thank my fellow graduate students whose friendships I value, and the faculty and staff of the Department of Veterinary Microbiology who have made my studies here interesting. I especially thank Brent Wagner who has provided invaluable assistance with many aspects of my research.

Peter Flood has been the source of many stimulating conversations related to muskoxen, other arctic ungulates, and the world in general. John Nishi was the source of great insight and guidance for methods and theories in field and Arctic biology. Eric Hoberg constantly challenged me, encouraging a global perspective. He has been a source of great inspiration, an extraordinary role model and a great friend. Lydden Polley has been a dedicated and enthusiastic supervisor. He has allowed me the freedom to pursue my personal goals and explore the Arctic as well as the world of research; for this I am very grateful. The remaining members of my advisory committee have provided valuable guidance throughout my research: Alvin Gajadhar, John Gordon, Vikram Misra, Gary Wobeser.

Finally, I would like to thank my parents for providing me with a solid foundation on which I've been able to build, and my brother, my sisters, and Peter Jelfimow for their unending support.

Financial assistance for this research has been provided by an Interprovincial Graduate Student Scholarship, the Department of Resources, Wildlife and Economic Development (Government of the Northwest Territories), the Northern Scientific Training Program (Department of Indian Affairs and Northern Development), Merial Ltd., the Wildlife Disease Association, the WCVN Wildlife Health Fund.

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LIST OF ABBREVIATIONS

aL3	early third-stage larvae
bL3	intermediate third-stage larvae
cL3	late third-stage larvae
eL3	emerged third-stage larvae
BL	body length
BW	body width
CS1	first cuticular sheath
CS2	second cuticular sheath
CT	computed tomography
DD	degree-days
DH	definitive host
DPI	days post-infection
ED	distance to the base of the esophagus
EIJ	esophageal-intestinal junction
EPD	distance to the excretory pore
EW	esophagus width
GP	genital primordium
GPL	length of the genital primordium
GPW	width of the genital primordium
id	inside diameter
IH	intermediate host
K	thermal constant
KW	Kruskal-Wallis
L1	first-stage larvae
L2	second-stage larvae
L3	third-stage larvae
LPG	larvae per gram of feces (wet weight)
LS	lake shore
L VI	lake shores on Victoria Island
ML	mainland
MSM	mesic sedge meadow
mSS	mean of the soil and surface temperatures
NRD	distance to the nerve ring
PI	post-infection
RB	river bank
T ₀	threshold temperature
TL	tail length
VI	Victoria Island
W	Wilcoxon-Mann-Whitney test
WSM	wet sedge meadow

1. INTRODUCTION

1.1 Background

In 1988 an unusual, unidentified nematode parasite was recovered from cysts in the lungs of a muskox (*Ovibos moschatus*) found dead west of Kugluktuk, Nunavut, Canada (67°49'N, 115°9'W) (Fig. 1) (Gunn et al., 1991). Subsequently, between November 1989 and November 1990, similar cysts were found in the lungs of 49 of 53 hunter-killed muskoxen in the region of the Rae and Richardson rivers west of Kugluktuk (Gunn et al., 1991). Older bulls appeared to be the most heavily infected, with more than 250 cysts in an individual animal, but all age and sex classes were infected (Gunn et al., 1991; Gunn and Wobeser, 1993). The parasite had not been reported on the mainland east of Kugluktuk or on Victoria or Banks islands where numerous animals had been examined in the course of commercial muskox harvests and other research (Gunn et al., 1989; Gunn et al., 1991), suggesting that the geographic distribution of the parasite was restricted. The origin, identity, life cycle, and significance of this apparently new parasite were not known.

1.2 Parasite description

In 1995, *Umingmakstrongylus pallikuukensis* Hoberg, Polley, Gunn and Nishi 1995 was named as a new genus and species within the family Protostrongylidae (Hoberg et al., 1995). The generic name was derived from the Inuinnaqtun name for muskox 'Umingmak', and the Latin 'strongylus' for the strongylate nematodes. The specific name was derived from the Inuinnaqtun name 'Pallik' for the geographic location in which the parasite was first found, and 'kuuk' referring to river (Hoberg et al., 1995). *Umingmakstrongylus pallikuukensis* is a member of the subfamily Muelleriinae (Carreno and Hoberg, 1999), morphologically most similar to the genus *Cystocaulus* (Hoberg et al., 1995). It represents the northern-most geographic occurrence reported for a protostrongylid in North America (Anderson, 1992).

Umingmakstrongylus pallikuukensis is an exceptionally large parasite (females measuring up to 47 cm in natural infections) and the adults live in well defined cysts 9-40 mm in diameter in the lung tissue. These lesions are considered pathognomonic for the parasite, differing significantly from those caused by species in the genera *Muellerius* or *Cystocaulus* (Hoberg et al., 1995). Lung pathology associated with *U. pallikuukensis* appears to be localized to the cysts. In naturally infected muskoxen, reduced exercise tolerance has been observed and this may increase susceptibility to predation (Gunn et al., 1991). The discovery of *U. pallikuukensis* coincided with a decline in the size of the affected muskox population between 1988 and 1994 (Fournier and Gunn, 1998), but the role of the parasite in this decline is undetermined.

As a member of the Protostrongylidae it was expected that *U. pallikuukensis* would require a gastropod intermediate host to complete its life cycle. Experimental infections in the laboratory demonstrated that first-stage larvae (L1) from muskox feces or lungs could develop to third-stage larvae (L3) in the slug *Deroceras reticulatum* (Hoberg et al., 1995). A life cycle similar to other protostrongylids was inferred. Hoberg et al. (1995) recognized that many factors may affect the development of larvae and subsequent transmission of the parasite in the arctic environment (*e.g.* temperature and moisture at the level of the microhabitats, distribution of potential gastropod intermediate hosts). A narrow window for larval development and transmission in the Arctic was predicted, and it was postulated that increased global temperatures could affect parasite epidemiology at these latitudes. These authors suggested that the apparently limited geographic distribution of *U. pallikuukensis* could reflect historical isolation of muskox populations during the Pleistocene, or more recent isolation resulting from local extirpations on the mainland in the early 1900's.

1.3 Rationale for further research

Research on *U. pallikuukensis* was pursued for a number of reasons. (1) The parasite could be a significant pathogen for muskoxen. These animals are important cultural and economic resources as well as as sources of food for many residents of the Arctic (Gunn et al., 1990). Personal accounts of wild muskoxen demonstrating exercise intolerance, and mortality of adult muskoxen caused by grizzly bear (*Ursus arctos*) predation or possibly by pneumonia (Case and Stevenson, 1991; Gunn et al., 1991), suggested that *U. pallikuukensis* may have played a role in the population decline. To facilitate informed management decisions with respect to the parasite it is necessary, therefore, to understand aspects of the life cycle, including transmission, pathogenicity,

and host specificity. (2) *Umingmakstrongylus pallikuukensis* itself deserved attention. The parasite fauna of arctic ruminants is poorly documented and the transmission dynamics are not well understood (Hoberg et al., 1999; Brooks and Hoberg, 2000). It is important to understand the biology of this unique new genus and species within the context of the Protostrongylidae and as an apparently successful, but geographically restricted, parasite in the Arctic environment. (3) *Umingmakstrongylus pallikuukensis* could be used to establish a model system for investigating protostrongylid larval development and transmission in the arctic ecosystem, including the effects of a changing climate on parasite epidemiology and geographic distribution.

In 1994, an interdisciplinary, multi-agency team was established including scientists from: the Department of Resources, Wildlife and Economic Development, Government of the Northwest Territories (NWT) (Nunavut and NWT since April 1, 1999); the Centre for Animal Parasitology, Canadian Food Inspection Agency, Government of Canada; the Departments of Veterinary Microbiology, Veterinary Anatomy and Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan; and the Biosystematics and National Parasite Collection Unit, Agriculture Research Service, United States Department of Agriculture. It was within this supportive framework that this thesis was conceived, with the broad objectives of determining the life cycle of *U. pallikuukensis* and understanding some of the many factors affecting development and transmission of this unusual parasite in a unique host in the Canadian Arctic.

2. THE PROTOSTRONGYLIDAE

2.1 Phylogeny of the Protostrongylidae

The Protostrongylidae is a family of nematode parasites that, as adults, occur in mammalian hosts. Under prevailing classifications, protostrongylids belong to the Class Secernentea, Order Strongylida, Superfamily Metastrongyloidea. They are parasites mainly of the pulmonary, nervous, and skeletal-musculature systems of the Cervidae and Bovidae, but have also colonized Leporidae. They have indirect life cycles that require molluscan intermediate hosts for larval development. Recent phylogenetic analyses based on morphological characters of the genera and species of the Protostrongylidae diagnose 2 major clades (Carreno and Hoberg, 1999). One clade consists of 2 subfamilies: the Muelleriinae Skrjabin, 1933 and the Elaphostrongylinae Boev and Schulz, 1950. Phylogenetic studies among elaphostrongylinae that also examine a history for host-associations and biogeography have been conducted by Platt (1984) and Carreno and Lankester (1994). The second clade is more diverse, consisting of the Protostrongylinae (Boev and Schulz, 1950), the Varestrostrongylinae Boev, 1968, the Skrjabinocaulinae Boev and Sulimov, 1963, and the genera *Neostromgylus* Gebauer, 1932, *Orthostromgylus* Dougherty and Goble, 1946, and *Pneumocaulus* Schulz and Andreeva, 1948. *Umingmakstrongylus pallikuukensis* is a member of the Muelleriinae, and is the putative sister-group of *Cystocaulus* (Carreno and Hoberg, 1999).

Comprehensive reviews of the Protostrongylidae are available (Boev, 1975; Kontrimavichus, 1975; Anderson, 1992) and, as a consequence, this review is focused to provide a brief overview of the phylogenetically related Muelleriinae and Elaphostrongylinae. Using these sub-families as examples, the life cycles of the Protostrongylidae and many factors affecting development and transmission will be discussed. Where appropriate, examples from other subfamilies will be used. It is intended that this will serve as the framework for understanding the rationale for the research presented in the thesis.

2.2 General description of genera phylogenetically related to *U. pallikuukensis*

2.2.1 The Muelleriinae

The Muelleriinae consist of the genera *Cystocaulus*, *Muellerius*, and *Umingmakstrongylus*. *Cystocaulus* and *Muellerius* are parasites of the lung parenchyma, primarily in the Caprinae. There are 3 species of *Cystocaulus*: *C. ocreatus* (Railliet and Henry, 1907) has been reported from Europe, Asia, North America, and Africa; *C. ovis* (Sarwar, 1955) Boev, 1957 is known primarily from Asia; and *C. vsevolodovi* Boev, 1946 from Asia and Europe (Boev, 1975). There are 2 species of *Muellerius*: *M. capillaris* (Mueller, 1889) is the best known and has a cosmopolitan distribution; *M. tenuispiculatus* Gebauer, 1932 is less well known and is found in Europe (Boev, 1975). *Cystocaulus ocreatus* and *M. capillaris* commonly are found in mixed lungworm infections in sheep and goats in Europe (Gerichter, 1951; Rose, 1955; Boch and Nurnberg, 1962). There is an extensive literature on *M. capillaris* but less information is available for *C. ocreatus*. These parasites are usually found in sub-pleural nodules, that are sometimes extensive, in the obtuse margin of the diaphragmatic lobes of the lungs (Rose, 1961; Sedlmeier et al., 1969). Both species have been associated with pneumonia and decreased weight gains in domestic sheep (Rose, 1959; Berrag and Cabaret, 1998). *Muellerius capillaris* has been reported in wild North America bighorn sheep (*Ovis canadensis*) in South Dakota and in Colorado, USA (Pybus and Shave, 1984 and Demartini and Davies, 1977, respectively). In Colorado, this parasite was implicated in a die-off of wild-caught bighorn sheep kept in captivity (Demartini and Davies, 1977).

2.2.2 The Elaphostrongylinae

The Elaphostrongylinae consist of the genera *Parelaphostrongylus* Boev and Schulz, 1950 and *Elaphostrongylus* Boev and Schulz, 1950. The parasites of this sub-family live as adults in the musculature and/or central nervous system of their definitive hosts, which are normally cervids. The genus *Parelaphostrongylus* consists of 3 species which are reported only in North America. The genus *Elaphostrongylus* consists of 3 species that appear to have originated in Europe.

Parelaphostrongylus odocoilei (Hobmaier and Hobmaier, 1934) is a common muscle-dwelling nematode described originally in mule deer (*Odocoilei hemionus hemionus*) and black-tailed deer (*O. h. columbianus*) from western North America. It

has also been found in naturally infected woodland caribou (*Rangifer tarandus caribou*), mountain goats (*Oreamnus hemionus*) and most recently in Dall's sheep (*Ovis dalli*) (Gray and Samuel, 1986; Pybus et al., 1984; Kutz et al., 2000c). Moose (*Alces alces*) have been experimentally infected, but patent infections have not been established in white-tailed deer (*Odocoileus virginianus*) (Platt and Samuel, 1978; Pybus and Samuel, 1984b). Hemorrhage and a mild myositis are generally associated with the adults of *P. odocoilei* and mild to severe interstitial pneumonia has been associated with the eggs and larvae (Pybus and Samuel, 1984a).

Parelaphostrongylus andersoni Prestwood, 1972 is a muscle dwelling nematode first detected in white-tailed deer. It has a disjunct distribution throughout the USA and Canada. It is known in woodland and barren ground caribou (*R. t. groenlandicus*) in Canada and was recently found in caribou from the northern Alaskan peninsula (Lankester and Hauta, 1989; R. Zarnke, pers. comm.). Experimentally it can infect mule deer, fallow deer (*Dama dama*), and probably moose (Pybus and Samuel, 1984b; Lankester et al., 1990; Lankester and Fong, 1998). As with *P. odocoilei*, *P. andersoni* can cause hemorrhage, focal myositis and interstitial pneumonia (Pybus and Samuel, 1981). Clinical signs have been seen only in animals experimentally infected with massive numbers of larvae (Nettles and Prestwood, 1976).

Parelaphostrongylus tenuis (Dougherty, 1945) is common in white-tailed deer from eastern North America. Adult parasites occur most commonly in the cranial subdural space and the venous sinuses of the cranium. Although it rarely causes significant disease in white-tailed deer, *P. tenuis* can cause severe neurological disease in moose, caribou, reindeer (*R. t. tarandus*), mule deer, wapiti (*Cervus elaphus*) and fallow deer (Anderson, 1992). It has also been reported as a cause of neurological disease in non-cervids including domestic sheep and goats, llamas (*Llama glama*), eland (*Taurotragus oryx*), sable antelope (*Hippotragus niger*), and pronghorn antelope (*Antilocapra americanus*) (Anderson, 1992).

Species of the genus *Elaphostrongylus* are parasites of the central nervous system and skeletal muscles and occur primarily in cervids in Europe and Asia, but have been translocated to New Zealand (*E. cervi*) (Mason and McCallum, 1976), and Canada (*E. rangiferi*) (Lankester and Fong, 1989).

Elaphostrongylus rangiferi Mitskevich, 1960 is common in reindeer in northern Fennoscandia and Russia and in caribou in Newfoundland (Mason and McCallum, 1976; Lankester and Fong, 1989). Adult parasites are found both in the muscle (primarily beneath the epimysium) and in the central nervous system, usually in the arachnoid or subdural space over the spinal cord and brain (Lankester and Northcott,

1979; Hemmingson et al., 1993; Handeland, 1994). These parasites can cause severe neurological disease as well as interstitial pneumonia (Roneus and Nordkvist, 1962; Lankester and Fong, 1998). Natural infections are known in moose, domestic sheep and goats (Handeland and Sparboe, 1991; Handeland, 1991; Lankester and Fong, 1998), and immature *Elaphostrongylus*-like nematodes were recovered from the central nervous system of ataxic muskoxen in northern Norway (Holt et al., 1990). Because of the severe outbreaks of clinical disease and mortality associated with *E. rangiferi*, it is of great importance to consider this parasite in the management of reindeer and caribou. There is an extensive literature on the biology and epidemiology of *E. rangiferi*, in an environment similar to that in which *U. pallikuukensis* occurs.

Elaphostrongylus cervi Cameron, 1931 can also cause severe neurological disease and interstitial pneumonia in a variety of cervid hosts, but primarily red deer (*Cervus elaphus*) (Kontrimavichus, 1975). It is found in the musculature and central nervous system of these hosts from Europe, Asia and New Zealand. The potential risk of this parasite to Canadian cervids has resulted in strict importation regulations for red deer entering Canada, as well as considerable research on immunological tests and molecular techniques to detect infections and to develop effective diagnostic tests to differentiate among the Elaphostrongylinae (Gajadhar et al., 1994; Ogunremi et al., 1999).

Elaphostrongylus alces Steen, Chabaud and Reh binder 1989 is a recently described elaphostrongyline known only in moose from Sweden, Norway and Finland (Steen et al., 1989; Gibbons et al., 1991; Steen et al., 1998a). Adult parasites are found immediately beneath the epimysium, in fasciae between muscle bundles, or in the epidural space of the central nervous system. The parasite is known to cause neurologic disease and has been associated with emaciation and death in moose (Steen and Reh binder, 1986; Steen and Roepstoff, 1990). Experimentally the parasite can establish in reindeer, but natural infections have not been reported (Halvorsen et al., 1989).

2.3 The life cycle of protostrongylids

2.3.1 General life cycle

Depending on the species, protostrongylids live as adults in the lungs, muscles and fascia, or central nervous system. Adult parasites lay eggs which, for lung dwelling protostrongylids remain in the lungs and, for tissue protostrongylids, are transported via the blood stream to the lungs. Eggs hatch in the lungs to first-stage larvae (L1), migrate to the airways and are carried up the respiratory tree to the pharynx. They are then

swallowed and passed in the feces. First-stage larvae may be most common either inside the fecal pellet (e.g., *Protostrongylus* spp.) or on the surface (e.g., *P. tenuis*) (Forrester and Lankester, 1997b). Once in the external environment, L1 must contact and infect a suitable gastropod intermediate host, within which they undergo 2 moults to become infective third-stage larvae (L3). Infected gastropods, or perhaps L3 that have left the gastropods, are ingested by the definitive host, probably accidentally, and the L3 migrate from the digestive tract to their site of reproduction.

2.3.2 Parasite development in the definitive hosts

The route of L3 migration to the final site of reproduction may differ among genera and even among congeners. A direct migration has been suggested for *E. cervi*, whereas there is evidence for haematogenous spread for *E. rangiferi* (Handeland, 1994; Olsson, et al., 1998). For *M. capillaris* and *C. ocreatus*, migration appears to occur via the lymphatics (Boev, 1975). Also, for some species (e.g., *E. rangiferi*), larvae may migrate first to 1 anatomical location to mature (e.g., central nervous system) and may then move to another location (skeletal muscles) to reproduce (Hemmingsen et al., 1993).

The prepatent period is the time from when L3 are ingested to when L1 are first present in the feces. In the typical host this varies considerably among different protostrongylid species, even within a genus. For example, the prepatent period of *P. odocoilei* is 45-62 days but that of *P. tenuis* is 82-137 days (Platt and Samuel, 1978; Pybus and Samuel, 1984; Rickard et al., 1994). The prepatent period typically is shorter for the lungworms (Muelleriinae) than the tissue worms (Elaphostrongylinae) (Rose, 1959; Gerichter, 1951; Anderson, 1992). It may also be inversely related to the dose of L3 (Platt and Samuel, 1978; Pybus and Samuel, 1984b; Rickard et al., 1994), and may be extended when animals are infected with repeated low doses of L3 compared to with a single high dose (Prestwood and Nettles, 1977). Parasites in atypical hosts may have longer prepatent periods (Platt and Samuel, 1978).

The patent period is the length of time L1 are shed after a single infection event. This again differs among protostrongylid species, but can be up to 5 (*C. ocreatus*) or 6 years (*P. odocoilei*) (Boev, 1975; Pybus and Samuel, 1984a). Typically, after a single infection event, the quantity of larvae shed per gram of feces (LPG) increases logarithmically during the first 2 to 6 or 8 wk of patency and then, depending on the species of protostrongylid, may plateau and then decrease (e.g., *P. andersoni*) or may remain relatively high, although somewhat erratic (e.g., *P. odocoilei*) (Nettles and

Prestwood, 1976; Platt and Samuel, 1978; Pybus and Samuel, 1984a; Lankester and Hauta, 1989). In nature there is evidence of an acquired immunity to some protostrongylids (e.g., *P. tenuis* and *P. andersoni*) (Kontrimavichus et al., 1976; Slomke et al., 1995), but for other species little immunity seems to develop and infections are cumulative over time (e.g., *P. odocoilei* and *M. capillaris*) (Boev, 1975; Platt and Samuel, 1978).

Seasonal variations in larval production have been reported for most protostrongylids in naturally infected hosts. For *E. rangiferi* in reindeer an increase in LPG is seen in bulls during the rut, and cows from January until July (Halvorsen et al., 1985). Increased larval production associated with stress (e.g., parturition), has been reported for *Protostrongylus* spp. in bighorn sheep (Forrester and Senger, 1964; Uhazy et al., 1973) and maximum LPG of *P. odocoilei* from mule deer is observed in March and April (Samuel et al., 1985).

2.3.3 Protostrongylid development in the environment

2.3.3.1 Survival of first-stage larvae

Depending on environmental conditions, L1 of protostrongylids may survive in the environment for extended periods (Rose, 1957; Beresford-Jones, 1966; Lorentzen and Halvorsen, 1986). Resistance to different environmental extremes may differ among species and may account for, or be a response to, the different geographic locations in which the various species are found (Shostak and Samuel, 1984; Cabaret et al., 1991; Morrondo-Pelayo et al., 1992; Diez-Banos et al., 1993). First-stage larvae survive longer in water than if desiccated, and longer at constant cool temperatures than at fluctuating temperatures, particularly repeated freezing and thawing. Survival generally increases with decreasing temperatures (Forrester and Senger, 1963; Shostak and Samuel, 1984) and, when L1 of *E. rangiferi* are maintained frozen in feces or water for 360 days there is no decrease in survival (Lorentzen and Halvorsen, 1986). The type of vegetation on which feces are deposited also affects the survival of the L1 (Diez-Banos et al., 1993). Exposure of L1 to various environmental conditions is not, however, without consequences. Although L1 may survive adverse conditions, the subsequent infection success in gastropods may be reduced (Shostak and Samuel, 1984; Morrondo-Pelayo et al., 1992).

2.3.3.2 *Infection of gastropods*

Larvae typically enter gastropods by actively penetrating the superficial epithelium, usually in furrows of the foot tissue, but have also been reported entering the mantle and antennae (Kassai, 1958; Platt and Samuel, 1984; Samson and Holmes, 1985a; Rezac et al., 1994). Although less common, passive entry whereby larvae are ingested by the gastropod is known or suspected (Anderson, 1963; Platt and Samuel, 1984; Samson and Holmes, 1985b; Rezac et al., 1994) and may represent a less energetically costly method for entering the intermediate host (Samson and Holmes, 1985b).

The rate and success with which L1 infect gastropods may vary depending on the environment, the L1, and the gastropods. In the laboratory, infection rates depend on many environmental factors such as the substrate on which larvae and gastropods are placed during infection, moisture content of the substrate (the larvae are probably dependent on a thin layer of moisture to move effectively), size of exposure chamber, density of L1, temperature, and exposure time (Lankester and Anderson, 1968; Sauerlander, 1979; Skorping, 1982, 1985; Rezac et al., 1993). Characteristics of the L1, such as age and conditions under which they have been maintained (see section 2.3.3.1) will also affect infection rates (Lankester and Anderson, 1968; Skorping, 1982, 1985). Finally, the gastropods themselves critically influence infection rates. In the laboratory, species of gastropods differ in their susceptibility to various species of protostrongylids (Gerichter, 1948; Kassai, 1958; Lankester and Anderson, 1968; Solomon et al., 1996). Within a species, the age, size, and activity of individual gastropods, as well as the quantity and quality of mucus produced, may influence infection rates (Skorping, 1985; Cabaret, 1987; Rezac et al., 1993; Manga-Gonzalez and Morrondo-Pelayo, 1994).

Larvae are often aggregated in a few individual gastropods within a population, even after identical laboratory infection procedures (Kassai, 1958; Skorping, 1985). Similar aggregation has been observed in field collections (Lankester and Anderson, 1968; Maze and Johnstone, 1986).

2.3.3.3 *Factors affecting development in gastropods*

Development of larvae in intermediate hosts depends on several factors. First, development cannot occur in all species of gastropods; there is a continuum of suitability. Some gastropods will not support development at all, in other species development occurs, but at a slow rate, and yet in other species, larvae develop rapidly

(Gerichter, 1948; Lankester and Anderson, 1968; Manga-Gonzalez and Morrondo-Pelayo, 1994).

Environmental temperatures significantly affect the rate of larval development for protostrongylids in intermediate hosts. Typically, there is a linear relationship between development rate and temperature, although at the extremes the curve tapers (Halvorsen and Skorping, 1982). At the lower end of the temperature range there is a theoretical threshold below which minimal detectable development occurs. First-stage larvae of *Protostrongylus* spp. in gastropods maintained below this threshold for extended periods of time will, however, develop more quickly than expected when subsequently placed at temperatures above the threshold (Samson and Holmes, 1985b). At higher temperatures, development may reach a maximum rate not increased by further increases in temperature (Halvorsen and Skorping, 1982). The temperature thresholds differ among species of the Protostrongylidae and among species of intermediate hosts (Rose, 1957; Halvorsen and Skorping, 1982; Samson and Holmes, 1985b). Gastropod age and size, physiological condition, nutritional status and infection intensity also influence rates of larval development (Lankester and Anderson, 1968; Skorping, 1984; Solomon et al., 1996).

2.3.3.4 *Protostrongylids in naturally infected intermediate hosts*

The prevalence and intensity of infection of wild gastropods with protostrongylids is generally low (prevalence < 10%, intensity usually 1-5 larvae, but up to 97) and varies depending on the parasite species, definitive host population densities and habitat use, time of year, climatic conditions, habitat types, and gastropod species, density and life history traits (Lankester and Anderson, 1968; Samuel et al., 1985; Maze and Johnstone, 1986; Rowley et al., 1987; Kraika and Samuel, 1990; Berrag and Urquhart, 1996; Lankester and Peterson, 1996). The seasonal patterns of larval occurrence in gastropods may differ depending on parasite species and geographic location, or more specifically, climate (Samuel et al., 1985; Berrag and Urquhart, 1996). For some protostrongylid species intensity of infection of gastropods may not vary during the summer season (Kraika and Samuel, 1990).

2.3.3.5 *L3 emergence*

The phenomenon of emergence of L3 from gastropod intermediate hosts has been reported anecdotally for some species of protostrongylids. Emerged L3 of *P. boughtoni* were infective to a domestic rabbit, but this mode of transmission was not

considered significant for the epidemiology of this parasite (Kralka and Samuel, 1984). In contrast, some authors have considered emergence of L3 to be important in transmission of *Cystocaulus* spp. and *Muellerius* spp. (Boev, 1975). There are no published reports of L3 emergence among the Elaphostrongylinae, but it has been observed for *P. odocoilei* originating from Dall's sheep (S. J. Kutz, unpubl. data). Factors affecting L3 emergence and the patterns of emergence have not been investigated in detail.

2.3.4 Protostrongylid transmission

Transmission of protostrongylids typically occurs when definitive hosts ingest gastropods containing infective L3. If emergence of the L3 is discounted, transmission is dependent on the temporal and spatial overlap of infected gastropods and definitive hosts. The availability of L3 within intermediate hosts is influenced by seasonality of L1 production and habitat use by definitive hosts (environmental contamination), intrinsic development characteristics of the parasites in gastropods, life span of intermediate hosts, and climatic conditions (Samuel et al., 1985). Detailed investigations of some protostrongylid host-parasite systems, considering many of these factors, have led to predictions of the most probable season and/or location for transmission to occur (Halvorsen et al., 1980; Samuel et al., 1985). A second mode of transmission is transplacental, which has been confirmed only for *Protostrongylus* spp. of bighorn sheep (Hibler et al., 1972, 1974; Gates and Samuel, 1977; Kistner and Wyse, 1979).

2.4 Discovery *U. pallikuukensis*

2.4.1 Hoberg et al., 1995

The discovery of *U. pallikuukensis*, an exceptionally large, conspicuous nematode in a large, economically and culturally important arctic ungulate, was surprising. Description of the parasite in 1995 demonstrated that *U. pallikuukensis* is an unusual parasite apparently thriving in a unique host in the Arctic. Hoberg et al. (1995) raised many questions and hypotheses with respect to the origin, life cycle and geographic distribution of this parasite as well as its significance to the infected muskox population, other uninfected muskox populations, and other arctic ruminants.

2.4.2 Brief overview of thesis

A series of integrated experiments was designed to provide basic and, at times, more detailed information on the life cycle of *U. pallikuukensis* and factors influencing its development, transmission, and geographic distribution in the arctic environment.

In Chapter 3 aspects of the life cycle of *U. pallikuukensis*, including prepatent and patent periods, and patterns of larval shedding are described. The adult parasite populations in the lungs are characterized and the pulmonary pathology is described.

In Chapter 4 the use of standard radiography to examine lung pathology and cyst characteristics in live muskoxen is described. Standard radiography and computed tomography are then used to investigate the parasitic lesions at post mortem.

In Chapter 5 the phenomenon of emergence of L3 from the intermediate hosts is addressed. Patterns of emergence from 3 different intermediate host species in the laboratory are described and observations on L3 emergence in the field and the significance for parasite transmission are discussed.

In Chapter 6 a detailed description of the larval stages in the intermediate host is presented, identifying morphological and morphometric changes in larvae as they develop from L1 to L3. In addition, the effect of temperature on development rates is determined in 2 intermediate host species and the threshold temperatures and degree-days required for development to L3 are calculated for both species. This provides the basis for comparison of larval development patterns, threshold temperatures and thermal constants among examples of the Muelleriinae and Elaphostrongylineae.

In Chapter 7 the use of an enclosure system to examine development of *U. pallikuukensis* on the tundra is described. Larval development rates in the Arctic are compared to those predicted on the basis of a variety of temperatures (microhabitat and air) and on the temperature thresholds and degree-day requirements established in Chapter 6. Larval emergence and overwinter survival of larvae in slugs and subsequent larval development are also examined.

In Chapter 8, the gastropod fauna on the mainland near Kugluktuk, Rae River and Basil Bay, and on Victoria Island near Simpson Bay, is surveyed to gain insight into one of the factors which may affect the geographic distribution of *U. pallikuukensis*. The various gastropod species recovered are exposed to L1 of *U. pallikuukensis* to determine their suitability as intermediate hosts.

In Chapter 9, the findings from the previous chapters are integrated to develop a descriptive model for the seasonal pattern of development and transmission of *U. pallikuukensis* in the Arctic. In addition, hypotheses for the apparently limited geographic distribution are presented. Unanswered questions related to *U.*

pallikuukensis are discussed and directions for future research are suggested. Finally, the significance of this research with respect to the investigation of parasites under changing climatic conditions is discussed.

3. EXPERIMENTAL INFECTIONS OF MUSKOXEN (*OVIBOS MOSCHATUS*) AND DOMESTIC SHEEP WITH *UMINGMAKSTRONGYLUS PALLIKUUKENSIS* (NEMATODA: PROTOSTRONGYLIDAE): PARASITE DEVELOPMENT, POPULATION STRUCTURE, AND PATHOLOGY

3.1 Abstract

Three muskoxen and 2 domestic sheep were experimentally infected with third-stage larvae of *U. pallikuukensis* derived from the slugs *Deroceras reticulatum* and *Deroceras laeve*. The course of parasite development and patency was followed up to 26 mo post-infection (PI) using fecal examinations and radiography. The prepatent period in muskoxen was 91-97 days and the patent period extended to the time of euthanasia, 14 and 26 mo PI. Larval production peaked 13-14 mo PI. At post mortem of 1 muskox on day 97 PI not all adult parasites were within typical pulmonary cysts; 2 were found free in interlobular septa. By 14 and 26 mo PI, adult parasites were found only within cysts. Cysts were randomly distributed between left and right lungs in 2 muskoxen. Dimensions of cysts were positively correlated with the number of adult parasites they contained. Lung pathology appeared localized and was associated with adult nematodes. Adult parasites did not establish in sheep. Patterns of parasite development, patency and pathology associated with *U. pallikuukensis* differ considerably from other lung-dwelling protostrongylids.

3.2 Introduction

In 1988 pulmonary cysts containing protostrongylid lungworms were found in muskoxen (*Ovibos moschatus*) from a population west of Kugluktuk, Northwest Territories, Canada, 67°54'N, 116°38'W (Gunn and Wobeser, 1993). This constituted the first definitive report of protostrongylids in bovidae from sub-arctic and arctic North America (Goble and Murie, 1942; Boev, 1975). Subsequent surveys, based on

examination of lungs from this population in 1989 and 1990, demonstrated a prevalence of these lungworms of 92% in adult muskoxen and a maximum intensity of 258 cysts in a single animal (Gunn and Wobeser, 1993). Coincident with the discovery of the parasite there was an estimated 50% decline in this muskox population from approximately 1800 muskoxen in 1988 to approximately 970 in 1994 (Fournier and Gunn, 1998). The role of this parasite in the host population decline is not known, but the high prevalence and intensity of infection, together with preliminary histopathologic evaluations, indicated that it may have a deleterious effect on infected muskoxen. Hoberg (1997) suggested that the apparent recent emergence of this nematode may represent the response of an indigenous parasite to changing ecological or climatic conditions.

Adult nematodes were collected from muskoxen from the affected population and described by Hoberg et al. (1995), establishing a new genus and species, *Umingmakstrongylus pallikuukensis* Hoberg Polley Gunn and Nishi, 1995. Morphologically, specimens of *U. pallikuukensis* are most similar to those of species in the genus *Cystocaulus* Schulz Orlov and Kutass, 1933. Results of phylogenetic analysis at the generic level within the Protostrongylidae place *Umingmakstrongylus* + *Cystocaulus* as the sister-group of the genus *Muellerius* Cameron, 1927 within the subfamily Muelleriinae (Carreno and Hoberg, 1999).

Umingmakstrongylus pallikuukensis is remarkable among the Muelleriinae. Reproductively active adult parasites are exceptionally large, with females in excess of 47 cm in length. The adults live in cysts up to 40 mm in diameter in the lung parenchyma of the muskoxen (Hoberg et al., 1995). In the laboratory Hoberg et al. (1995) demonstrated that first-stage larvae (L1) of *U. pallikuukensis* develop to third-stage larvae (L3) in the slug *Deroceras reticulatum* Müller. The life cycle was thought to be completed when infective L3 were ingested by muskoxen and subsequently established in the lungs (Hoberg et al., 1995).

The objectives of the research reported in this chapter were to: (1) complete the life cycle of *U. pallikuukensis* in captive muskoxen; (2) describe the pathology and population structure of adult parasites in the lungs; (3) determine whether L3 that spontaneously emerged from gastropods were infective to muskoxen; and (4) determine whether this parasite could develop to maturity in domestic sheep (*Ovis aries*). This work constitutes a component of a broader investigation of the biology, distribution and significance of *U. pallikuukensis* in the Canadian Arctic.

3.3 Materials And Methods

The life cycle of *U. pallikuukensis* was studied under laboratory conditions at the Western College of Veterinary Medicine, Saskatoon, Canada. Gastropods were infected with L1 and 3-6 wk later L3 were recovered from these gastropods and given to muskoxen and sheep in an attempt to complete the life cycle (Table 3.1).

3.3.1 Infection of gastropods

The slugs *D. reticulatum* and *D. laeve* were used as experimental intermediate hosts (Table 3.1). They were obtained from protostrongylid-free laboratory cultures established at the Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, and a composting box in Ottawa, Canada (*D. reticulatum*) and from a greenhouse in Saskatoon (*D. laeve*). *Deroceras laeve* is the only slug reported from the North American Arctic (Mozley, 1937; Pilsbry, 1946, 1948; La Rocque, 1953) and had previously been observed in the Kugluktuk region (J. Nishi and G. Wobeser, pers. comm.).

Gastropods were infected as described by Hoberg et al. (1995). Exposure to L1 was repeated daily for 1-5 days during which period each slug was exposed to a maximum total of approximately 2000 L1. Between successive and after the last exposure, gastropods were housed overnight in clean plastic containers with water, lettuce and carrots. The day following the final exposure, slugs were transferred to an autoclaved, moistened 50:50 soil/vermiculite mixture in Rubbermaid® containers (8 cm by 20 cm by 34 cm). They were maintained at room temperature (20 C +/- 2 C) and fed washed lettuce, carrots, potatoes and chalk on a weekly basis.

3.3.2 Recovery of third-stage larvae

Two methods were used: (1) recovery of L3 from the slugs by artificial digestion at 24-38 days post-infection (PI) as described by Hoberg et al. (1995); and (2) recovery of L3 that had spontaneously emerged from the slugs. To recover emerged L3, gastropods were transferred from the Rubbermaid® containers to moistened petri dishes containing carrots and lettuce beginning approximately 7-14 days PI. Every 2-5 days, the internal surface of each dish was examined for larvae using a dissecting microscope. At the same time the food material from each dish was suspended in warm tap water at 30 C +/- 5 C in glass funnels (75 mm inside diameter (id) and 77 mm stem, 145 mm total height) at room temperature in light. After 16-24 hr the sediment was examined for larvae.

Table 3.1: Experimental infections of muskoxen and sheep with *Umingmakstrongylus pallikuukensis*.

Animal ID	Age	Sex ^a	Date infected	Source of L1	IH ^b and number ^c	L3 Source ^d (days PI of IH)	Dose of L3	Euthanasia (days PI)
Muskox 1	4 yr	m(c)	1 March 1995	feces wild ^e	Dr 5	D (24-29)	850	791
					Dr 6	E (26-38)	220 ^f	
Muskox 2	13 yr	f(h)	4 April 1995	feces wild ^e	Dr 2 (47) ^g	D (25-27)	97	441
					DI 2 (50) ^g			
Muskox 3	14 yr	m(c)	21 Feb. 1996	feces Muskox 1	Dr 13	E (20 - 38)	175	97
Lamb A	3 mo	m	22 Dec. 1994	lungs wild ^e	Dr 1	D (22-26)	200	78
Lamb B	4 mo	m	28 July 1995	feces Muskox 1	Dr 5	D (31-38)	175	187

^ac-Castrated, h-Hysterectomized.

^bIH-Intermediate host, Dr - *D. reticulatum*, DI - *D. laeve*.

^cNumber of gastropods used for L1 to L3 development.

^dD-L3 recovered by artificial digestion of slugs, E-L3 recovered after emerging from slugs.

^eSamples from muskoxen killed by hunters near Kugluktuk in which adult parasites were identified as *U. pallikuukensis*.

^fNumber of L3 used from each species of IH.

^gSecond dose of emerged L3 given 96 days after the first dose.

Larvae recovered were held in tap water at 4 C for up to 24 hr (digested larvae), or 18 days (emerged larvae) before being used for experimental infections. With both techniques for larval recovery, L3 still within the second cuticular sheath and those that had shed the sheath were used to infect muskoxen and lambs.

3.3.3 Experimental infections of muskoxen and lambs

Animals: Three muskoxen from the Western College of Veterinary Medicine Muskox Research Herd and 2 Dorset-cross lambs from a commercial farm near Saskatoon were used (Table 3.1).

Baermann examinations (Gajadhar et al., 1994) and routine fecal flotations (Foreyt, 1994) were performed on the feces of each animal during the 2 wk preceding experimental infection. All animals were negative for protostrongylid L1 as determined by Baermann examinations. Eggs of *Trichuris* sp., *Nematodirus* sp. and other trichostrongylids were found on the flotations in the feces of Muskox 2. This animal was treated with a single oral dose of fenbendazole (10 mg/kg) (Panacur suspension[®], Hoechst Roussel Vet) 11 days before infection.

Animals were housed indoors according to Canadian Council on Animal Care Guidelines (Olfert et al., 1993). Muskoxen were fed an alfalfa brome or mixed grass hay ad libitum as well as 500 grams of specially formulated muskox pellets (University of Saskatchewan Feedmill) and 250 grams chopped oats daily. Lambs were fed alfalfa pellets and an autoclaved mixed grass hay. Salt blocks and water were available ad libitum. Unconsumed feed and unexamined fecal wastes from the pens were incinerated.

Infection with U. pallikuukensis: Animals were given L3 suspended in 20 ml tap water by gastric tube, followed immediately by a minimum of 120 ml water and 60 ml air. Muskox 1 received a second dose of approximately 220 emerged L3 96 days after the initial infection. All other animals were infected only once (Table 3.1). Throughout this chapter results for Muskox 1 are reported as days or months after the first infection. Clinical monitoring consisted of daily observations as well as hematology (complete blood counts and total body chemistries) and thoracic radiographs approximately every 2-3 mo.

3.3.4 Monitoring parasite development

Fecal examinations began on days 16, 45 and 63 PI for muskoxen 1, 2 and 3, respectively. Feces from Muskox 1 were examined every 1-2 days until patency; from Muskox 2 weekly until day 75 PI, then daily until patency; and from Muskox 3 every 7-10 days from days 63 to 89 PI, then daily until euthanasia on day 97 PI. Following patency, fecal larval output was quantified weekly or bi-weekly until months 19 and 15 PI in muskoxen 1 and 2, respectively. Feces of Muskox 1 were sampled irregularly from months 20-25 PI and larval output was again quantified during month 26 PI. Feces from Lamb A were examined daily from day 9 PI until euthanasia and from Lamb B every 1-2 wk from day 60 PI until euthanasia.

During the prepatent periods, feces voided by an individual animal over a 24 hr period were pooled and a minimum of 3, but usually 6-9, subsamples were examined. The Baermann technique was used, modified as follows: 20 to 30 grams of feces were lightly crushed (squeezed once between the thumb and forefinger) and suspended in a plastic strainer (110 mm id, mesh size 1 mm) containing 2 layers of cheesecloth, and placed in a glass funnel (144 mm id., 100 mm stem, 225 mm total height) with an attached tube and clamp; the funnel was filled to the brim with warm tap water (30 C \pm 5 C) and samples were left at room temperature with the lights on; after 16 to 24 hr, 40 to 50 ml of fluid were drawn from the stem of the funnel, centrifuged 10 min at 1500 rpm and all the sediment was examined for L1 in a scored petri dish.

Following patency, all feces voided by each animal during a 24 hr period were collected and mixed. Three 10 gram samples were removed, the fecal pellets in each sample counted and all 3 samples examined using the modified Baermann technique described above. At first, larvae were quantified by examining all the sediment from each of the three samples from each animal. As larval numbers increased, larvae in 3 0.01 ml (Muskox 1) or 0.1 ml (Muskox 2) aliquots from the suspended sediment in each funnel were counted. From these counts, the mean number of larvae per gram (LPG) wet feces in each 10 gram sample was calculated. The LPG for the 24 hr period was calculated from the means of the 3 10 gram samples and the monthly LPG reported is the mean of the daily counts performed within the specified month.

For Muskox 1, LPG of dry feces was determined from months 16-26 PI by drying (48 hr at 70 C) 2 10 gram samples from the pooled feces collected on a single day for larval counts. To avoid variation in dry weight due to different pellet sizes, the 10 gram samples to be dried contained the same number of pellets as the wet samples. Daily fecal output from Muskox 1 was measured over 5-24 hr periods during month 26 PI (April 1997).

3.3.5 Post mortem examination

Muskoxen: Animals were euthanized using intramuscular xylazine hydrochloride (Rompun[®], 20 mg/ml, Haver/Mobay) followed by intravenous sodium pentobarbital (Euthanyl-Forte[®], 50 mg/ml, MTC). At post mortem a gastric tube was tied into the trachea of muskoxen 1 and 2, the lungs were inflated and the tube clamped closed. The inflated lungs from Muskox 1 were examined within 3 hr of death by computed tomography (CT), those of Muskox 2 were examined radiographically. A post mortem bronchial wash was performed on Muskox 3. Details of the CT examination have been reported elsewhere (see Chapter 4 and Kutz et al., 1999a).

The fresh lungs of all 3 muskoxen were carefully examined by dissecting along the bronchi and removing overlying pulmonary tissue in strips 3-5 mm thick. Each strip was meticulously examined both visually and by palpation. All lung tissue from each animal was examined in this way during the 48 hr following euthanasia. Cyst location was noted for Muskoxen 1 and 2 and then the cysts were removed and classified by palpation as soft or mineralized. For Muskox 1, because of initial difficulty in accurately defining the separation between the right apical and middle lobes, data for cyst location from these lobes were combined. Cyst locations were not recorded for Muskox 3. Cyst length and width were measured, and the mean of these 2 measurements was used as the cyst diameter. The cyst volume was then calculated using the formula for volume of a sphere. All cysts from Muskox 2 were measured and dissected while only subsets of cysts from muskoxen 1 and 3 were examined.

An attempt was made to recover intact adult parasites and to characterize the parasite population within each cyst examined. The walls of some cysts were carefully incised and the entire cysts then placed in saline at 37 C for 10 min to several hours to encourage movement of adult parasites from the cysts. The total number of adult parasites recovered from all cysts in Muskox 2 was counted. In muskoxen 1 and 3, however, where only subsets of cysts were dissected, the mean number of parasites per cyst was multiplied by the total number of cysts physically recovered from the lungs to estimate the total adult parasite population. Intact adult parasites recovered were measured to determine total length while alive and then preserved in steaming 70% ethanol/5% glycerine. Parasite fragments were similarly fixed, but at room temperature. Cysts were dissected up to 5 days after euthanasia at which point the parasites began to deteriorate and could not be removed whole. Morphometric data and descriptions of structural characters for adult parasites recovered at days 97, 441 and 791 PI were compiled but are not included in this thesis.

Intact cysts from muskoxen 1 and 3 were fixed in 10% formalin, embedded in paraffin blocks, stained with Hematoxylin and Eosin, Masson's Trichrome, Perl's Prussian Blue Reaction Stain, Von Kossa and periodic acid-Schiff's reagent and examined histologically (Lillie, 1965; Carson, 1990). Eleven cysts from Muskox 1 were submitted for bacteriological examination.

To estimate the mean proportional volume that each of the 5 lung lobes contribute to the total lung volume of an adult muskox the lungs of 5 hunter killed wild adult animals were dissected and the lobes weighed individually.

Lambs: Complete post mortem examinations were performed on both lambs. Lung and hepatic lesions detected macroscopically were fixed in 10% formalin, embedded in paraffin blocks and stained with Hematoxylin and Eosin, Von Kossa and periodic-acid Schiff's reagent and examined histologically (Lillie, 1965; Carson, 1990).

Statistical analyses were performed using StatView™ SE + Graphics (Abacus Concepts Inc., 1988), Zar, 1984 and Siegel and Castellan, 1988.

3.4 Results

3.4.1 Monitoring parasite development - muskoxen

First-stage larvae were first recovered from feces of muskoxen 1 and 2 on days 95 and 91 PI, respectively, and were shed up to the time of euthanasia at days 791 and 441 PI (Table 3.2). No larvae were found in the feces of Muskox 3, but gravid adult female worms, eggs and L1 were recovered by dissection of some cysts at post mortem on day 97 PI.

First-stage larvae were recovered from all fecal samples examined from muskoxen 1 and 2 following patency. Numbers of larvae began to increase 3-4 mo following patency, and peaked 13-14 mo PI (Fig. 3.1). Changes in larval numbers associated with the second infection of Muskox 1 were not apparent. The maximum mean monthly L1 production by Muskox 1 was 5942 LPG (SD +/- 2419) during month 13 PI (March); the daily maximum of 8953 LPG (SD +/- 3268) occurred within this month on day 391 PI. The maximum mean monthly L1 production from Muskox 2 was 39 LPG (SD +/- 28) during month 14 PI (May); the daily maximum of 71 LPG (SD +/- 12) also occurred within this month on day 414 PI. Larval production per gram of feces dry weight from Muskox 1 was approximately twice that of the LPG wet weight.

Table 3.2: Development of *Umingmakstrongylus pallikuukensis* in experimentally infected muskoxen.

Muskox ID	Prepatent period (days)	Patent period (days)	Total cysts	Cyst volume in cm ³ ±1SD	M:F ratio	Median adults /cyst	Range of adults/cyst	Total adults recovered	% Recovery L3/adults
1	95	696	183	1.40 ± 0.99 (22) ^a	0.86 (22)	3 (24)	0-6	595 ^b	56%
2	91	350	16	0.99 ± 0.54 (13)	1.3 (16)	2 (16)	2-5	41 ^c	42%
3	>97	N/A	13	0.09 (1)	1.2 (5)	2 (6)	1-5	34 ^b	19% ^d

^aNumber of cysts on which the data are based.

^bExtrapolated from the mean of a subset of 24 (Muskox 1) and 6 (Muskox 3) dissected cysts.

^cActual number recovered based on dissection of all 16 nodules detected.

^dLikely an underestimate because of early stage of infection.

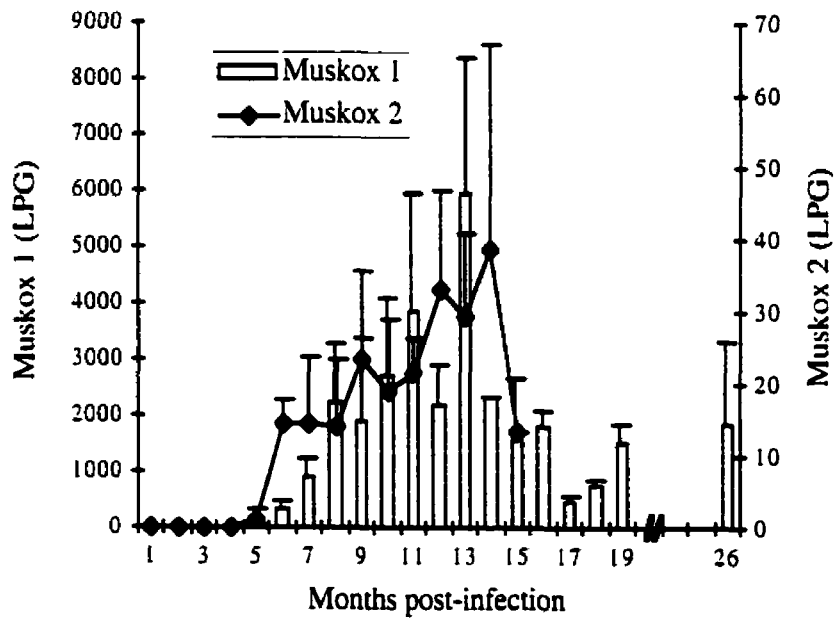


Figure 3.1. Mean monthly production of *Umingmakstrongylus pallikuukensis* first-stage larvae by muskoxen 1 and 2. Larvae per gram (LPG) are based on wet mass of feces (Error bars = 1 SD).

Muskox 1 produced an average of 880 grams feces/day wet weight (SD +/-164) over 5 24 hr periods in April 1997. Based on a mean count of 1872 LPG (range 700-3898) recorded during this month (26 mo PI), a mean of 1,647,360 L1 (range 616,000-3,430,240) were shed in the feces of this animal each day.

Lesions attributable to *U. pallikuukensis* were visible radiographically in muskoxen 1 and 2 by days 191 and 178 PI, respectively (Fig. 3.2). There was an increase in the number of lesions detected radiographically between days 191 and 252 PI in Muskox 1. Lesions increased in size from days 252 to 415 PI but there was little subsequent change in size by day 789 PI. Radiographic changes consistent with lungworm infection were not apparent in Muskox 3 immediately prior to euthanasia on day 97 PI. Radiographic findings have been described in more detail elsewhere (see Chapter 4 and Kutz et al., 1999a). No abnormalities specific to the lungworm infection were found in the complete blood counts, full body chemistry panels, or physical examinations of any of the animals.

3.4.2 Post mortem examination - muskoxen

Lesions of parasitological significance in the muskoxen were limited to the lungs. Cysts of *U. pallikuukensis* contained apparently viable parasites and there was no gross evidence of mineralization.

Cyst distribution: The majority of cysts were found deep in the lung parenchyma with only 24 of 183, 1 of 16, and 0 of 13 cysts visible at the lung surface of muskoxen 1, 2 and 3, respectively (Figs. 3.3-3.5). On gross dissection, most cysts in muskoxen 1 and 2 were located centrally in the lung parenchyma and adjacent to bronchi. The cyst distributions were confirmed by CT examination (Fig. 3.6) (Kutz et al., 1999a). Cysts were found in all lung lobes of Muskox 1 but only in the diaphragmatic lobes of Muskox 2 (Table 3.3). In both animals they were distributed randomly between left and right sides of the lungs (Muskox 1, Chi-square = 0.911, df = 1, $P = 0.3398$; Muskox 2, Chi-square = 0.206, df = 1, $P = 0.6496$) and in Muskox 1 randomly among the lobes of the left lung (Chi-square = 0.59, df = 1, $P = 0.4424$). In the right lung of Muskox 1 there were more cysts in the apical/middle lobe and less in the diaphragmatic lobes than would be expected based on estimated lung volumes (Chi-square = 11.403, df = 2, $P = 0.0033$).

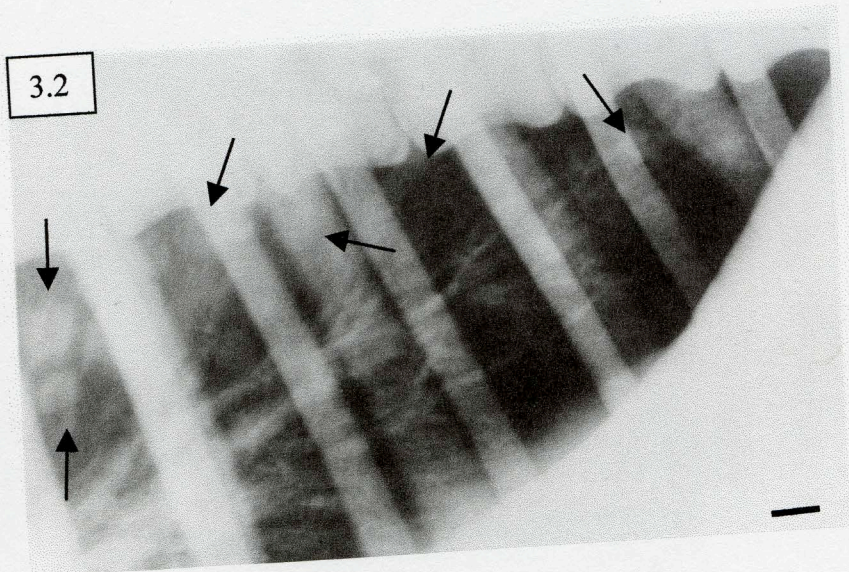


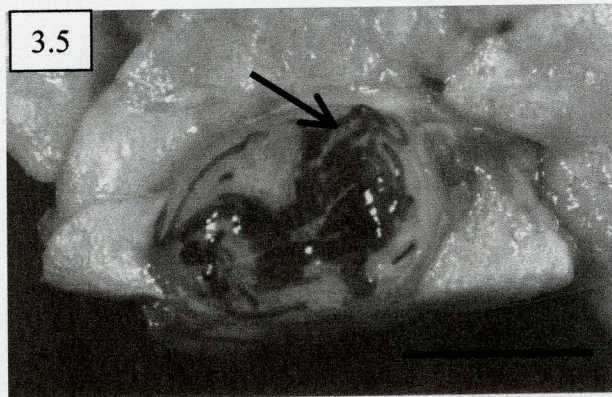
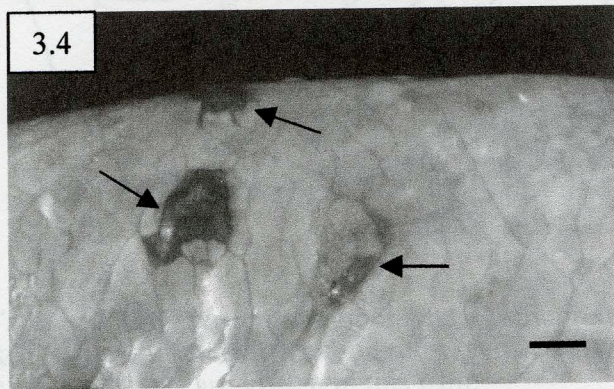
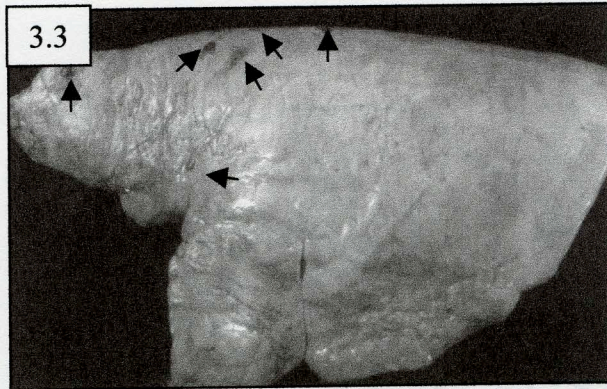
Figure 3.2. Lateral caudal thoracic radiograph of Muskox 1 at day 317 post-infection showing several cysts of *Umingmakstrongylus pallikuukensis* (arrows) in the dorsal part of the diaphragmatic lobes. Bar = 60 mm.

Figures 3-5. Cyst of *Umingmakstrongylus pallikuukensis* from Muskox 1 at day 791 post-infection.

Figure 3.3. Lateral view of left lung showing 5 cysts (arrows) beneath the pleural surface.

Figure 3.4. Close up of 3 subpleural cysts (arrows) from Figure 3.3. Bar = 10 mm.

Figure 3.5. Cross-section of the lung showing a typical cyst of *U. pallikuukensis* containing adult parasites (long arrow) surrounded by the cyst wall (short arrow). Bar = 5mm.



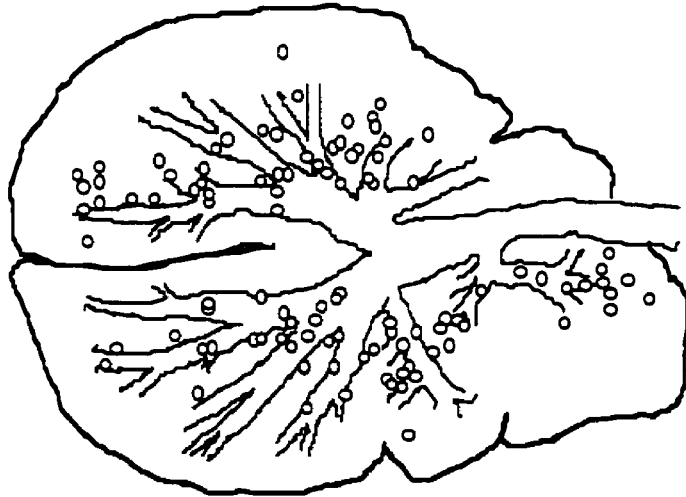


Figure 3.6. Tracing from a post mortem computed tomographic scout film of the lungs of Muskox 1 showing the distribution of cysts of *Umingmakstrongylus pallikuukensis*. Not all cysts are visible in this film.

Table 3.3: Distribution of cysts of *Umingmakstrongylus pallikuukensis* among lung lobes of 2 experimentally infected muskoxen.

Muskox ID	Percentage (number) of cysts in each lung lobe and in each lung							
	Right Ap/mid ^a	Right Diaph	Acc	Right total	Left Ap	Left Diaph	Left total	
1	30 (55)	24.6 (45)	5.5 (10)	60.1 (110)	9.8 (18)	30.1 (55)	39.9 (73)	
2	0 (0)	62.5 (10)	0 (0)	62.5 (10)	0 (0)	37.5 (6)	37.5 (6)	
Percentage of lung ^b (SD)	21.3 (2.4)	32.0 (2.6)	3.3 (0.5)	56.6 (3.1)	12.4 (2.3)	31.0 (2.8)	43.4 (3.1)	

^aAp/mid = Apical and middle lobes combined; Diaph = Diaphragmatic; Acc = Accessory; Ap=Apical.

^bEach lobe reported as percentage of total lung weight (n=5).

Cyst characteristics: All cysts in muskoxen 1 and 2 had tough, gray, well defined walls. In Muskox 3 not all parasites were in discrete cysts; 1 male and 1 female were found unencapsulated in different locations within interlobular septa. Relative to muskoxen 1 and 2, cysts in Muskox 3 were considerably smaller with poorly defined walls. The cysts and individual parasites in Muskox 3 were extremely difficult to locate and this precluded analysis of the adult parasite associations in this animal.

With the exception of a single empty cyst in Muskox 1, all cysts examined in muskoxen 1 and 2 contained at least 1 male, 1 gravid female, eggs and larvae. The ratio of female to male parasites is presented in Table 3.2. Among the 22 cysts examined from Muskox 1, 10 contained more females than males, 2 more males than females, and 10 the same number of males and females. In Muskox 2 all cysts recovered were examined. None contained more females than males, 5 had more males than females, and 11 had the same numbers of males and females. In Muskox 3, of 5 parasitic foci examined, there were none with more females than males, 1 cyst with more males than females, 2 cysts with the same number of males as females, and 1 single male and 1 single female (not in cysts). The median number of nematodes per cyst was significantly greater in Muskox 1 than 2 (Table 3.2) (Wilcoxin-Mann-Whitney test, $P = 0.0148$). A strong positive correlation between cyst size and number of parasites/cyst was observed in muskoxen 1 and 2 (Fig. 3.7) (Muskox 1, $n = 22$, $r^2 = 0.650$, $P < 0.001$; Muskox 2, $n = 13$, $r^2 = 0.636$, $P < 0.002$). When mean cyst size was compared for cysts containing 2 parasites there was no difference at $P < 0.05$ between muskoxen 1 and 2 ($df = 12$, $t = 1.77$, $P = 0.102$). The sample sizes for cysts containing 3-6 parasites were too small for statistical comparison.

Histopathology: Lung tissues containing parasitic lesions from muskoxen 1 and 3 were examined histologically. The most significant lesions were restricted to areas containing adult *U. pallikuukensis*. Results will be presented in chronological order based on age of infection.

In Muskox 3 on day 97 PI, 2 separate lung sections containing adult parasites were examined histologically. In the first there were 2 groups of cross sections through a parasite(s) separated by a few alveoli (Fig. 3.8). One of these groups was near an interlobular septum, the second near a bronchiole. It was not possible to determine whether these 2 groups of parasites were a single nematode spread across several alveoli or 2 or more individual parasites. The alveoli were distended by the parasites and there was mild hemorrhage into the alveoli and mild infiltration of surrounding alveolar septa with neutrophils, lymphocytes and few eosinophils. The adjacent bronchiole was

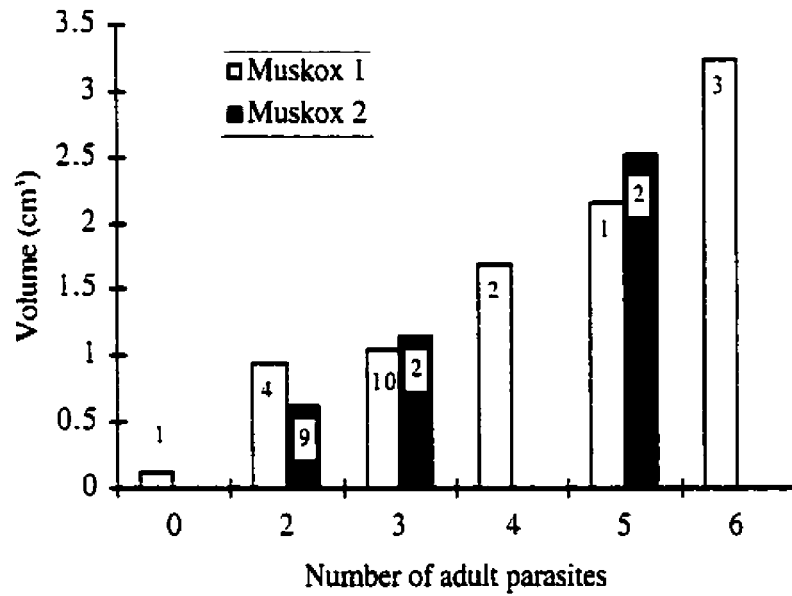


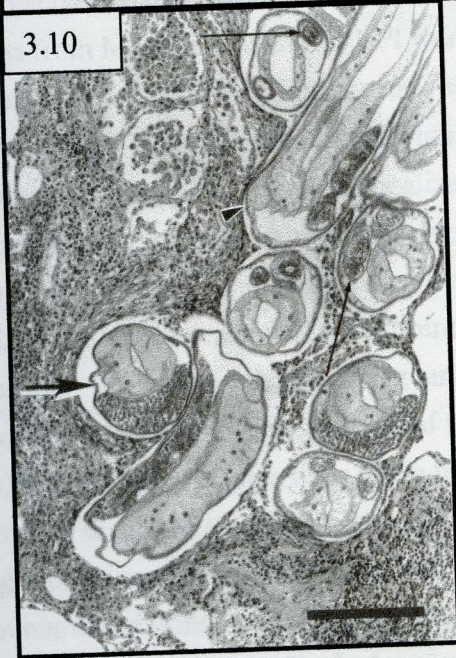
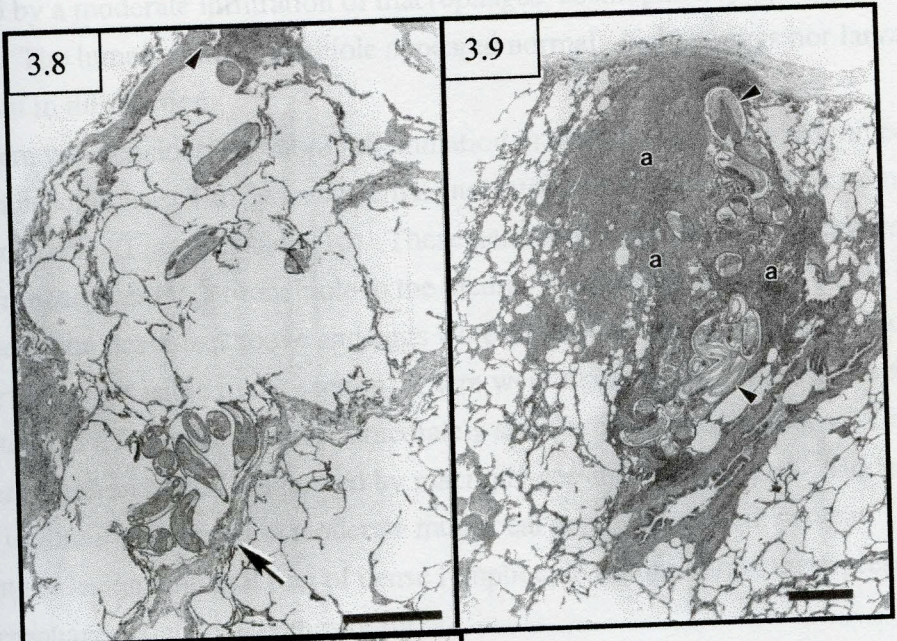
Figure 3.7. Relationship between cyst size and adult *Umingmakstrongylus pallikuukensis* populations in muskoxen 1 and 2. Numbers within bars represent the number of cysts examined.

Figures 3.8 - 3.10. Histological sections from the lungs of MuskoX 3 at day 97 post-infection.

Figure 3.8. Two groups of nematodes in cross-section; 1 adjacent to an interlobular septum (arrow), the other to a bronchiole (arrowhead). Bar = 500 μm .

Figure 3.9. Nematodes (arrowheads) surrounded by numerous inflammatory cells (a). Bar = 500 μm .

Figure 3.10. Male (arrow) and female (arrowhead) nematodes. Eggs are visible within the female (thin arrow). Bar = 200 μm .



surrounded by a moderate infiltration of macrophages, eosinophils, lymphocytes and fibroblasts. The lumen of the bronchiole appeared normal. Neither eggs nor larvae were present in this section.

There was considerably more inflammation in the second section examined. Cross-sections of at least 1 male and 1 female nematode were surrounded by numerous inflammatory cells (Figs. 3.9 and 3.10). These nematodes and the surrounding reaction extended from adjacent to a bronchiole to the pleura. Nematodes appeared larger than those in the lesion described above and eggs were present in at least 1 female. No free eggs or larvae were visible in the section. There were many eosinophils, macrophages, lymphocytes and a few plasma cells immediately surrounding the nematodes. Adjacent alveoli were filled and often obliterated by macrophages and lymphocytes. Moderate fibroblast infiltration and mild to moderate multifocal hemorrhage were present throughout the lesion. Several foci of dense lymphocyte accumulation were present and many macrophages contained hemosiderin.

Cysts from Muskox 1 at day 791 PI were better defined and less variable than those in Muskox 3 at day 97 PI. Four cysts were examined histologically, 3 associated with the pleura and 1 deeper in the parenchyma; histological lesions were similar in all 4.

Most of the lung pathology was localized to the cysts. These typically contained a densely packed core of adult nematodes, eggs and L1 (Fig. 3.11). A matrix of macrophages, fibroblasts, capillaries and remnants of alveolar walls extended among the nematodes. There were occasional foci of mineralization that were not detected macroscopically. There were degenerating larvae in some cysts, but no evidence of degeneration of the adult parasites. A wall of macrophages, multinucleated giant cells, fibroblasts, lymphocytes, plasma cells and a few eosinophils surrounded the central core of each cyst and separated the adult parasites from adjacent lung tissue. Many of the macrophages contained hemosiderin. Alveoli and compressed bronchioles, several of which communicated directly with the core of the cyst, were present throughout the wall and often contained L1 (Fig. 3.12). In 1 cyst a section of an adult worm appeared incorporated into the inner layer of the wall. Multifocal, moderate hemorrhage was present within the core of the cyst and the surrounding wall.

Several of the parasite-associated cysts completely filled the lung lobules in which they were located. Often the surrounding interlobular septa and adjacent lobules were compressed or distorted but there was no evidence that the cysts invaded these adjacent lobules. In cases where cysts did not occupy the entire lobule, there was often moderate inflammatory cell infiltration throughout the remaining area of the lobule. The lung parenchyma adjacent to the lobules containing cysts was generally unaffected

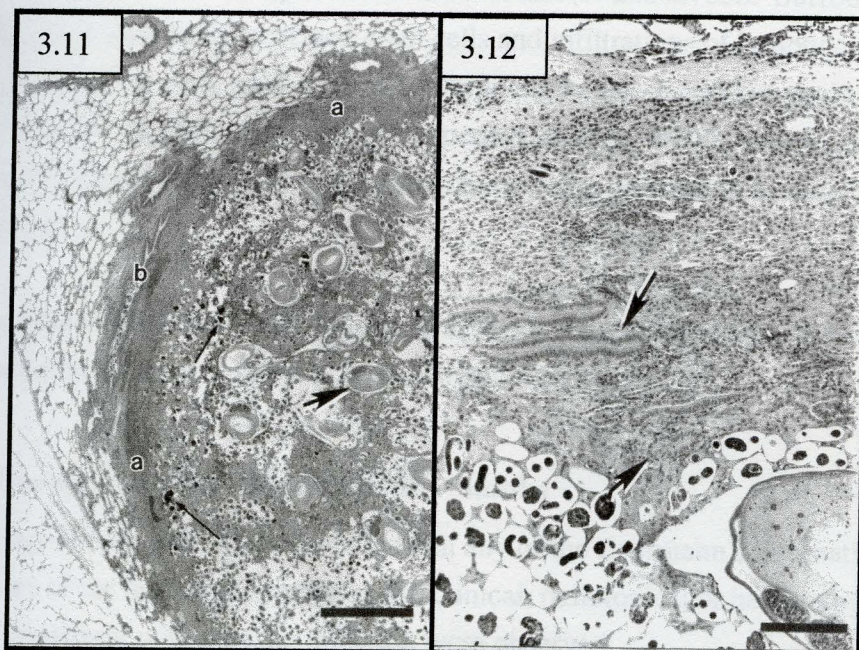


Figure 3.11, 3.12. Histological sections from the lungs of Muscox 1 at day 791 post-infection.

Figure 3.11. Part of a cyst containing adults of *Umingmakstrongylus pallikuukensis* (arrows), eggs and L1 surrounded by a thick cyst wall (a). First-stage larvae are visible in an adjacent bronchiole (b). Small foci of mineralization are visible within the cyst (thin arrows). Bar = 1000 μ m.

Figure 3.12. Bronchioles (arrows) are visible extending through the inner part of a cyst wall. Bar = 200 μ m.

although there were occasional L1 present in the bronchioles and alveoli. Surrounding a minority of these L1 were multinucleated giant cells and infiltrations of lymphocytes and eosinophils. In the same areas mild increases in bronchiole-associated lymphoid tissue were observed, but the lumina of the airways appeared normal. Small, multifocal accumulations of inflammatory cells in the parenchyma were not always associated with identifiable larvae.

Bacteriology: No bacteria of clinical significance were isolated from the cysts with either anaerobic or aerobic cultures in enrichment broths.

3.4.3 Monitoring parasite development - lambs

First-stage larvae were not recovered from any of the Baermann examinations of feces from either of the lambs ante mortem. No clinical, radiological or hematological abnormalities consistent with parasite infection were observed.

3.4.4 Post mortem examination - lambs

No parasites were recovered from the experimentally infected lambs at post mortem on days 78 and 187 PI. In Lamb A there were 7 firm, white foci, 1-7 mm diameter, in the subpleural regions of the left diaphragmatic lobe and right apical and middle lobes. In the liver there was a firm white 3 mm diameter, irregularly shaped lesion which extended into the liver parenchyma. In Lamb B there were 2 firm white 3 mm diameter subpleural lesions on the dorsal and ventral aspects of the left middle lung lobe. In the liver there was a raised, white 1 mm diameter lesion on the diaphragmatic surface extending into the parenchyma.

Histopathology: Histological lesions consistent with parasitic migration were detected in both animals. Large subpleural foci of cellular infiltration extended into the pulmonary parenchyma in both lambs. At the centre of these foci in Lamb A (78 days PI) there were areas of necrosis. Surrounding the foci were numerous eosinophils, lymphocytes and macrophages which extended beneath the pleura. There was generalized increased cellularity of the lungs of Lamb A and a moderate, multifocal increase in bronchiole-associated lymphoid tissue. There was also mild, multifocal hemorrhage into the alveoli, mild smooth muscle hypertrophy of the arterioles and multifocal accumulation of eosinophils surrounding the bronchioles and arterioles.

In the liver of Lamb A a large subcapsular focus consisting of severe cellular infiltration extended into the hepatic parenchyma. Cells consisted primarily of lymphocytes, eosinophils and multiple foci of multinucleated giant cells. Within this lesion there were 3 foci of necrosis containing degenerate eosinophils and surrounded by numerous giant cells, eosinophils and macrophages. Fingers of fibrous tissue extended into the lesion. There was moderate biliary hyperplasia and smooth muscle hypertrophy of the arterioles in this area. Elsewhere in the liver there were multifocal, mild to moderate, periportal and subcapsular infiltrations of eosinophils, lymphocytes and macrophages.

The pulmonary and hepatic lesions in Lamb B were similar to that of Lamb A but there were fewer distinct foci of cellular infiltration and less involvement of adjacent tissue. More fibroblasts and fewer eosinophils were present in the lesions than in Lamb A.

3.5 Discussion

In this study, development of *U. pallikuukensis* in experimentally infected muskoxen is documented for the first time. Consistent with other protostrongylids, the life cycle was found to be heteroxenous, involving a gastropod intermediate and a ruminant definitive host. Observations on parasite ontogeny, structure of adult parasite populations and pulmonary pathology in the natural definitive host has provided the basis for comparisons among genera of the Muelleriinae and other Protostrongylidae. Additionally, results of the current study represent basic biological data critical for developing a context for understanding the factors that may influence the epidemiology of this nematode in the Arctic (Hoberg et al., 1995).

3.5.1 Prepatent period

The prepatent period of 91-97 days for *U. pallikuukensis* is longer than that typically reported for any other protostrongylid that lives as adults in the lungs of its normal ruminant definitive host (Boev, 1975). In other Muelleriinae the prepatent periods range from 25-59 days for *Cystocaulus ocreatus* (Raillet and Henry, 1907) and *Muellerius capillaris* (Mueller, 1889) (Davtyan, 1949; Gerichter, 1951; Rose, 1959; Svarc and Zmoray, 1960; Azimov et al., 1973). In the Protostrongylinae of bighorn sheep (*Ovis canadensis* Shaw), *Protostrongylus stilesi* Dikmans 1931 and *P. rushi* Dikmans 1937, prepatent periods range from 45-54 days (Fougere-Tower and Onderka, 1988). In contrast to the above mentioned genera in which the adults reproduce in the

lungs of ruminants, the Elaphostrongylinae, which reproduce in the central nervous system or musculature, generally have longer prepatent periods. These range from 46, 49-69 and 91 days for *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier, 1934), *P. andersoni* Prestwood 1972, and *P. tenuis* (Dougherty, 1945), respectively, to approximately 120 days for *Elaphostrongylus rangiferi* Mitskevich 1960 and *E. cervi* Cameron 1931 (Anderson, 1963; Prestwood, 1972; Pybus and Samuel, 1984b; Gray and Samuel, 1986; Handeland, 1994; Gajadhar and Tessaro, 1995). Longer prepatent periods may reflect extended periods of larval migration through tissues and organs instead of relatively rapid transport via the vasculature (Anderson, 1992; Olsson et al., 1998). Alternatively, developing larvae may use an indirect migration route on their way to the sites of sexual reproduction, as may occur with *E. rangiferi* (Handeland, 1994).

The distribution of cysts containing *U. pallikuukensis* in the lungs of muskoxen is consistent with arrival of nematodes via the blood stream. It has yet to be determined, however, if these parasites follow a direct route from the gastrointestinal tract to the lungs or if migration occurs first through other sites in the body. A sister-group relationship for the Elaphostrongylinae and the Muelleriinae (Carreno and Hoberg, 1999) may suggest that an extended prepatent period is ancestral for this subclade.

The prolonged prepatent period of *U. pallikuukensis* may be an adaptation that ultimately enhances transmission in cool arctic environments where the window for transmission is likely very narrow. Larvae of *U. pallikuukensis* infecting gastropods in the Arctic during the early summer require 4-6 wk to develop to the infective stage (see Chapter 7). If we assume that the major source of infective larvae is that within gastropods, the most likely time for infection of muskoxen would be during late summer. The 3 mo prepatent period, together with a gradual increase in larval production, may result in high larval production by the following spring when mild, moist conditions and the presence of active gastropods may be conducive to larval survival, development and transmission. In contrast, a shorter prepatent period with larval production beginning in late autumn/early winter under cold, desiccating conditions and in the absence of gastropods may result in poor larval survival and a wasted reproductive effort by the parasites. These factors clearly demonstrate the seasonally defined dynamics of host-parasite transmission in high-latitude systems.

The approximate 6 day range of prepatent periods among the 3 experimental muskoxen was narrow despite differences in host characteristics and in numbers and sources of infective larvae. Gray and Samuel (1986) reported that the prepatent periods for *P. odocoilei* in 2 mule deer *Odocoileus hemionus* were inversely proportional to the

dose of L3. A similar relationship has been reported for bighorn sheep infected with *P. stilesi* (Fougere-Tower and Onderka, 1988) and with *E. cervi* in mule deer (Gajadhar and Tessaro, 1995). Also, Olsson et al. (1998) found the time required for arrival of *E. cervi* in the central nervous system of experimentally infected guinea pigs was inversely proportional to the dose of infective L3. We do not have sufficient data to assess the effect of L3 dose of *U. pallikuukensis*, or of various host factors, on the prepatent period.

3.5.2 Patency and larval production

In the present study, larval output was quantified using a modified Baermann technique. Recently, Forrester and Lankester (1997a) reported that for *P. tenuis* the standard Baermann technique recovers only 13-14% of the L1 in a sample and provides an unreliable estimate of actual numbers of larvae present in the feces. In our study, the daily LPG was based on the mean from 3 samples and the methods used were consistent over time and between animals. Although we recorded large standard deviations in our mean daily larval numbers, the pattern of larval production observed with low numbers for 2-4 mo following patency followed by an increase and then fluctuating numbers, was similar in both muskoxen 1 and 2. It seems reasonable to assume, therefore, that although the total larval counts may have been underestimated, the temporal pattern is representative of the overall trend in larval production by *U. pallikuukensis* in these animals. The relatively high larval production in Muskox 1 extended over approximately 6 mo (Fig. 3.1). It would have been difficult, therefore, to detect any influence of the second infection (96 days after the first) on larval production.

Numbers of L1 in feces peaked in March (13 mo PI) for Muskox 1, and May (14 mo PI) for Muskox 2. It was not possible, however, to determine whether the patterns of larval production were seasonally influenced. The animals were housed under constant, artificial lighting throughout the entire study period, with some natural light from small windows high in the stalls. Tedesco (1996) demonstrated that muskoxen maintain an internal biological clock that is synchronized by the environment and even in a constant environment (unchanging photoperiod) this clock will induce seasonal physiological changes within 2-4 wk of those occurring in animals outdoors. If the larval production from the experimental muskoxen was influenced by this internal clock and/or by the natural light, the peak of the larval production could have been delayed compared to natural infections where muskoxen may be more likely to be infected in late summer than in early spring.

Increased larval output has also been associated with host stress, particularly rut and parturition as has been reported in bighorn sheep infected with *P. stilesi* (Yde et al., 1988) and reindeer (*Rangifer tarandus tarandus*) infected with *E. rangiferi* (Halvorsen et al., 1985). This could not be investigated in our animals as they had been surgically sterilized. Further field studies are necessary to assess any seasonal patterns of larval production.

The patent period of *U. pallikuukensis* in experimentally infected muskoxen was lengthy: at least 23 mo in Muskox 1 and 11 mo in Muskox 2. Muskox 1 was a healthy young adult in excellent physical condition, yet the parasites were able to successfully establish and remain viable for a long period. In this animal the presence of live adult parasites, eggs and L1 at post mortem, together with the minimal mineralization and absence of adult parasite degeneration in the sections examined histologically, suggest that patency would have extended well beyond the date of euthanasia. In the related genera, *C. ocreatus* and *M. capillaris*, patent periods are typically 3-5 mo although 4-5 yr have been reported (Koprynin et al., 1953; Davtyan and Shul'ts, 1949; Kassai, 1962-1963; Rose, 1959). The patent periods of the Elaphostrongylinae have not been extensively studied experimentally (Platt and Samuel, 1978), but on the basis of epidemiological data, may extend beyond 1 yr. For example, *E. rangiferi*, a muscle nematode in reindeer of northern Norway, is believed to have a life span of several years (Halvorsen et al., 1985). The extended patent periods (basically extended longevity and reproductive activity by adults) of this northern protostrongylid and of *U. pallikuukensis* are suggestive of selection pressure for survival and transmission of parasites in harsh, unstable and relatively unpredictable environments. Prolonged life spans for adult nematodes may ensure long-term survival of the parasite population over 1 or more years when climatic conditions in any given year may not be suitable for survival and development of larval stages in the environment.

3.5.3 Cyst distribution and characteristics

The distribution of cysts of *U. pallikuukensis* within the lung lobes contrasts markedly with that for species in the related genera *Cystocaulus* and *Muellerius* in sheep (Gerichter, 1951; Kassai, 1957; Rose, 1959; Rose, 1961; Boch and Nurnberg, 1962; Sedlmeier et al., 1969). Whereas these parasites form primarily subpleural nodules most often along the obtuse margin of the diaphragmatic lobes, the majority of the cysts of *U. pallikuukensis* were found deeper in the parenchyma and often associated with the bronchi. Localization of cysts within a larger sample size of lungs is necessary to

determine whether the pattern of distribution among lobes observed in Muskox 1 is typical for this parasite. Hoberg et al. (1995) reported a dorsal distribution of cysts within the lung parenchyma in 2 naturally infected muskoxen, but did not differentiate among different lobes.

Based on these experimental infections, once established, *U. pallikuukensis* produces clearly demarcated lung cysts in which reproductively active male and female parasites, eggs and L1 are enclosed by a cyst wall. These findings, along with the comparison between lesions caused by *U. pallikuukensis* and those caused by *M. capillaris* or *C. ocreatus*, are consistent with the preliminary observations of Hoberg et al. (1995).

Despite the 1 year difference in age of infection at the time of euthanasia and differences in numbers of L3 given, there was no significant difference in cyst size between muskoxen 1 and 2. Radiographic findings in Muskox 1 suggested growth of cysts from days 252 to 415 PI with very little change in cyst size by day 789 PI (Kutz et al., 1999). These findings suggest that growth of the cysts is not indeterminate. Although the number of cysts recovered from experimentally infected animals was within the range reported in wild muskoxen (Gunn and Wobeser, 1993), the size of the cysts did not approach the maximum diameter of 40 mm observed in wild animals (Hoberg et al., 1995). The strong correlation of cyst size with number of parasites in the cyst suggests that fewer adult parasites may have been present in cysts of experimentally infected muskoxen than in the larger cysts observed in wild animals. The patterns of infection with L3 may also be a factor in the cyst size differences. Experimental animals received either 1 or 2 relatively large doses of L3 (approximately 100, 200 and 1100 in muskoxen 1, 2 and 3, respectively) while wild muskoxen may be more likely to ingest smaller numbers of L3 over a period of time.

3.5.4 Lung pathology and cyst development

This study provided an opportunity to examine lung lesions at days 97 (Muskox 3) and 791 PI (Muskox 1, 2 infections). Previous examinations of lesions attributable to *U. pallikuukensis* have been limited to animals collected from the wild where infections are of unknown age but probably represent an accumulation of many infection events over time (Hoberg et al, 1995). Considerable variability in cyst size, mineralization and adult parasite condition has been described in naturally infected muskoxen (Gunn and Wobeser, 1993; Hoberg et al., 1995), but was not observed in

our experimentally infected animals. These differences may have been caused by differences in age of infection, temporal patterns of infection, or in host immunity.

Histological examination of parasitic lesions and surrounding lung tissue at days 97 and 791 PI provided insight into the development of the cysts. In Muskox 3 at day 97 PI it is possible that the more severe inflammatory response surrounding the male and female adult parasites in one of the lesions was caused by interactions between the parasites, perhaps releasing antigens into the host tissue in association with copulation or the release of eggs and their development to L1 (Lightowers and Rickard, 1996). This host reaction may represent the initial stages of cyst wall formation. Alternatively, parasite death could have induced these severe tissue changes.

The cyst wall itself may act as a selective barrier. Once formed it limits the extent of the inflammatory response within the lung tissue and provides some protection for the parasites in an environment that supports their continued reproductive activity. Bronchioles and alveoli containing eggs and larvae extend through the wall providing communication with larger airways, and a route for L1, parasite wastes and host inflammatory products to be rapidly cleared from the lung tissue (Svarc, 1984). The lung pathology associated with reproductively active *U. pallikuukensis*, which is restricted to the cysts, contrasts with the diffuse changes reported for other lung nematodes of ruminants such as *Protostrongylus*, *Muellerius*, *Cystocaulus*, and *Dictyocaulus* (Rose, 1961; Beresford-Jones, 1967; Stockdale, 1976; Seese and Worley, 1993).

Umingmakstrongylus pallikuukensis did not establish in 2 domestic sheep although histological lesions consistent with parasitic migration were present in the livers and lungs of both animals. The sample size is too small to eliminate the possibility of infection of sheep but these results may discourage their use as an experimental model for this parasite.

3.5.5 Life cycle and transmission

We have confirmed in 2 muskoxen that L3 of *U. pallikuukensis*, artificially digested from the gastropod intermediate hosts *D. reticulatum* or *D. laeve*, can develop to reproductively mature adults in the natural definitive host. In a third muskox we have demonstrated that L3, which have spontaneously emerged from *D. reticulatum*, are also viable and infective. Although L1 were not found in the feces of this animal by day 97 PI, cysts recovered from the lungs contained gravid adult females and free eggs and larvae. On this basis, this animal can be considered to have been at the point of patency.

Successful infection by L3 that have emerged from the intermediate host is intriguing. Emergence of L3 from live or dead gastropod intermediate hosts has been reported for species of several genera of the Protostrongylidae: *Protostrongylus stilesi*, *P. boughtoni* Dougherty and Goble, 1943, *Muellerius capillaris* and *Cystocaulus ocreatus*; and for *Angiostrongylus costaricensis* Morera and Cespedes, 1971 (Davtyan, 1950; Rose, 1957; Monson and Post, 1972; Ubelaker et al., 1980; Kralka and Samuel, 1984a). Kralka and Samuel (1984a) showed that emerged L3 of *P. boughtoni* produced patent infections in domestic rabbits (*Oryctolagus cuniculus* (L.)). They considered, however, that such larvae were an unlikely source of natural infection because of low larval intensities in typical intermediate hosts in the field and the probability of reduced survival of the L3 in the desiccating environments into which they would emerge in nature. With the exception of *A. costaricensis*, larvae of which emerge into an aquatic environment, and perhaps species of *Cystocaulus*, emergence has been considered of questionable epidemiological significance (Davtyan, 1950; Ubelaker et al., 1980; Kralka and Samuel, 1984a).

In the Arctic, L3 both within gastropods and those that have emerged into the environment may be significant sources of *U. pallikuukensis* infection for muskoxen and may represent alternative but complementary routes for transmission. The potential for alternative pathways for transmission may provide some level of plasticity in this host-parasite system that would promote parasite survival in harsh environments. Thus, where the activity and availability of infected intermediate hosts may be substantially limited by ecological factors associated with seasonal variability and annual cycles (Danks, 1992; Hoberg et al. 1995), larval emergence may result in enhanced availability of L3 beyond the life and/or activity of the gastropods. More detailed information on larval emergence from various intermediate hosts in the laboratory and in the field are reported in Chapter 5.

3.5.6 Parasite host biology

The effect of *U. pallikuukensis* on muskox populations is unknown. *Dictyocaulus viviparus* has significant metabolic costs for the host (Verstegen et al., 1989) and mixed infections with Protostrongylid lungworms (*Muellerius*, *Cystocaulus*, *Protostrongylus* and *Neostrongylus* Gebauer, 1932) can result in impaired respiratory gaseous exchange in domestic goats (Berrag and Cabaret, 1996). Pulmonary compromise caused by *Protostrongylus* sp. in bighorn sheep has been implicated as a predisposing factor in bacterial and viral pneumonia outbreaks (Forrester, 1971; Uhazy,

1972; Spraker, 1984) and infection with these parasites may decrease alveolar macrophage viability in vitro (Silflow and Foreyt, 1988). *Umingmakstrongylus pallikuukensis* differs from these lungworms in that, in established infections, pathology seems localized to the cysts, leaving the majority of the lung tissue in adult muskoxen unaffected. No clinical signs of pulmonary disease were observed in experimental animals but exercise intolerance in naturally infected muskoxen (C. Adjun and G. Atatahak, pers. comm.) suggests pulmonary compromise. Hoberg et al. (1995) suggested that the greatest effect of the parasites may be that of a space occupying lesion with the cysts causing displacement and compression of lung tissue. Additionally, it is reasonable to speculate that there are significant metabolic costs associated with heavy infections with *U. pallikuukensis*. These factors, in combination with other infectious agents, predation and limiting environmental conditions in the Arctic, could result in increased mortality rates in muskox populations where the parasite is present. We have no data, from wild or captive muskoxen, on the effects of the parasite on immature animals.

Umingmakstrongylus pallikuukensis is an intriguing parasite differing considerably from its putative sister-taxon *Cystocaulus* and from the related *Muellerius*. It occurs at a high prevalence and intensity in the wild and successfully established in experimentally infected muskoxen, with 55% of the L3 given developing to reproductively active adults in one animal. The cyst architecture may protect adult nematodes from the host's immune system while allowing rapid exodus of first stage larvae into airways. The long patent period, combined with high larval output, probably produce extensive environmental contamination over an extended time period. Yet, to complete its life cycle, the parasite still must survive under harsh arctic conditions, penetrate a susceptible gastropod intermediate host, develop to infective third-stage larvae at cool summer temperatures and then be ingested by a muskox. The high prevalence and intensity of *U. pallikuukensis* in muskoxen of the Kugluktuk region, together with the localized lung pathology, is indicative of a parasite well adapted to its current host and the rigors of Arctic environments and suggests a relatively long term host-parasite association in contrast to a contemporary host switch. Current phylogenetic evidence, however, suggests that the origin of *Umingmakstrongylus* can be linked to colonization of muskoxen by a protostrongylid nematode, probably from a caprine source in the Pleistocene or late Tertiary (Hoberg et al., 1995; Carreno and Hoberg, 1999).

Wildlife is an extremely important resource for people in remote communities of the Northwest Territories and across high latitudes of the Holarctic. Through

subsistence hunting, sport hunting, commercial harvests, and tourism, wildlife provides considerable cultural, nutritional, and economic benefits (Gunn et al., 1990). Yet, despite the integral role of wildlife, relatively little is known about the biodiversity of the parasite fauna of arctic bovids and cervids, the potential impact of parasitism on populations of ruminants in northern ecosystems, or the link between global warming and parasite transmission dynamics in the Arctic (Hoberg, 1997; Hoberg et al., 1999). This paucity of data has been illustrated by the recent discovery of *U. pallikuukensis*. The unique pathology and life history characteristics of this parasite, its sensitivity to ecological and climatic influences, its potential regulatory effects on muskox populations, and the possibility that, like other protostrongylids, it may establish in more than one definitive host species, warrant further study (Hoberg, 1997).

4. A LUNG NEMATODE IN CANADIAN ARCTIC MUSKOXEN: STANDARD RADIOGRAPHIC AND COMPUTED TOMOGRAPHIC IMAGING

4.1 Summary

Medical imaging was used ante mortem to follow the development of *U. pallikuukensis* infection in muskoxen and post mortem to investigate the distribution and characteristics of parasite-associated pulmonary cysts. In 2 experimentally infected animals, lesions were not visible radiographically until days 178 and 191 post-infection (PI), 3 mo after the parasites became patent. Serial radiographs taken throughout the period of patency of 1 animal showed an initial increase in lesion size by day 415 PI, but by day 789 PI lesions had stabilized or decreased in size. Although not all lesions detected at post mortem were visible radiographically during life, the films did provide an indication of the relative severity of infection. In contrast to other parasitic pneumonias there was no evidence of pulmonary disease outside the discrete parasitic cysts. Radiographs of lungs at post mortem proved to be an effective tool for locating parasitic cysts in a lightly infected muskox and demonstrated a broncho-vascular cyst distribution. Post mortem computed tomography provided a more rapid and detailed assessment of the number, size and distribution of cysts in the lungs of one muskox.

4.2 Introduction

Wildlife resources are important sources of food and income in northern communities throughout the Arctic. Muskoxen (*Ovibos moschatus*) (Fig. 4.1), through subsistence and sport hunting, commercial harvests and tourism, provide food as well as cultural and economic benefits for northern people (Gunn et al., 1990). In the Northwest Territories (NT), Canada, 3 federally or territorially inspected commercial harvests are held once or twice each year. One harvest is held on Banks Island near Sachs Harbor, a second near Holman and a third near Cambridge Bay on Victoria Island



Figure 4.1. Adult female muskox. Bar = approximately 30 cm.

(Fig. 4.2). These harvests supply muskox meat to territorial, national and international markets. In the Northwest Territories a total quota of approximately 13,000 muskoxen can be harvested each year for commercial, subsistence and sport purposes (Fournier and Gunn, 1998).

Muskoxen have not always been so plentiful in northern Canada. Excessive hunting in the early 1900's almost extirpated muskoxen from the mainland Northwest Territories and their numbers on the arctic islands were greatly reduced. In 1917 muskoxen became protected by law and populations gradually increased and their range expanded. By 1969 restricted muskox hunting was again permitted (Gunn et al., 1990). Populations on the arctic islands have recovered remarkably well, with approximately 65,000 on Banks Island and 37,000 on Victoria Island (Fournier and Gunn, 1998). Mainland populations have also recovered and expanded their range, but to a much lesser degree and there is evidence that the muskox population west of Kugluktuk, which increased during the early 1980's, has since decreased (Gunn et al., 1991; Fournier and Gunn, 1998). The reasons for the slower recovery of mainland populations and the recent decline in the Kugluktuk population are not known.

Umingmakstrongylus pallikuukensis, a protostrongylid lungworm of muskoxen, was described in 1995 by Hoberg et al. (1995). The parasite is found in the mainland muskox population of the watersheds of the Rae and Richardson rivers west of Kugluktuk, and has also been found in a single animal near Norman Wells, NT (Fig. 4.2) (Gunn and Wobeser, 1991; Hoberg et al., 1995; S.J. Kutz, unpubl. obs.). Parasite prevalence in the affected muskox population is at least 93% (Gunn et al., 1991; Gunn and Wobeser, 1991). The very large adult parasites (females up to 47 cm in length) live in well defined cysts in the lung parenchyma (Fig. 4.3) and up to 258 of these cysts have been found in the lungs of naturally infected animals (Gunn et al., 1991; Gunn and Wobeser, 1991; Hoberg et al., 1995). Cysts range from 1-4 cm in diameter, have a distinct fibrous wall and contain adult parasites, eggs and first-stage larvae (L1) in a dense matrix (Fig. 4.4). Cysts examined from naturally infected muskoxen have varying degrees of mineralization (Hoberg et al., 1995).

Umingmakstrongylus pallikuukensis is a member of the Protostrongylidae. It is related to *Muellerius capillaris* and *Cystocaulus ocreatus*, lungworms of sheep and goats, which have been reported to cause varying degrees of pathology in these hosts (Rose, 1955, 1961; Beresford-Jones, 1967; Carreno and Hoberg, 1999). Other protostrongylid species, *Protostrongylus stilesi* and *P. rushi*, can cause severe verminous pneumonia with secondary bacterial and viral infections in bighorn sheep and have been implicated in all-age die-offs of these hosts (Forrester, 1971). Muskoxen

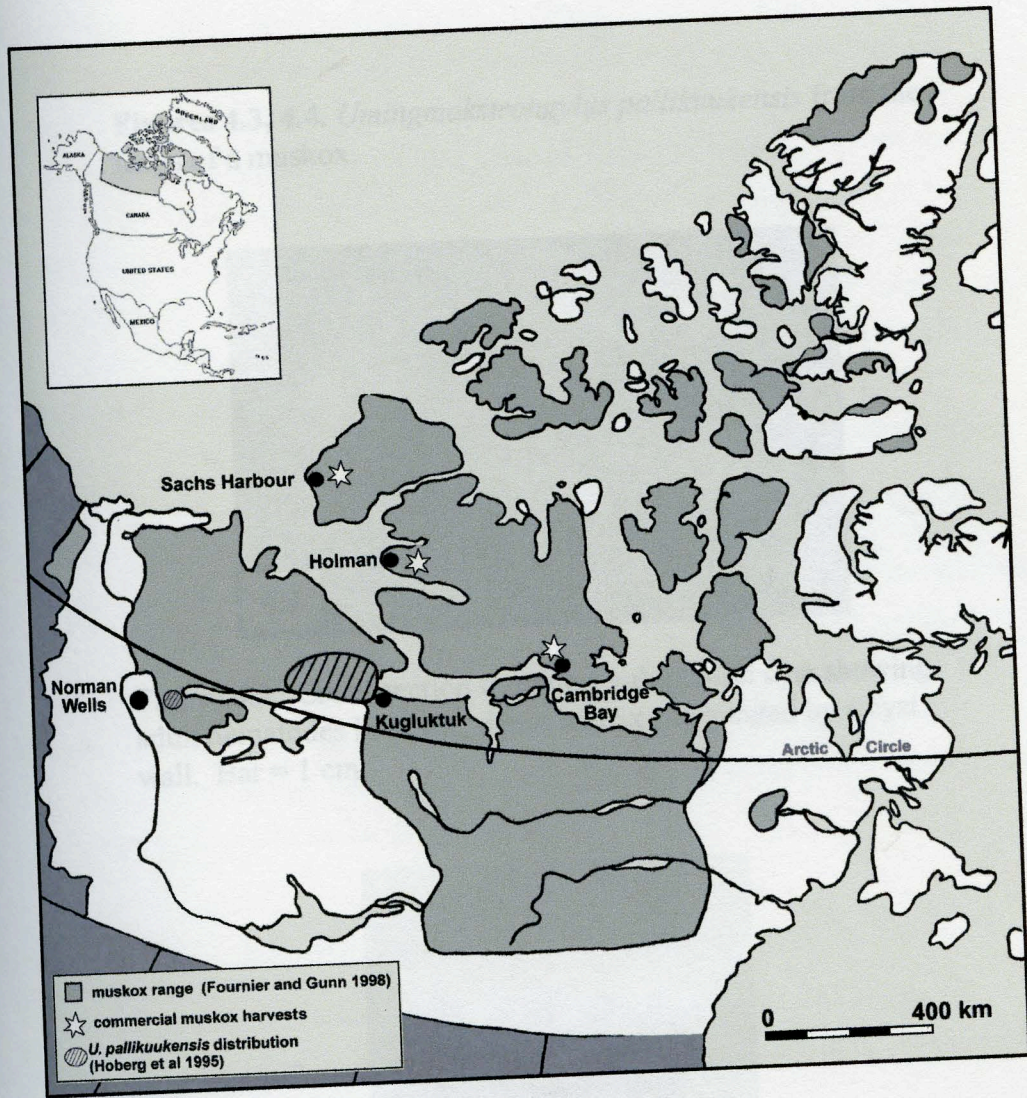


Figure 4.2. Distribution of muskoxen in the Northwest Territories (shaded areas)(from Fournier and Gunn, 1998), locations of commercial muskox harvests (stars), and known distribution of *U. pallikuukensis* (hatched areas).

Figures 4.3, 4.4. *Umingmakstrongylus pallikuukensis* from the lungs of a muskox.

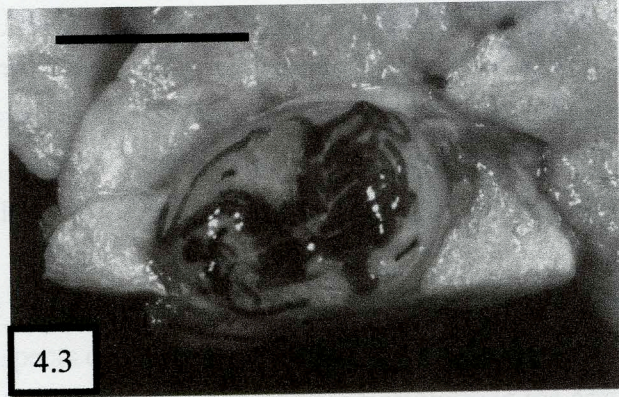


Figure 4.3. Cross-section through a sub-pleural cyst showing adult nematodes in a pale, dense matrix surrounded by a cyst wall. Bar = 1 cm.

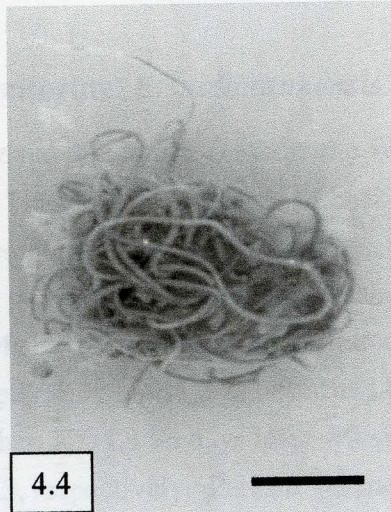


Figure 4.4. Coiled adult parasites with cyst wall removed. Bar = 1 cm.

heavily infected with *U. pallikuukensis* may exhibit exercise intolerance and epistaxis, detectable by hunters, but the parasite's effects on host survival at individual and population levels are not well understood (Hoberg et al., 1995).

Protostrongylid parasites require a gastropod intermediate host to complete their life cycle. First-stage larvae shed in the feces of definitive hosts develop to infective third-stage larvae (L3) in appropriate gastropods. This development is influenced by environmental temperature (generally more rapid development at warmer temperatures) as well as gastropod species and physiological condition (Halvorsen and Skorping, 1982; Solomon et al., 1996). Hoberg et al. (1995) demonstrated that L1 of *U. pallikuukensis* could develop to the L3 in the gastropod *Deroceras reticulatum* (Fig 4.5).

Following the initial description of *U. pallikuukensis* in 1995 we have investigated several aspects of this new parasite's biology, particularly its development in the mammalian and gastropod hosts and its epidemiology in the arctic ecosystem. The work reported in this chapter is based on infections of muskoxen with L3 of *U. pallikuukensis* recovered from *D. reticulatum* and *D. laeve* (Kutz et al., 1999b). Medical imaging techniques were used to enhance our understanding of the development and pathology of *U. pallikuukensis* in muskoxen.

4.3 Establishing and Monitoring *U. pallikuukensis* in Muskoxen

Muskoxen for experimental infection were from the Western College of Veterinary Medicine Research Herd at the University of Saskatchewan, Saskatoon, Canada. In total 3 animals, free of protostrongylid parasites, were infected. At the time of infection Muskox 1 was a 4 year old castrated male, Muskox 2 a 13 year old hysterectomized female, and Muskox 3 a 14 year old castrated male.

Muskoxen were infected orally with L3 as described by Kutz et al. (1999b). Muskox 1 was given 850 L3 then 220 L3 96 days later. Muskoxen 2 and 3 were infected with 97 L3 and 175 L3, respectively. Parasite patency was monitored by examining feces daily to biweekly for L1 using a modified Baermann technique (Kutz et al., 1999b). Muskox 1 became patent at 95 days PI, Muskox 2 at 91 days PI and Muskox 3 was not patent at the time of euthanasia at 97 days PI (Kutz et al., 1999b).

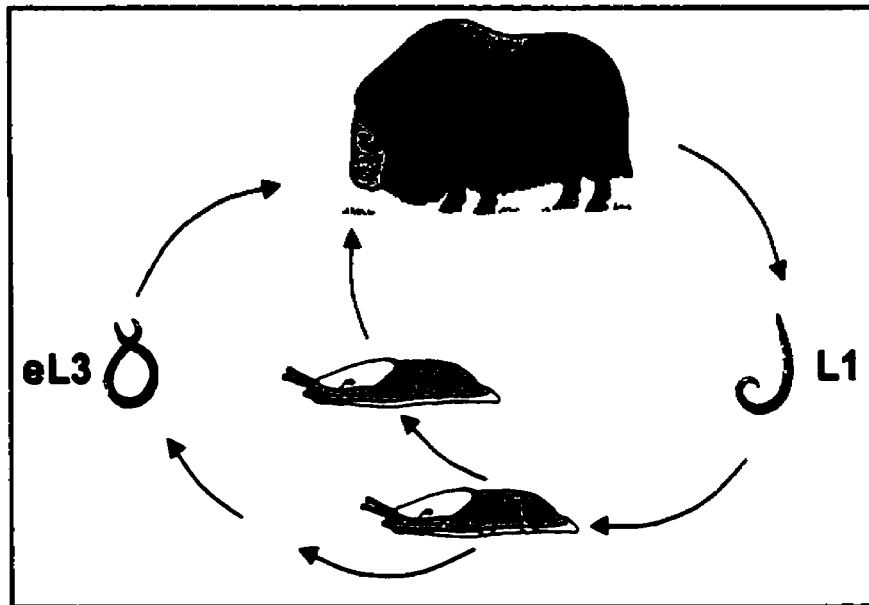


Figure 4.5 The proposed life cycle of *U. pallikuukensis* in muskoxen. Adult parasites live in the lungs and lay eggs that hatch to first-stage larvae (L1). Larvae are swallowed and passed in the feces. They develop in the tissue of susceptible slugs or snails to third-stage larvae (L3). Third-stage larvae within gastropods, or those which have emerged from gastropods and are on the vegetation (eL3), are ingested by muskoxen, migrate to the lungs and develop to adult parasites.

4.3.1 Live animal lung radiography

4.3.1.1 Techniques

Pre-infection thoracic radiographs were available for muskoxen 2 and 3, but not for Muskox 1. The radiographs of muskoxen 2 and 3 showed evidence of chronic obstructive pulmonary disease. The underlying lesions in these 2 animals may have interfered with the subsequent detection of more subtle lesions associated with the parasitic infection.

Muskox 1 was radiographed at day 76 and day 191 PI and Muskox 2 at day 178 PI. Subsequently both animals were radiographed approximately every 90 to 180 days until euthanasia. Radiographs of Muskox 3 were taken only at euthanasia on day 97 PI.

Lateral views of the chests of standing animals were taken using a ceiling mounted Picker GX 1500 (Picker International Canada, Brampton, Ontario) with Ultravision G Medical Xray Film and Ultravision UV intensifying screens, a 400 speed system (Sterling Diagnostic Imaging, Mississauga, Ontario, Canada). Muskox 1 was sedated with intramuscular xylazine (Rompun) prior to radiographic examinations. In order to view cranial and caudal aspects of the lungs a minimum of 2 and sometimes 3 films were necessary. It was frequently impossible to obtain good views of the cranio-ventral thorax because of difficulty positioning the animals with their front legs forward.

To assess the change in cyst size in Muskox 1, a subset of cysts was identified in the radiographs taken at day 252 PI and their sizes measured in these and subsequent radiographs. Calipers were used to measure the maximum width of each cyst at day 252 PI, then, in the same plane at days 415 and 789 PI. For cysts that were visible only at days 415 and 789, the maximum dimension at day 415 was used to determine the plane of measurement. To allow for differences in the distances between the films and the muskox in each radiograph the length of the third thoracic vertebra in each film was measured and if it differed from that in radiographs at day 252 PI, a correction factor was applied to the cyst measurements. The cyst sizes reported are those measured from the radiographs. These are magnified because of the distance of the cysts from the film and do not represent the actual sizes of the lesions. A Wilcoxon signed-rank test was used to compare cyst sizes at different times PI (StatView™ SE + Graphics, 1988 Abacus Concepts Inc.).

Cysts were quantified on days 252, 415 and 789 PI in Muskox 1. This was done by counting all cysts visible within the area bordered anteriorly by a vertical line drawn from the anterior margin of the third thoracic vertebra, ventrally by the ventral

surface of the trachea, caudally by the caudal aspect of the fifth vertebra and dorsally by the ventral surfaces of the vertebral bodies.

4.3.1.2 Findings

In Muskox 1 at day 76 PI there was no radiographic evidence of lung pathology (Fig. 4.6). At day 191 PI, multiple discrete, round to oval, homogenous regions of increased opacity were visible in the dorsal and caudal areas of the lung (Fig. 4.7). This film was taken on expiration thus the ability to detect cysts may have been decreased. Sixty-one days later, also in an expiratory film, more cysts were visible and appeared larger than at day 191 (Fig. 4.8). Numerous cysts were visible in all subsequent radiographs and were present in the apical, middle, diaphragmatic and accessory lobes. Twelve cysts that were identified at day 252 PI had a mean diameter of 14.4 mm. These same cysts at day 415 PI had significantly increased in size to a mean diameter of 16.0 mm (Wilcoxon signed-rank test, $n=12$, $P=0.0029$) (Figs. 4.9, 4.10). Thirteen cysts common to films at days 415 and 789 PI were identified and measured. By day 789 PI, cyst sizes had decreased to a mean diameter of 15.4 mm but did not significantly differ from day 415 PI (Wilcoxon signed-rank test, $n=13$, $P=0.242$) (Figs. 4.10, 4.11). The numbers of cysts visible radiographically in the cranio-dorsal lung field (defined above) were very similar at days 252 ($n=18$), 415 ($n=18$) and 789 ($n=16$) PI. The slight variations in animal positioning and radiographic technique could account for these small differences in counts.

In Muskox 2 the first radiographic lesion associated with *U. pallikuukensis* was seen in the diaphragmatic lobes at day 178 PI (Fig. 4.12). The radiographic changes associated with this lesion was subtle and only detected in retrospect after 3 cysts were identified at day 276 PI (Fig. 4.13). Two (possibly 3) cysts were visible at day 440 PI (Fig. 4.14). Although the mean cyst size appeared to increase over time, the sample size was too small to statistically compare individual cysts in this animal. In contrast to Muskox 1, cysts in Muskox 2 were visible only in the diaphragmatic lobes. Throughout the course of infection in muskoxen 1 and 2 there was no radiographic evidence of lung pathology outside the cysts or of mineralization of the cysts.

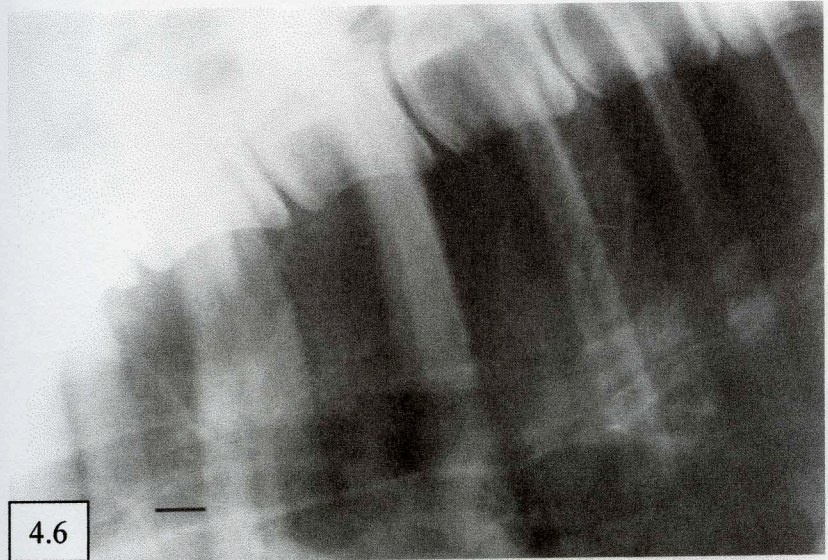


Figure 4.6. At day 76 PI there are no lesions consistent with parasitic infection.

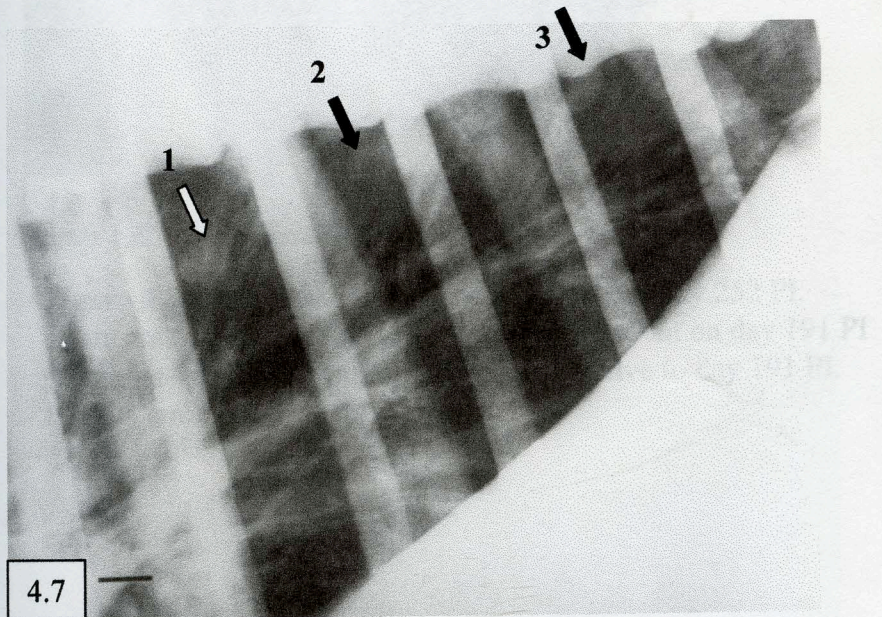


Figure 4.7. At day 191 PI there are 3 homogenous, round to oval lesions visible in the caudal lung fields.

Figure 4.8. Lateral radiographs of craniodorsal views of the
horns of Muskox 1 showing changes in cyst size over time. Bar =
2 cm. Cysts in different radiographs represent the same
cyst.

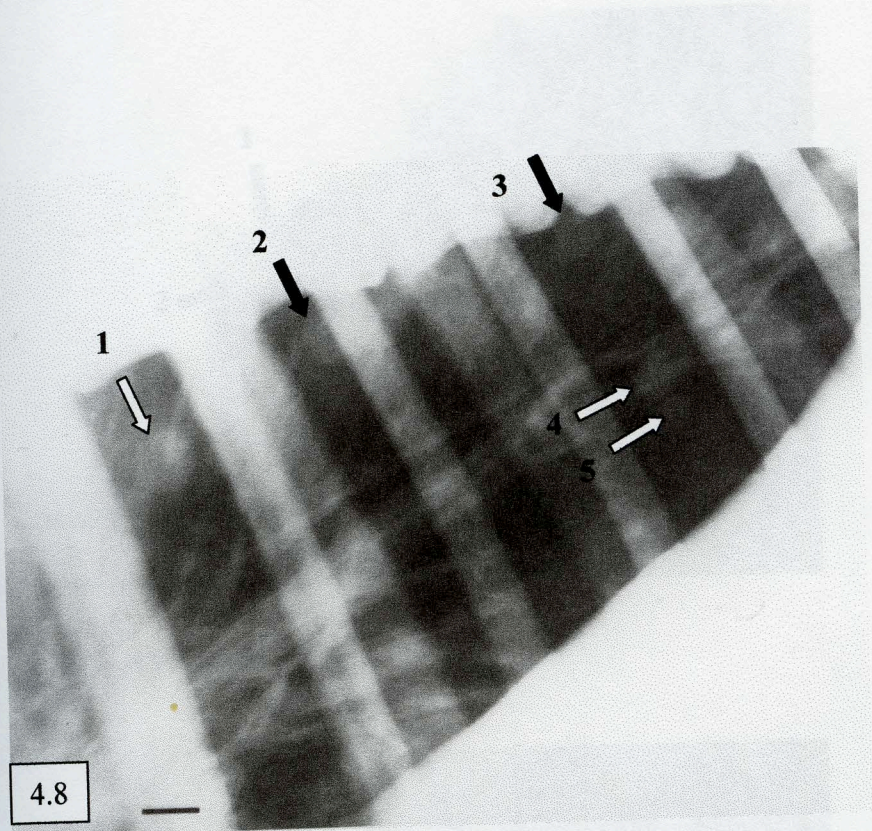


Figure 4.8. Lateral radiographs of Muskox 1 at day 252 PI. There are 5 cysts visible, 2 that were not apparent on day 191 PI (numbers 4 and 5). Cysts appear larger relative to day 191 PI. Bar = 2 cm.

Figure 4.10. At day 415 PI.

Figures 4.9-4.11. Lateral radiographs of craniodorsal areas of the lungs of Musko^x 1 showing changes in cyst size over time. Bar = 2 cm. Numbered cysts in different radiographs represent the same cyst.

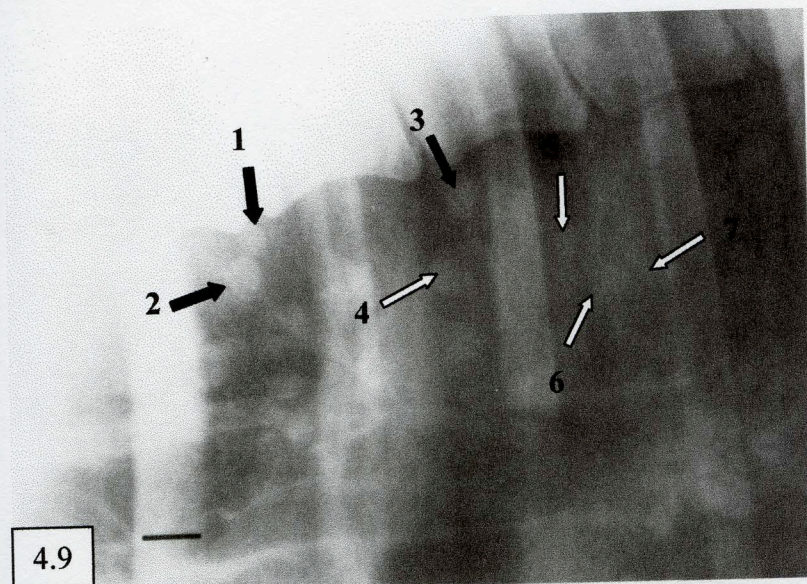


Figure 4.9. At day 252 PI.

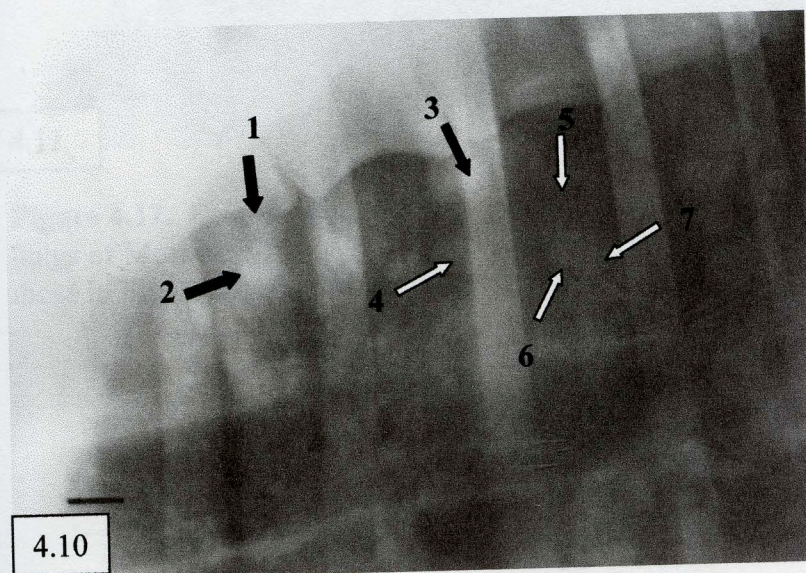


Figure 4.10. At day 415 PI .

Figure 4.11-4.14. Lateral radiographs of craniodorsal areas of the lungs of Muskox 2. Small arrows indicate cysts; heavy arrows indicate probable cysts. Bar = 2 cm.

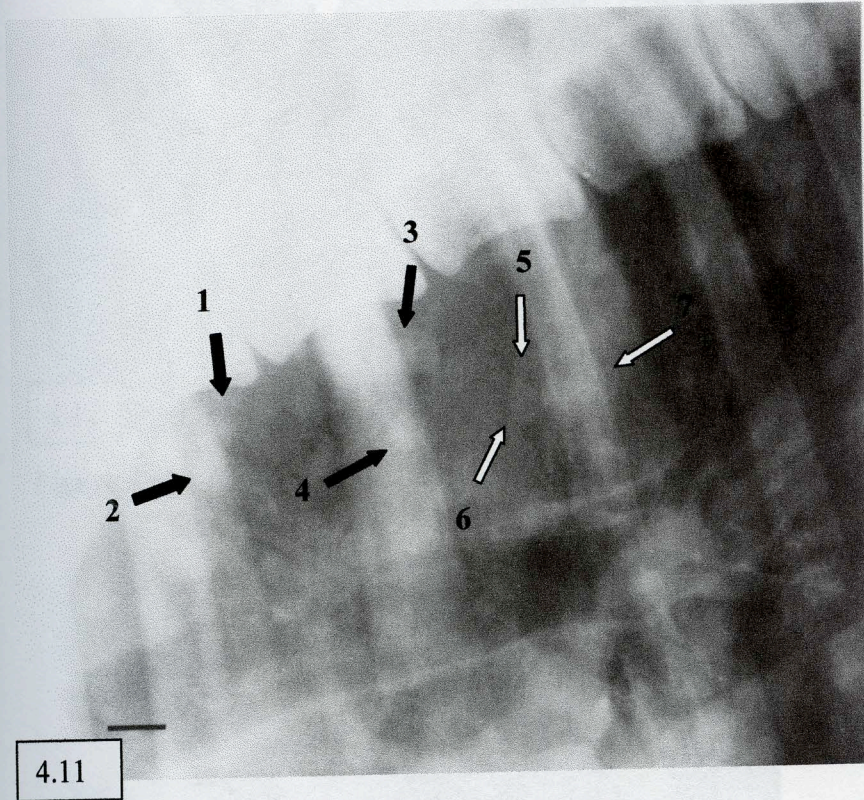


Figure 4.11. Lateral radiographs of craniodorsal areas of the lungs of Muskox 1 at day 789 PI . Numbered cysts correspond to those in Figures 4.9. and/or 4.10. Bar = 2cm.

Figures 4.12 -4.14. Lateral radiographs of caudodorsal areas of the lungs of Musko^x 2. Small arrows indicate cysts; heavy arrows indicate probable cysts. Bar = 2 cm.

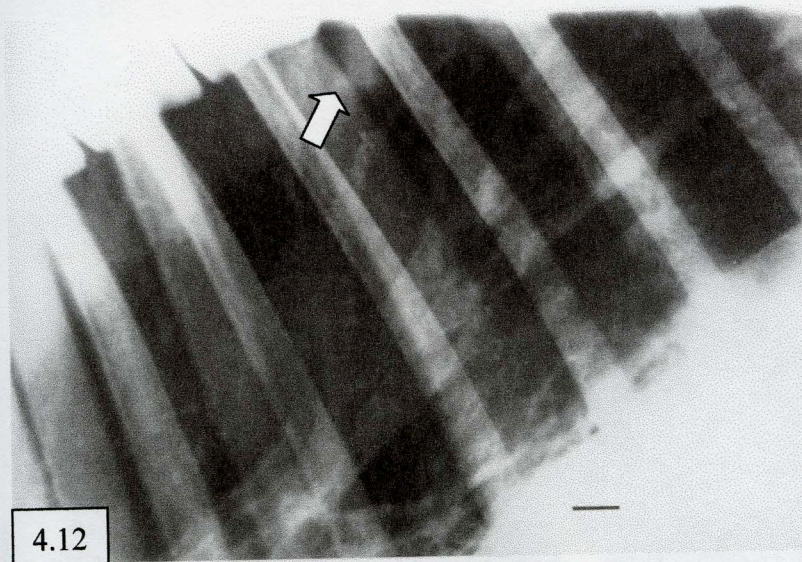


Figure 4.12. At day 178 PI 1 cyst is apparent.

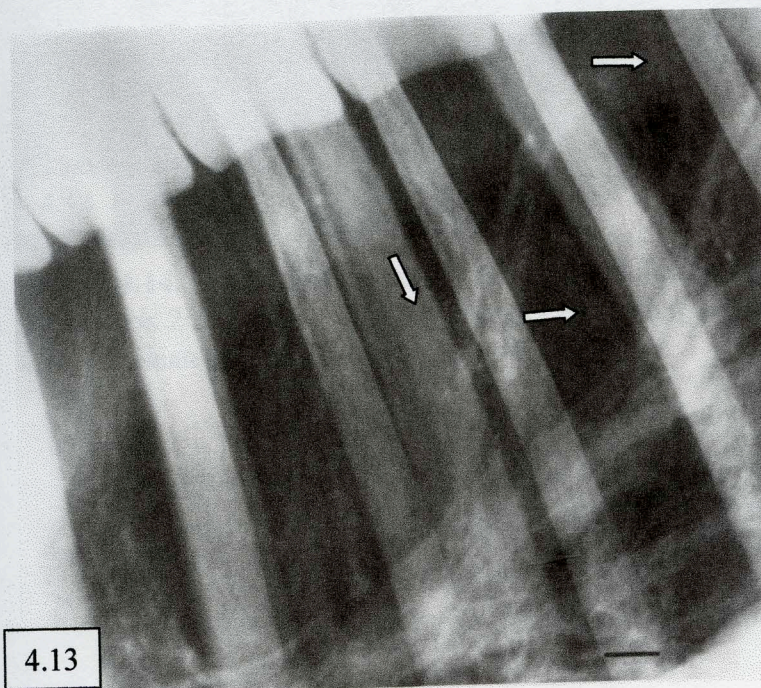


Figure 4.13. At day 276 PI 3 cysts are visible.

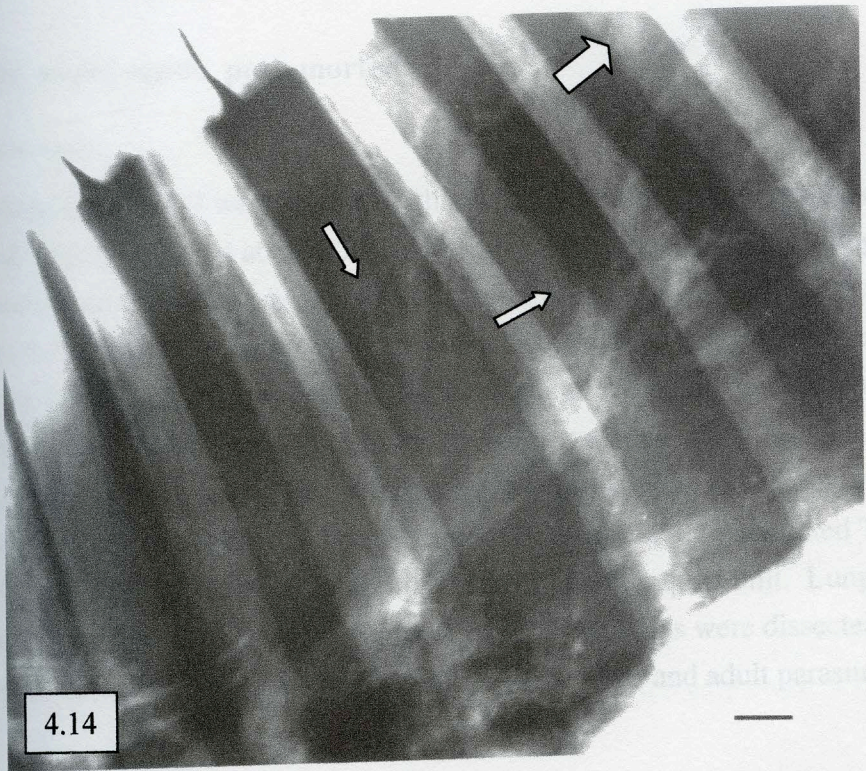


Figure 4.14. Lateral radiograph of caudodorsal areas of the lungs of Muskox 2 at day 440 PI. Small arrows indicate cysts; heavy arrow indicates probable cysts. Bar = 2 cm.

Cysts associated with *U. pallikuukensis* were not detected in radiographs of Muskox 3 immediately prior to euthanasia on day 97 PI. It is possible, however, that parasite-induced changes may have been obscured by the pre-existing pulmonary pathology.

In films of the diaphragmatic lobes of muskoxen 1 and 2, cysts often appeared to be associated with the major airways and blood vessels. Unfortunately, without dorsal-ventral views it was not possible to define the exact relationship.

4.3.2 Lung examination post mortem

4.3.2.1 Techniques

Muskoxen 1, 2 and 3 were sedated with xylazine (Rompun) and euthanized with pentobarbital (Euthanyl-Forte) at days 791, 441 and 97 PI respectively. The lungs and tracheas of muskoxen 1 and 2 were removed intact and inflated artificially using a gastric tube tied into the trachea. The lungs of Muskox 1 were evaluated using CT performed with a Hi Speed CT/i[®] spiral scanner (GE Medical Systems, Milwaukee, Wisconsin). Images were obtained using 3 mm sections every 3 mm at a pitch of 1.3, and evaluated on the lung setting. Axial images were reformatted into the sagittal and coronal planes. Lungs from Muskox 2 were examined radiographically using a ceiling mounted Transix 800 S (Picker International Canada, Brampton, Ontario) radiographic unit. Lungs from Muskox 3 were not examined by radiography or by CT. All lungs were dissected until up to 48 hr post mortem to locate and recover all parasitic cysts and adult parasites.

4.3.2.2 Findings

The airways of Muskox 2 at post mortem contained aspirated rumen contents which interfered with the interpretation of the radiographs. Nevertheless, in dorso-ventral films several cysts were identified in both diaphragmatic lobes. Cysts were often associated with the main bronchi of these lobes, but were not detected in other lung lobes (Fig. 4.15). There was no evidence of cyst mineralization.

Computed tomographic examination of the lungs from Muskox 1 showed the cysts of *U. pallikuukensis* very clearly, appearing as well defined high density nodules (Fig. 4.16). These ranged in size from 5-20 mm, average being approximately 15 mm. A total of 191 cysts were counted. In general they were centrally located with abronchovascular distribution and very few cysts were at or near the pleural surface

Figures 4.15, 4.17. Post mortem computed tomographic
scans of the lungs of Muskox 1. Bar = 2 cm.

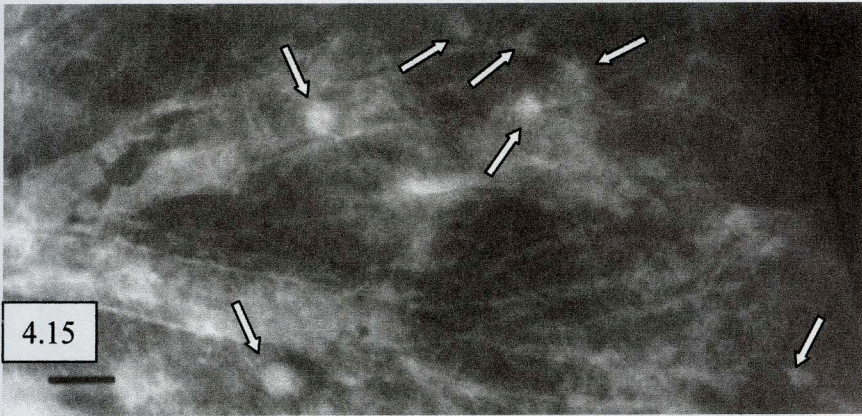


Figure 4.15. Post mortem radiograph of the artificially inflated right diaphragmatic lobe of Muskox 2. Arrows indicate cysts in lung adjacent to main bronchi. Bar = 2 cm.

Figure 4.17. Reformatted sagittal view of the left diaphragmatic
lobe demonstrating cysts located primarily dorsally and
associated with the bronchi. Small arrows indicate cysts, heavy
arrows indicate airways branching from main bronchi.

Figures 4.16, 4.17. Post mortem computed tomographic examination of the lungs of Muskox 1. Bar = 2 cm.

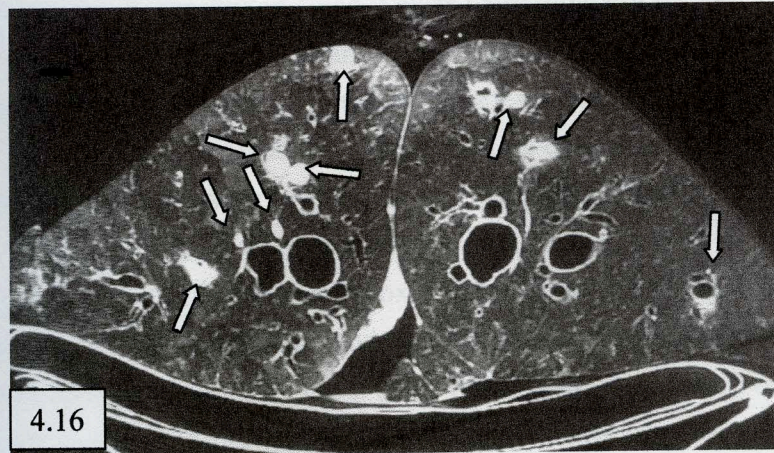


Figure 4.16. Axial section of lungs at the midregion of the diaphragmatic lobes; parasitic cysts are visible in the parenchyma, near airways and rarely near the pleural surface.

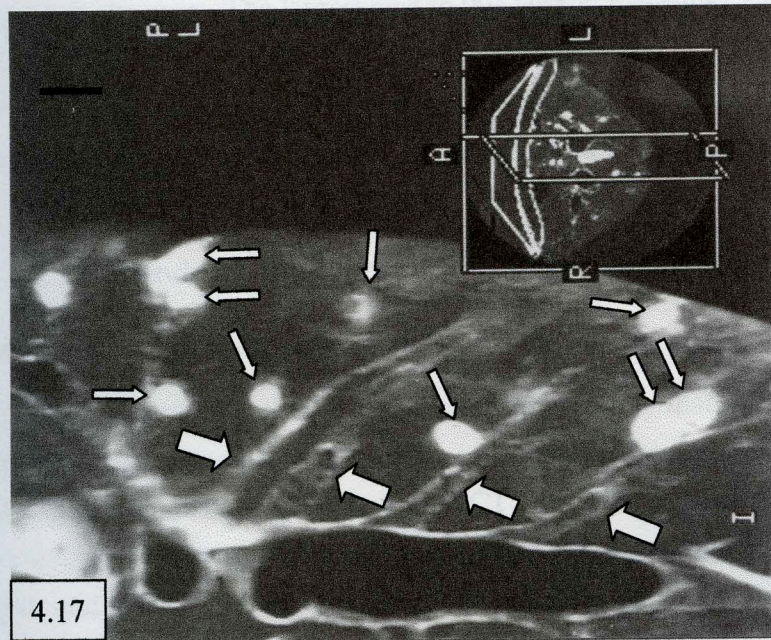


Figure 4.17. Reformatted sagittal view of the left diaphragmatic lobe demonstrating cysts located primarily dorsally and associated with the bronchi. Small arrows indicate cysts; heavy arrows indicate airways branching from main bronchus.

(Fig. 4.17). In some of the airways adjacent to cysts there was mild inflammatory change with thickening of the bronchial walls.

On dissection of the lungs from muskoxen 1, 2 and 3 a total 183, 16 and 13 cysts were recovered. In muskoxen 1 and 2 cysts were typically located in the lung parenchyma adjacent to major bronchi and blood vessels. Cysts were present in all lung lobes of Muskox 1 but only in the diaphragmatic lobes of Muskox 2. Cyst locations in Muskox 3 were not recorded. In all 3 muskoxen, more cysts were found on post mortem dissection than were seen in ante mortem radiographs, and more cysts were found by CT of Muskox 1 than were detected by dissection.

Cysts of *U. pallikuukensis* in muskoxen 1 and 2 had tough, well defined capsules, were round to oval, soft, and had no evidence of mineralization on dissection. They ranged in diameter from 10-25 mm (Fig. 4.3). Cysts contained 2 or more adult parasites as well as eggs and first-stage larvae (Fig. 4.4). Adult parasites were not found in the lung tissue outside the cysts. Histologically within the cysts adult parasites, eggs and first-stage larvae were located within a dense matrix which also contained a variety of inflammatory cells. In Muskox 3 adult nematodes were found both inside and outside cysts. The largest cyst in this animal measured 7 mm in diameter.

Other than the parasitic cysts, the lungs of Muskox 1 appeared grossly normal. Mild to severe pulmonary emphysema was grossly apparent in muskoxen 2 and 3.

4.4 Synthesis of Standard Radiographic, CT and Dissection Results

4.4.1 Medical imaging for investigation of a lung nematode

The findings described in this chapter demonstrate clearly that medical imaging has a useful role in monitoring the development of *U. pallikuukensis* in the lungs of experimentally infected muskoxen. Post mortem CT is, at least in animals with long-standing infections, uniquely useful for quantifying and characterizing parasite-associated cysts and for determining their distribution within the lungs.

There was no radiographic evidence of parasitic infection at 76 days PI in Muskox 1 or at 97 days PI in Muskox 3. Cysts were first detected radiographically in muskoxen 1 and 2 at days 191 and 178 PI, 3 mo after patency. It is interesting that the largest cyst dissected from Muskox 3 at day 97 PI was only 7 mm in diameter. This cyst was considerably smaller than those recovered from the other two muskoxen at post mortem and it, as well as the smaller cysts, may have been obscured in the radiographs by ribs. It is likely, therefore, that the pathological changes associated with *U.*

pallikuukensis are not sufficient to be detected radiographically in a live animal until between 3-6 mo PI. In MuskoX 1 we have shown that, as the infection aged, the cyst sizes increased until at least day 415 PI and then stabilized or decreased by day 789 PI. Had this animal not been euthanized this trend may have become more apparent.

Radiographic examination of the animals while they were alive provided an indication of the course of infection over time. In MuskoX 1 the appearance at day 191 PI, and increase in size of visible cysts from days 252 to 415 PI, coincided with a continuous increase in first-stage larval production from this animal (see Fig. 3.1 and Kutz et al., 1999b). Although there was no increase in the number of visible cysts in the area of lung in which cyst development was monitored radiographically, it is possible that increase in larval production also may have been associated with establishment of additional cysts in other parts of the lungs.

Radiographic examination of the lungs of MuskoX 2 at post mortem provided useful data on cyst size and on distribution of cysts within lung lobes and their relation to airways and vasculature. This facilitated subsequent dissection and recovery of the cysts.

Post mortem CT was a very valuable tool for determining cyst size, character, and spatial distribution within the lungs. It was more rapid and accurate than gross dissection for this long standing infection but its usefulness at postmortem for detecting earlier lesions caused by this parasite is not known. Computed tomography produced a clear image of the spatial relationships of the cysts to the airways and vasculature, and also provided a permanent electronic record. If feasible in a live animal, CT technology would be superior to standard radiographs for monitoring cyst size, characteristics and distribution during the course of the infection.

The pulmonary pathology viewed radiographically in the experimentally infected animals appeared to be primarily space occupying. There was no evidence of diffuse parenchymal involvement or effusions with CT, gross dissection and histological examination, but evidence of focal airway thickening and inflammation near some cysts was seen with CT. No clinical signs were noted in the experimental animals, but they were confined in pens and not exposed to the stresses of a natural environment.

4.4.2 Lung changes associated with related nematodes

Although there are no published reports of radiographic lesions caused by related nematodes, the absence of pathology outside the discrete cysts of *U. pallikuukensis* contrasts with what would be expected with lung protostrongylids such as *Muellerius*

capillaris or *Protostrongylus* spp. For example, in sheep *Muellerius capillaris* produces numerous nodules a few millimeters to several centimeters in diameter, usually immediately beneath the pleural surface. These may be either worm nodules, which are small, often calcified and do not contain reproductively active parasites; or brood nodules, which are larger, not calcified and contain reproductively active parasites (Sedlmeier et al., 1969; Polley, 1987). Radiographically, we would expect the calcified worm nodules to have increased opacity and be smaller than the cysts of *U. pallikuukensis* observed in this study. The brood nodules would probably appear as less opaque, irregularly shaped, interstitial lesions. *Protostrongylus stilesi*, found in the lung parenchyma of bighorn sheep, forms unencapsulated, diffuse nodules in the caudo-dorsal aspects of the diaphragmatic lobes (Forrester and Senger, 1964). Radiographically these may appear similar to *M. capillaris* brood nodules.

4.4.3 The host-parasite system in the Arctic

In order to ensure continued health of muskoxen, which are culturally and economically important in the Arctic, the presence of *U. pallikuukensis* must be considered in management plans and potential translocations. As well, because of the effects of temperature on larval survival in the environment and on development in the gastropod intermediate host, the effects of global climate change (with predicted warming of the Arctic) on parasite distribution and significance should also be addressed (Hoberg et al., 1995; Hoberg, 1997). *Umingmakstrongylus pallikuukensis* has not yet been reported in commercially harvested muskoxen from Victoria or Banks islands. It is possible that the parasite is not able to develop on these islands because of climatic conditions or insufficient suitable gastropod intermediate hosts. Alternatively it may not yet have been transported to these islands by natural host dispersal.

The potential for *U. pallikuukensis* to infect various species of domestic or wild ungulates from more temperate regions of North America is not known. Lung protostrongylids typically do not infect the Bovinae, but are common in both wild and domestic sheep and goats, frequently causing severe pulmonary disease. It seems unlikely that *U. pallikuukensis* could infect cattle, and preliminary work suggests it does not infect domestic sheep (see Chapter 3). The ability of this parasite to infect wild sheep, goats and cervids is not known, but *D. laeve*, a suitable gastropod intermediate host for the development of *U. pallikuukensis*, is present throughout most of North America, including the Arctic (Pilsbry, 1946, 1948), and climatic conditions in many areas of the continent may be suitable for parasite development and transmission.

Much remains to be learned about these host-parasite systems. Medical imaging of muskoxen infected with *U. pallikuukensis* before and after death has proven to be a very useful tool for investigating this relationship. This preliminary work may have wider application in the study of lung parasites in wild animal populations.

5. EMERGENCE OF THIRD-STAGE LARVAE OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS* FROM THREE SPECIES OF GASTROPOD INTERMEDIATE HOSTS

5.1 Abstract

We investigated the emergence of third-stage larvae (L3) of *Umingmakstrongylus pallikuukensis* from the slugs *Deroceras laeve*, *D. reticulatum* and the snail *Catinella* sp. in the laboratory and from *D. laeve* on the tundra. Third-stage larvae emerged from 8 of 8 *D. laeve* and 8 of 8 *D. reticulatum* housed at 20 C in darkness and from 9 of 10 *D. laeve* and 5 of 5 *Catinella* sp. housed at 21 C with 10-12 hr of light/day. Larvae emerged from *D. laeve* and *D. reticulatum* over a wide range of infection intensities (2-179 and 20-65, respectively) and patterns of emergence were independent of intensity. The majority of the L3 emerged from most *Deroceras* spp. slugs by 58 or 60 days post-infection (PI). Lower rates of emergence were observed for *Catinella* sp. Larvae emerged from *D. laeve* on the tundra by 10 wk PI and were recovered from the vegetation of some experiments the following year. Live L3 survived 13 mo in tap and distilled water at 0 - 4 C. Emergence of L3 of *U. pallikuukensis* from the intermediate host may increase the temporal and spatial availability of L3 and enhance its survival and transmission.

5.2 Introduction

Umingmakstrongylus pallikuukensis is a protostrongylid lungworm in muskoxen from the Canadian Arctic (Hoberg et al., 1995). It is a member of the subfamily Muelleriinae, a sister group of the Elaphostrongylinae (Carreno and Hoberg, 1999). *Umingmakstrongylus pallikuukensis* is present at a high prevalence (approaching 100%) and intensity in the muskox population west of Kugluktuk, Nunavut, Canada (Gunn and Wobeser, 1993). It follows the typical indirect

protostrongylid life cycle requiring a gastropod intermediate host (IH) (Hoberg et al., 1995; Kutz et al., 1999b).

First-stage larvae (L1) of protostrongylids are shed in the feces of the definitive host (DH), invade the tissues of suitable gastropods and develop to third-stage larvae (L3). Typically, transmission occurs when the DH ingests gastropods containing infective L3 (Anderson, 1992). In some species within the Protostrongylidae and the related Angiostrongylidae, however, L3 emerge from the live gastropod IH and transmission may occur when these emerged L3 are ingested by the DH with vegetation or water (Heyneman and Lim, 1967; Boev, 1975). There is an extensive European and Russian literature on the protostrongylids which has been summarized by Boev and subsequently translated to English (Boev, 1975). It is this literature in which the majority of the reports on larval emergence are found. Among the Muelleriinae and Protostrongylinae, emergence of L3 from living gastropods has been reported for *Muellerius capillaris*, *Cystocaulus ocreatus*, *Protostrongylus rufescens*, *P. davtyani*, *P. tauricus* and *P. pulmonalis* and/or *P. kamenskyi* (Boev, 1975), *P. stilesi* (Monson and Post, 1972) and *P. boughtoni* (Kralka and Samuel, 1984a). Rose (1957) and Boev (1975) reported emergence of L3 of *M. capillaris* from dead IH. There have been no reports of larval emergence among the Elaphostrongylinae (Kontrimavichus et al., 1976; Anderson, 1992), although emergence of L3 of *Parelaphostrongylus* sp. (recovered from Dall's sheep (*Ovis dalli*) from the slug *Deroceras laeve* has been observed in the laboratory (S. J. Kutz, unpubl. obs.). Among the Angiostrongylidae it is well known that the L3 of *Angiostrongylus cantonensis* and *A. costaricensis* emerge from their IH (Heyneman and Lim, 1967; Ubelaker et al., 1980).

Experimentally, emerged L3 of *P. boughtoni* and *A. cantonensis* can produce patent infections in their respective DH (Heyneman and Lim, 1967; Richards and Merritt, 1967; Kralka and Samuel, 1984a). Kutz et al. (1999b) reported that the L3 of *U. pallikuukensis* emerge from the gastropod *Deroceras reticulatum* and, through experimental infections, demonstrated that the emerged L3 were infective to a muskox. Larval emergence is considered to be important in the epidemiology of *M. capillaris*, *C. ocreatus* and *A. cantonensis* (Heyneman and Lim, 1967; Boev, 1975). Many authors, however, question the significance of larval emergence in parasite transmission and believe the only route of infection for the DH is by ingestion of infected gastropods (Anderson, 1992). Patterns of emergence over time for larval protostrongylids from gastropods, factors affecting this emergence and its epidemiological significance have not been investigated in detail.

The objectives of the present study were to: (1) investigate in the laboratory the patterns of L3 emergence of *U. pallikuukensis* from 2 potential natural IH, the slug *D. laeve* and the snail *Catinella* sp., as well as from the laboratory IH, *D. reticulatum*; and (2) determine whether L3 emerge from *D. laeve* under natural conditions in the Arctic. We propose that emergence of L3 of *U. pallikuukensis* from different gastropod species is a natural phenomenon that occurs consistently and is not a function of infection intensity. We also propose that larval emergence may be epidemiologically important in the successful maintenance of this parasite at a high prevalence and intensity in the naturally infected muskox population.

5.3 Materials and Methods

5.3.1 Laboratory experiments

Four laboratory experiments were designed to examine emergence of L3. Three species of gastropods, *D. laeve*, *D. reticulatum*, and *Catinella* sp., were evaluated under varying regimes of temperature, light, and intensity of infection (Table 5.1).

Infection of gastropods: First-stage larvae were obtained from the feces of an experimentally infected muskox using a Baermann technique (Gajadhar et al., 1994; Kutz et al., 1999b). *Deroceras laeve* and *Catinella* sp. were collected from the banks of the Coppermine and Rae rivers near Kugluktuk (67° 49'N, 115° 08'W), Nunavut, Canada and *D. reticulatum* were obtained from a protostrongylid-free laboratory colony (Kutz et al., 1999b). Only adult *D. laeve* and *D. reticulatum* (greater than 15 mg and 400 mg respectively) were used but, because few *Catinella* sp. were available, specimens with both 2 and 3 whorls were used. Prior to experimental infection with *U. pallikuukensis*, the feet of all wild-caught gastropods were examined for lesions which, based on previous experiments, were indicative of existing protostrongylid infections (S. J. Kutz, unpubl. obs.). No such lesions were seen on any of the specimens examined.

Gastropods were exposed to L1 on filter paper in plastic petri dishes over a single 3 hr period (Hoberg et al., 1995; Kutz et al., 1999). Gastropods not exposed to L1 were used as controls in the experiments with *D. laeve* and *Catinella* sp.

Gastropod maintenance and examination: Gastropods were housed initially in Rubbermaid® containers (Kutz et al., 1999b). At 12-22 days post infection, depending on the experiment (Table 5.1), they were removed from the containers and placed

Table 5.1: Laboratory experiments on emergence of third-stage larvae (L3) of *Umingmakstrongylus pallikuukensis* from three species of gastropods.

Experiment number	Gastropod species	Number of gastropods infected/control	Infection dose L1/gastropod	Temp. (C)	Days of experiment	Days between examination	L3/gastropod range
1 [†]	<i>D. laeve</i>	8/8	1000	20 ± 0.5	12 - 60 PI	2	38 - 179
2 [†]	<i>D. reticulatum</i>	8/0	1000	20 ± 0.5	14 - 58 PI	2-5	20 - 65
3 [†]	<i>D. laeve</i>	10/10	500	21 ± 1.0	20 - 60 PI	2-4	1 - 19
4 [†]	<i>Catinella</i>	5/6	500	21 ± 1.0	24 - 60 PI	2-8	2 - 12

[†]In total darkness in temperature controlled incubator.

[†]In 12-14 hr light/day at room temperature.

individually in glass petri dishes (50 mm inside diameter) containing a small amount of water, lettuce, carrots and chalk and examined for larval emergence on the days indicated in Table 5.1. Foot lesions and emerging larvae were detected by allowing gastropods to crawl in clean glass petri dishes and then inverting the dishes and examining the surface of the foot using a dissecting microscope with overhead lighting. They were then transferred to sterilized petri dishes with fresh food. The uneaten carrots and lettuce were removed from the old dishes, water was added to the lids and bases of the dishes and these were examined with a dissecting microscope. The food removed was placed in individual 100 ml glass jars or beakers containing approximately 80 ml tap water. Jars were shaken and the water in the beakers stirred vigorously in order to dislodge larvae from the food. After 16-24 hr in light at $21\text{ C} \pm 1\text{ C}$, food and supernatant were removed from the containers and the sediment examined for larvae. At the end of each experiment gastropods were killed, digested in a pepsin/hydrochloric acid solution (Hoberg et al., 1995), and the remaining larvae recovered, counted and classified to stage of development.

Analysis: It was assumed that all L3 produced in each gastropod were recovered by examining dishes and vegetation and by digestion. The total number of L3 for each gastropod was determined by adding the number of larvae which emerged during the experiment to the number of L3 recovered by digestion at the end of the experiment. The cumulative proportion of L3 that had emerged from an individual gastropod by a given sampling day was the sum of all the L3 that had emerged up to and including that day divided by the total number of L3 recovered from the gastropod. The mean cumulative proportion of emerged L3 in an experiment was calculated based only on gastropods from which at least one L3 had emerged during the experiment.

5.3.2 Field experiments

As part of an Arctic field study of the development of *U. pallikuukensis* we also investigated L3 emergence under natural conditions. Wild-caught *D. laeve* were experimentally infected with *U. pallikuukensis* (Kutz et al., 1999b) and placed in enclosures (10 slugs/enclosure) made from perforated plastic pails (150 mm high, 200 mm inside diameter) containing tundra and lined and covered with a fine netting. Enclosures were placed into the tundra so that the vegetation in the pails was at the same level as that outside. Experiments were established every 2 wk in a mesic sedge

meadow near Kugluktuk (Table 5.2). Multiple control enclosures containing 10 uninfected slugs were established for each experiment.

Slugs from the enclosures were examined by digestion (Hoberg et al., 1995) every 2 wk for larval development and, after the first L3 was recovered from a slug in an experiment, the vegetation was sampled at varying intervals for emerged L3 (Table 5.2). Some enclosures were left in the tundra over winter and were examined the following year. To recover emerged L3, vegetation was clipped to the level of the soil and placed in a glass flask or jar. Initially (vegetation examined 28 August and 11 September, 1997), approximately 250 ml of tap water was added to each container which was then shaken or stirred vigorously. The sediment was examined for larvae after 24 hours. On 11 September, 1997 the vegetation was re-examined by adding 1 drop of Ivory[®] liquid dish detergent to 5 L of tap water and using 250 ml of this solution for the larval recovery. The sediment was examined after approximately 24 hr. The detergent technique was used for all subsequent samples. Slugs were recovered from the remaining turf by placing it in unperforated pails and using a cold water bath technique (Kralka, 1986).

5.3.3 Survival of third-stage larvae

Live L3 that had emerged from *D. reticulatum* in a preliminary laboratory experiment were placed in approximately 50 ml of tap water (80 L3) or distilled water (164 L3) in 125 ml Erlenmeyer flasks with a foam cork and placed in the refrigerator at 0-4 C (water froze at least once during this period). Water was added as needed to prevent dehydration. These larvae were examined at room temperature 13 mo later to determine survival.

5.4 Results

5.4.1 Laboratory experiments

Larval emergence: Third-stage larvae of *U. pallikuukensis* emerged from all infected individuals of all 3 gastropod species with the exception of a single *D. laeve*. Only L3 emerged and these were found in the sediment from the uneaten vegetation as well as on the inside surfaces of the lids and bases of the petri dishes. Those found in water in the bases of the dishes, or in the sediment from the vegetation, were usually motile while those found in dry areas of the dishes were tightly coiled, coated with mucus and adhered to the dish surface. When dislodged and placed in water these

Table 5.2: Emergence of third-stage larvae (L3) from *Deroceras laeve* under field conditions.

Experiment	Start date	First L3*	Numbers of emerged L3 recovered from the vegetation					
			Aug. 28/97†	Sept 11/97	Sept 18/97	June 15/98	July 15/98	
1	June 19/97	July 17/97	1† (1/2)	2† (1/3)	—†	0 (0/6)	—	
2	July 3/97	July 31/97	—	27† (3/3)	—	3† (1/4)	—	
3	July 17/97	Aug 14/97	—	—	0 (0/3)	5* (1/6)	—	
4	July 31/97	July 15/98	—	—	—	—	0 (0/3)	
5	Aug 14/97	July 15/98	—	—	—	—	0 (0/3)	
Control	July 17/97	NA†	—	—	—	0 (0/3)	—	
Control	July 31/97	NA	—	—	—	—	0 (0/3)	

*Recovered by digestion of slugs.

†First value is the number of emerged L3 recovered, values in brackets are numbers of enclosures with emerged L3/total enclosures examined.

‡All live L3.

§Not examined.

¶1 live L3, 1 dead L3, 1 coiled L3.

*2 live L3, 3 dead L3.

†No larvae recovered by digestion.

larvae became motile. Early in the experiments a few L3 that had emerged were still within the second-stage cuticular sheaths, but most of the sheaths were broken at the anterior end. Second-stage sheaths were frequently observed free in the dishes. After day 30 PI, emerged larvae were usually free of the second-stage sheath. Larvae were not recovered from any of the control experiments with uninfected gastropods.

Third-stage larvae were observed emerging during examination of the feet of live *D. laeve*. In experiment 3 on day 28 PI, a partially coiled L3 within the second-stage sheath (broken at the anterior end), was expelled from the foot of the slug within a cloud of mucus. This was also observed on day 40 PI in experiment 1. Larvae actively emerging from the *D. laeve* in experiment 1 were observed on days 40 PI (2 L3 from 1 slug) and 46 PI (5 L3 from 1 slug). The cephalic extremities of these larvae were free from the slug tissue and moving vigorously while the posterior portions were still within the slug. Dark pigment was visible in areas of the slug from which the larvae were emerging.

Emergence patterns: Emerged L3 were first observed on day 22 PI in experiment 1 and day 20 PI in experiment 2. Emerged L3 were first observed on days 22 and 38 PI in experiment 3 and 4 respectively, but in these experiments examinations for larval emergence were not started until days 20 and 24 PI, respectively, and any L3 emerging earlier would not have been detected.

The pattern of emergence of L3 from *D. laeve*, *D. reticulatum* and *Catinella* sp. differed among the experiments (Figs. 5.1-5.4). In experiment 1 there was a relatively constant rate of emergence from day 24 until slugs were killed at day 60 PI (Fig. 5.1). In experiment 2 the pattern of larval emergence was described by a sigmoidal curve (Fig. 5.2). Few larvae emerged up to day 28 PI, a rapid rise in L3 emergence occurred from days 30 to 50 PI and then the rate decreased from days 52 to 58 PI. In experiment 3, larval emergence was greatest from days 22 to 26 PI and days 34 to 40 PI (Fig. 5.3) and in experiment 4 emergence began much later, and the level of emergence was lower (Fig. 5.4). The total number of foot lesions observed in *D. laeve* in experiment 1 varied from day to day and between individuals, but there was a trend for the lesion count to decrease as the proportion of emerged L3 increased (Fig. 5.1). A similar pattern was observed in experiment 3.

The total cumulative proportions of L3 that emerged from individual gastropods in each experiment relative to the intensities of infection are shown in Figures 5.5-5.8. There was 100% larval emergence from some *D. laeve* with both high (64 L3) and low (2 L3) intensities of infection (Figs. 5.5, 5.7). By the end of experiments 1, 2 and 3,

Figures 5.1-5.4. The mean cumulative proportions of third-stage larvae (L3) which emerged from gastropods during each experiment. Each bar represents the mean cumulative proportion of L3 that had emerged up to that day relative to the total L3 (total L3 = L3 emerged by end of experiment + L3 recovered from the gastropod by digestion at the end of experiment). Error bars = 1 SD of the mean of all gastropods (from which larvae emerged) within an experiment.

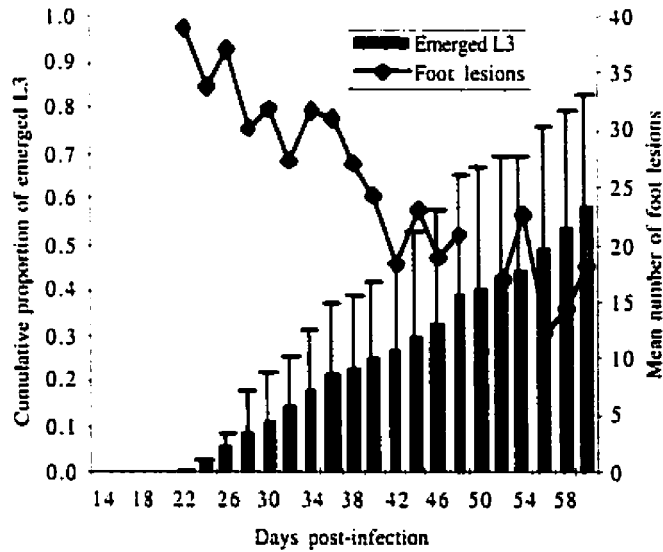
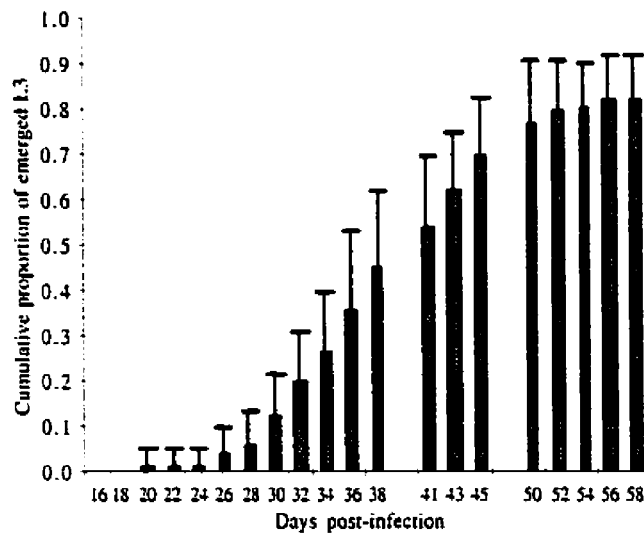


Figure 5.1. Experiment 1 with *Deroceras laeve*. Slugs were examined every second day. Also shown is the mean number of lesions in the feet of 8 slugs (not examined on day 50 post-infection).



Figures 5.2-5.4. Gastropods were examined only on the days indicated on the x-axis.

Figure 5.2. Experiment 2 with *D. reticulatum*.

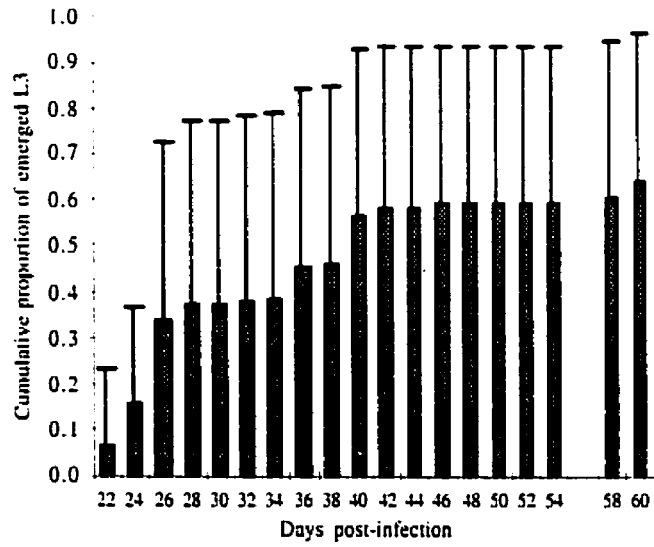


Figure 5.3. Experiment 3 with *D. laeve*.

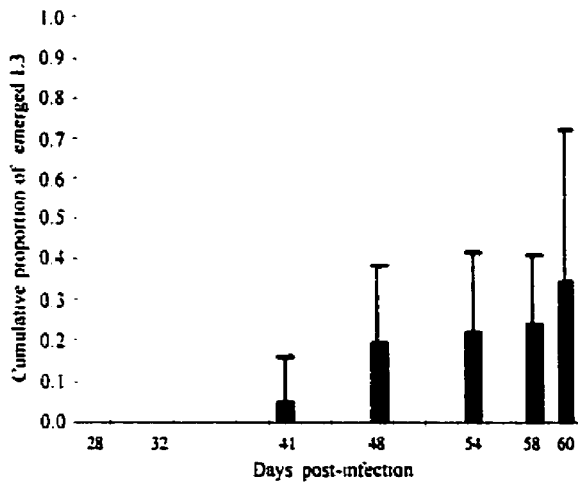


Figure 5.4. Experiment 4 with *Catinella* sp..

on days 58 or 60 PI, the majority of the L3 had emerged from more than half of the slugs (Figs. 5.5-5.7). Levels of larval emergence were lower in *Catinella* sp. (experiment 4) with only one snail having lost more than half of its L3 by day 60 PI (Fig. 5.8). Figure 5.9 demonstrates the patterns of L3 emergence from 6 individual *D. laeve* from experiments 1 and 3 with similar cumulative proportions of emergence (approximately 0.6) by the end of the experiments but with different intensities of infection. The patterns of emergence were generally similar. The emergence at lower intensities appears irregular because each L3 represents a higher proportion of the total than with higher intensities of infection.

5.4.2 Field experiments

The results for larval emergence in the field are shown in Table 5.2. All emerged L3 recovered in 1997 were alive while some in 1998 were dead or coiled and non-motile. More emerged L3 were recovered from the vegetation using the detergent technique after the water technique. In field experiment 2 on 11 September 1997, 7, 1 and 1 live L3 were recovered from the vegetation of each of 3 enclosures examined using tap water. The same vegetation was processed again using the detergent technique and 3, 1 and 14 more L3 were recovered, respectively, from each of the 3 enclosures. In 1997 infected slugs were recovered from all enclosures with L3 on the vegetation, but in 1998 slugs were not recovered from the enclosures with L3 on the vegetation.

5.4.3 Survival of third-stage larvae

Many of the emerged L3 held in water at 0 C to 4 C for over 1 yr were still alive. From the tap water, 21 of the original 80 L3 were recovered and of these 1 was dead, 3 were active and 17 were tightly coiled and in good condition. From the distilled water, 122 of the original 164 L3 were recovered and of these 7 were dead, 9 were active and 106 were tightly coiled and in good condition.

5.5 Discussion

We have demonstrated in the laboratory that L3 of *U. pallikuukensis* emerge from 3 species of gastropods while the gastropods are alive. Two of these gastropod species occur in the Arctic where muskoxen naturally infected with this parasite are found. We have also shown that L3 emerge from *D. laeve* on to the vegetation under natural conditions in the Arctic. We could not ascertain, however, on the basis of the field experiments, whether the L3 emerged from live or dead slugs.

Figures 5.5-5.8. Proportion of third-stage larvae (L3) that emerged relative to intensity of infection. Each bar represents the proportion of the total L3 within an individual gastropod that emerged during the experiment. The total number of L3 (as defined in Figs. 5.1-5.4) recovered from each individual gastropod is shown on the x-axis. Where numbers are present above the bars they indicate the number of days post-infection (PI) by which 100% of the L3 had emerged from that gastropod. Experiments 1, 3 and 4 ended on day 60 PI, experiment 2 on day 58 PI.

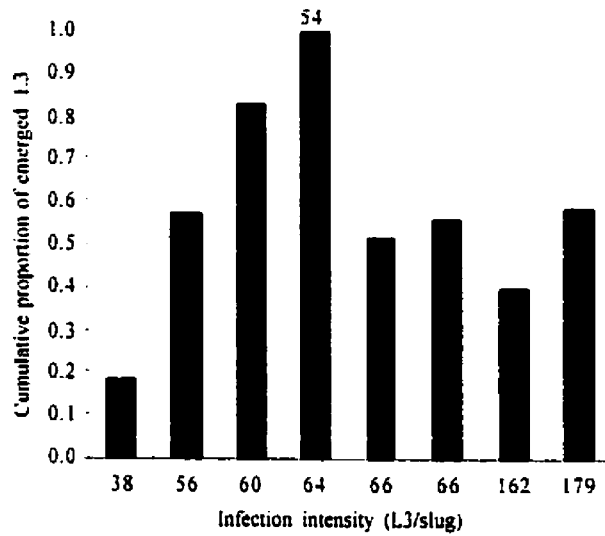


Figure 5.5. Experiment 1 with *D. laeve*.

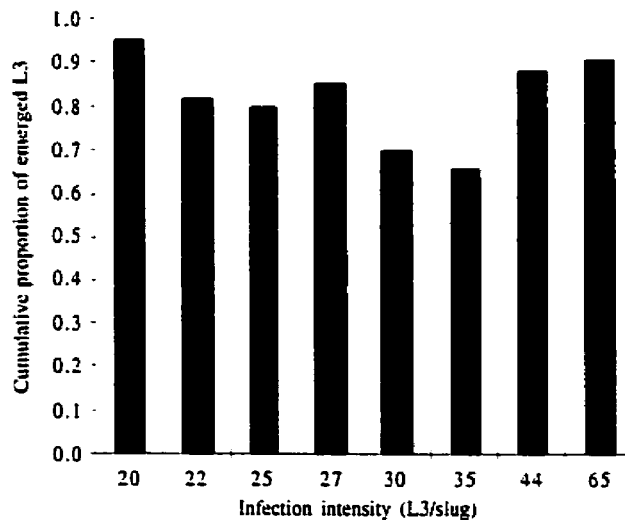


Figure 5.6. Experiment 2 with *D. reticulatum*.

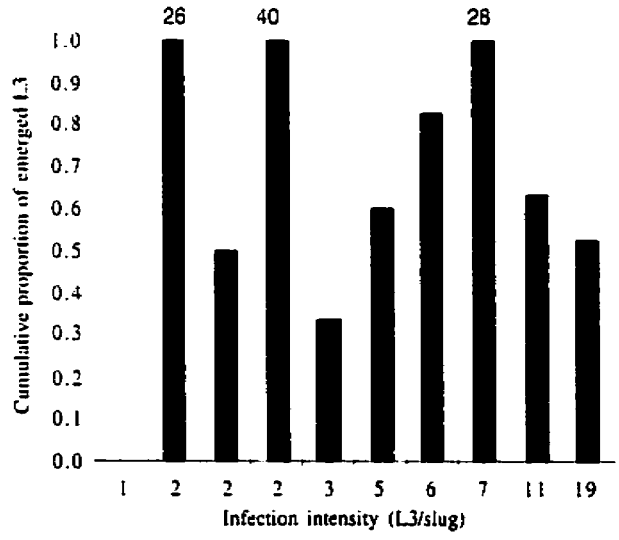


Figure 5.7 Experiment 3 with *D. laeve*.

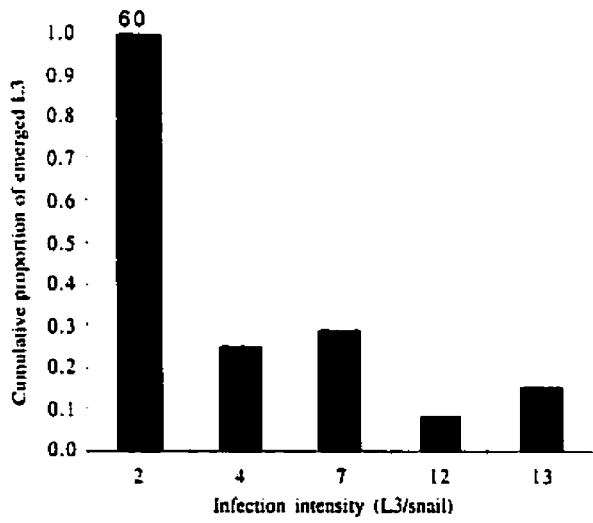


Figure 5.8. Experiment 4 with *Catinella* sp.

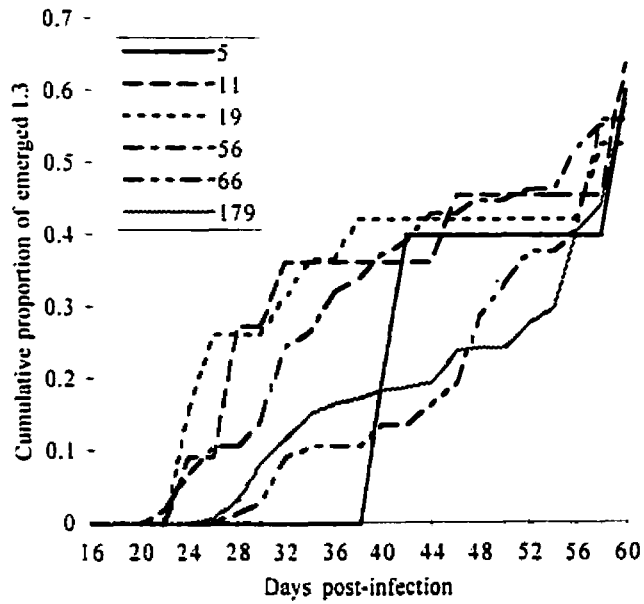


Figure 5.9 Patterns of emergence of third-stage larvae (L3) from individual *Deroceras laeve* with different intensities of infection (slugs from experiments 1 and 3) but similar proportions of emerged L3 by day 60 post-infection.

5.5.1 Larval emergence in the laboratory

In the laboratory experiments, emergence of L3 did not coincide with initial development of L3's in the gastropods. At 20 C motile L3 of *U. pallikuukensis* are present by days 12 PI and 15 PI in *D. laeve* and *D. reticulatum*, respectively (see Chapter 6). In the present studies, however, the L3 did not emerge from these IH until days 20-22 (*D. laeve*) or 18-20 PI (*D. reticulatum*). These data suggest that the L3 may need to reach a particular stage of development before emergence can occur. This suggestion is supported by the morphological and morphometric differences between early motile L3 in gastropods and those recovered 3 wk later from the gastropods or those that had emerged (see Chapter 6).

Differences in levels and patterns of emergence were observed among gastropod species. One hundred percent of the L3 had emerged from some *D. laeve* and *Catinella* sp. by day 60 PI (Figs. 5.5, 5.7, and 5.8). In contrast, although the highest overall mean proportion of emerged L3 occurred from the temperate species, *D. reticulatum*, none of these slugs lost all of their larvae by day 58 PI (Fig. 5.6). This difference may be related to the gastropod species (unlike *D. laeve* and *Catinella* sp., *D. reticulatum* is not an Arctic species (Pilsbry, 1948) and therefore is probably not a natural IH for *U. pallikuukensis*) or may be an artifact of the small number of gastropods in each experiment. The mean cumulative proportion of L3 emerging from *Catinella* sp. was considerably less than that from either of the slug species. Among the possible explanations for this difference are that many of these snails underwent short periods of aestivation during the study. Boev (1975) suggested that gastropod activity was necessary for larval emergence. Aestivating snails in this study would have had reduced activity relative to the slugs. In addition, larval development in *Catinella* sp. was not synchronous. At day 60 PI, when the snails were digested, some individuals contained L2 as well as L3. Solomon et al. (1996) demonstrated that development of larvae in aestivating snails (*Trochoidea seetzenii* and *Theba pisana*) was delayed compared to rates observed in active gastropods. Thus, aestivation may have been responsible for differential rates of larval development to L3 in some individual *Catinella* sp. and may have affected the rate of emergence.

Kralka and Samuel (1984a) suggested that larval emergence is a density dependent phenomenon resulting from unnaturally high infection intensities in the laboratory. Within the Protostrongylidae, naturally infected IH typically have low intensities of infection but occasionally higher intensities of infection occur (for example

75 and 97 larvae of *Parelaphostrongylus tenuis* have been reported in naturally infected *D. laeve* (Lankester and Anderson, 1968; Maze and Johnstone, 1986). Although we do not know what the natural intensities of *U. pallikuukensis* are in gastropods, the present laboratory experiments demonstrated larval emergence over a wide range of intensity of infection. Based on these experiments there is no evidence to suggest that the intensities of infection affected the cumulative proportion or rate of larval emergence from *D. laeve* and *D. reticulatum* (Figs. 5.5-5.7, 5.9). Insufficient data were available to determine the effect of intensity on emergence from *Catinella* sp. (Fig. 5.8).

Experiments comparable to the present study on the patterns of larval emergence from live IH of related Muelleriinae have not been reported. With *U. pallikuukensis* the rates of L3 emergence, the total numbers of larvae emerging and the proportions of gastropods from which L3 emerged, were high compared to *P. boughtoni* (Kralka and Samuel, 1984a), *P. stilesi* (Monson and Post, 1972), and *P. rufescens* (Boev, 1975), all members of the Protostrongylinae. Emergence of L3 of *A. cantonensis* and *A. costaricensis*, however, appears to be more common than among the Protostrongylinae (Heyneman and Lim, 1967; Ubelaker et al., 1980). Emergence of L3 among other Protostrongylidae has not been investigated in any detail.

5.5.2 Larval emergence in the field

Live L3 were recovered from the vegetation of 3 experiments established in 1997 both in the fall of that year and in the spring of 1998 after overwintering on the tundra. It was not possible, however, to determine whether the L3 had emerged from live or dead slugs because not all slugs originally put into the enclosures were recovered at the time of sampling, suggesting some gastropod mortality. Based on the high proportion of L3 which emerged from live gastropods in the laboratory, it is probable that at least some of the larvae emerged from live slugs. Additionally, it was not possible to determine if L3 found on the vegetation in 1998 had overwintered on the vegetation or had emerged more recently. The absence of L3 emergence in experiments 4 and 5 may have been because these larvae had only recently developed to L3 (second stage larvae were recovered from some slugs on digestion) and were not sufficiently mature to emerge.

The number of L3 that emerged from the gastropods in the field may have been underestimated by our sampling techniques. Firstly, only the vegetation was examined, not the sides of the enclosures or the soil within the enclosures. Slugs were frequently observed on the sides of the enclosures and occasionally on the soil and it is possible

that L3 emerged on to these surfaces. Secondly, the detergent technique seemed to improve larval recovery and it is likely, therefore, that not all L3 were recovered from the vegetation on 28 August 1997 when plain water was used. Detailed experiments, including refined sampling techniques, are required to describe the patterns of larval emergence in the field and factors influencing emergence.

5.5.3 Mechanisms for emergence

There are at least 2 different mechanisms for emergence of *U. pallikuukensis* from *D. laeve*: vigorous movements of the L3; and expulsion of L3 with mucus. These 2 mechanisms may be associated with the presence or absence of the second-stage cuticular sheath. Although larvae emerging early in the experiments were often ensheathed within the second-stage cuticle those emerging later were not. Protostrongylid L3 may have increased motility once released from the second-stage cuticular sheath (Gerichter, 1948; Rose, 1957; Beresford-Jones, 1966) and this may facilitate active larval emergence. Based on available literature there are differences among the Protostrongylids on whether they emerge with or without the second-stage cuticular sheath. *Muellerius capillaris*, *C. ocreatus* and *P. pulmonalis* emerge without their second-stage cuticular sheath (Boev, 1975), while *Protostrongylus boughtoni*, *P. tauricus*, *P. davtyani* and *P. stilesi* retain at least the second-stage cuticular sheath until after they emerge (Monson and Post, 1972; Boev, 1975). Kralka and Samuel (1984a) observed that L3 of *P. boughtoni* "did not clearly have sufficient mobility to actively crawl out of the feet encumbered by the extraneous cuticles" and proposed that emergence of the L3 may be a passive phenomenon associated with contractions of the gastropod's foot muscle, or an active expulsion caused by an immune response by the IH. Emergence of L3 of other *Protostrongylus* spp. may be brought about by similar mechanisms as *P. boughtoni*. The mechanisms of L3 emergence, including the presence or absence of cuticular sheaths when emerging, may be phylogenetically linked traits. Controlled experiments are required to test this hypothesis within the context of recent phylogenetic studies for the Protostrongylidae (Carreno and Hoberg, 1999).

In the present study we were not able to identify the factors that initiate and influence larval emergence. Emergence of L3's among metastrongyloids, including angiostrongylids and protostrongylids, has been observed after damage to the gastropod tissues. Cheng and Alicata (1964) found that the number of L3 of *A. cantonensis* that emerge from terrestrial snails increased when the snails were injured. Rose (1957) reported L3 emergence from damaged foot tissues after killing specimens of *D.*

reticulatum by cutting off the foot. Third-stage larvae of *P. rufescens* and *C. ocreatus* emerged during periods of high moisture (ie. during the rainy season) (Boev, 1975). Other factors influencing larval emergence have not been studied.

5.5.4 Survival of L3 in the environment

Third-stage larvae of *U. pallikuukensis* survived for up to 13 mo in tap or distilled water in the refrigerator. Although the coiled larvae were not active they were in good condition with no signs of degeneration. Without confirmed motility it cannot be determined whether they were alive, nor is it possible to assess their infectivity. It is known, however, that emerged L3 kept in tap water at 4 C for up to 18 days were infective to a muskox (Kutz et al., 1999b). Survival of L3 in cold water and subsequent infectivity is of significance because the potential terrestrial IH in the Arctic (*D. laeve* and *Catinella* sp.) live in moist habitats, often in mesic or wet sedge meadows, lakeshores or riverbanks (see Chapter 8). Rollo and Shibata (1991) reported that *D. laeve* is commonly found hatching and thriving in wet habitats and submerged and active in water during spring floods. In addition, *U. pallikuukensis* can develop in another arctic species, the freshwater snail *Aplexa hypnorum* (see Chapter 8). Third-stage larvae, therefore, could emerge from either of these species into the water. Larval emergence and subsequent survival in water, perhaps facilitated by the ability to feed when the second cuticular sheath is absent, strongly suggest that L3 in the environment may be a source of infection for muskoxen. Larvae emerging on to dry surfaces may use a different strategy for survival. Those found on dry surfaces were coiled tightly and in a thick mucous coating. This coating may provide protection against desiccation and extend the survival of the larvae in the natural environment.

Survival and mobility of free-living L3 of other species of protostrongylids have been only superficially investigated. Third-stage larvae of *M. capillaris* were resistant to desiccation but not to sunlight (Boev, 1975). Larvae of *M. capillaris* which had emerged from dead slugs did not survive longer than 17 days when maintained with the dead slugs (Rose, 1957). *Cystocaulus ocreatus* survived drying for 1 mo as well as repeated cycles of freezing and thawing associated with overwintering (Boev, 1975). Infective L3 of *Elaphostrongylus rangiferi* were highly resistant to the environment and could survive in water for 35 days as well as for several days after the death of the IH (Kontrimavichus et al., 1976) and L3 of *A. cantonensis* survived in water for up to 7 days (Richards and Merritt, 1967). Horizontal mobility of emerged L3, but no vertical mobility, was reported for *E. rangiferi* (Kontrimavichus et al., 1976). The survival and

mobility of emerged *U. pallikuukensis* should be further investigated to better understand the significance of this phenomenon.

5.5.5 Larval emergence and parasite transmission

Transmission of *U. pallikuukensis* depends on successful infection of a suitable gastropod IH with L1, development to infective L3 and subsequent ingestion by a muskox DH. This requires temporal and spatial overlap of L1 with gastropods and, according to the current understanding of protostrongylid life cycles, of gastropods containing infective L3 with the DH (Anderson, 1992). In the Arctic such transmission of *U. pallikuukensis* and other nematodes to the DH may be limited to seasonally defined periods (see, for example, Hoberg et al., 1999). The arctic summers are short and gastropods are active only from spring melt (usually the beginning of June) to early September. They then hibernate, probably sheltered in crevices or under stones (Dainton, 1989), and are unlikely to be ingested by muskoxen until they emerge from this hibernation. Emergence of larvae from gastropods may be a life history trait which increases the potential for transmission of the parasite to the definitive host on both a temporal and a spatial scale (Kutz et al., 1999b). Emerged L3 that survive ambient conditions on the vegetation may be available when the gastropods are not active or after they have died. In addition, emerged L3 may be more widely distributed in the environment than are the gastropods. This enhanced lifespan and dispersal of emerged L3 may reduce the necessity for direct gastropod-muskox contact.

Spatial overlap of muskoxen with high densities of gastropods may be limited by seasonal changes in the range and habitat use of muskoxen. For example, muskoxen in the region west of Kugluktuk migrate between winter range in the Rae-Richardson river valleys and summer range further north toward the arctic coast (Gunn and Fournier, 1999). These seasonal migrations may be significant in parasite transmission. Gastropod densities may be greatest in muskox wintering areas and yet availability of the gastropods to muskoxen in these areas would be restricted in the winter. If, however, L3 emerge and survive ambient conditions on the vegetation, they may be available during the winter when gastropods are hibernating but muskoxen are present. This could reduce or eliminate the necessity for spatial and temporal overlap of muskoxen and infected gastropods. Seasonal dietary shifts (and thus changes in habitat use) have been reported for muskoxen (O'Brian, 1988; Gunn and Sutherland, 1997) and may also influence their exposure to infected gastropods.

In areas where muskox habitat use overlaps with high densities of gastropods during the summer, L3 emergence could also increase the spatial and temporal distribution of the infective L3. For example, if L3 emerge over an extended time period (larval emergence was observed from day 18 to day 60 PI in the present study) they could emerge on to many different areas of the vegetation during normal summer gastropod activity. In temperate climates gastropods exhibit circadian activity patterns influenced by temperature, humidity and wind (Dainton, 1989; Kaufmann, 1990; Rollo and Shibata, 1991). Assuming gastropod activity is similarly influenced in the Arctic, they are probably not available to the muskoxen at all times during the day. Larval emergence, however, would provide multiple foci of infection, on vegetation or in the water, which would be available to muskoxen 24 hr a day, greatly increasing the probability for transmission compared to that dependent on ingestion of a single infected gastropod. This strategy may be particularly important in parasite transmission from heavily infected gastropods.

Larval emergence may also increase L3 availability after the death of the gastropod. It is possible, as has been proposed for *E. rangiferi* in Norway (Halvorsen and Skorping, 1982), that in some years, larvae of *U. pallikuukensis* may require 2 summer seasons to develop to L3. Nothing is known about the life history of *D. laeve* in the Arctic. It is known, however, that near Guelph, Canada, a more temperate region, large slugs (those which have overwintered) disappear from the population in August and September. If *D. laeve* has a life cycle in the Arctic similar to that in more temperate regions, and if, in some years, *U. pallikuukensis* requires two summers for development to L3, it would be the large slugs, infected the previous year, which would contain L3 by the following mid-summer. Without larval emergence all infective L3 within these large slugs would be lost in the second summer if the slugs died. If, on the other hand, larvae emerged on to vegetation or into the water before the death of the gastropod they may remain available for transmission. Observations in the present study and observations of L3 emergence from damaged gastropods by other authors (Rose, 1957; Cheng and Alicata, 1964) suggest that larval emergence may increase in unhealthy gastropods. Increased larval emergence with impending gastropod death (tissue degeneration) may be a strategy used by the parasite to increase its chances of transmission. If overwinter gastropod mortality is high, larval emergence from gastropods before they enter hibernation may also serve as a mode of maintaining L3 in the environment after the death of the gastropod.

In conclusion, based on laboratory and field data, emergence of L3 from the gastropod IH appears to be an inherent trait of *U. pallikuukensis*. This alternate

pathway for transmission may be of importance in the natural host-parasite system by increasing larval availability spatially and temporally, perhaps eliminating the narrow and seasonally defined windows of transmission. Recognition that emergence of L3's for *U. pallikuukensis* and perhaps other protostrongylids may be a natural and epidemiologically significant phenomenon serves to broaden our current understanding of transmission dynamics and life-history patterns for lungworms (e.g. Anderson, 1992). To further understand this phenomenon it is important to determine the extent of L3 emergence in the field, the survival of emergent larvae under natural conditions, the factors affecting emergence, as well as the life history and behavioral patterns of the gastropod intermediate hosts and how these relate to muskox life history strategies.

6. DEVELOPMENT OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS* WITHIN GASTROPODS: MORPHOLOGY, MORPHOMETRICS, AND THE INFLUENCE OF TEMPERATURE

6.1 Abstract

The morphological and morphometric aspects of larval development of *U. pallikuukensis* in *Deroceras laeve* were investigated under laboratory conditions. Larval stages were best defined by the separation of the cuticular sheaths, tail structure, and viability following digestion. There was some growth in body and esophagus width during the first-stage within the intermediate host, but the majority of the growth in body length and width occurred immediately following the second molt. Larval development in *D. laeve* and *D. reticulatum* occurred more rapidly at warmer temperatures. The calculated threshold temperatures were 8.5 C and 9.5 C in *D. laeve* and *D. reticulatum*, respectively, and 167 degree-days were required for development to third-stage larvae in both intermediate hosts. These thresholds are high compared to that based on published data for the closely related *Muellerius capillaris* (4.2 C), but similar to those for the more distantly related northern protostrongylid, *Elaphostrongylus rangiferi* (8.3 -10.3 C). Conversely, degree-days required for development to infective L3 were more similar among the Muelleriinae than between this group and the Elaphostrongyliinae. Development parameters for protostrongylid larvae may be influenced both by the environment and by features of the parasites and the intermediate hosts, including phylogeny.

6.2 Introduction

Umingmakstrongylus pallikuukensis is a protostrongylid lungworm of muskoxen first described by Hoberg et al. (1995) and found in muskoxen in the region of the Rae and Richardson River water sheds to the west of Kugluktuk, Nunavut, Canada (67°49'N, 115°08'W) (Gunn and Wobeser, 1993). Adult parasites live in cysts

5 to 40 mm in diameter in the lung parenchyma (Hoberg et al., 1995) and up to 258 of these cysts have been found in a naturally infected muskox (Gunn and Wobeser, 1993). Like other protostrongylids, *U. pallikuukensis* requires a gastropod intermediate host for development from the first (L1) to the third (L3) larval stage (Hoberg et al., 1995; Kutz et al., 1999b). The slug species *Deroceras reticulatum* and *Deroceras laeve* are both suitable laboratory intermediate hosts for this parasite (Kutz et al., 2000b).

Development of protostrongylid larvae in gastropods is influenced by a variety of factors including temperature, humidity, intensity of infection and the species, age, and physiological condition of the gastropod (Gerichter, 1948; Rose, 1957; Skorping, 1984; Samson and Holmes, 1985a; Cabaret, 1987; Solomon et al., 1996). Temperature has a critical influence with larval development rates increasing with temperature, up to a maximum temperature (Rose, 1957; Halvorsen and Skorping, 1982). It is generally accepted that there is a lower temperature threshold for protostrongylid larvae in intermediate hosts, below which development is minimal (Gerichter, 1948; Halvorsen and Skorping, 1982). These thresholds may differ among protostrongylid and intermediate host species (Gerichter, 1948; Halvorsen and Skorping, 1982), and some larval development may occur below the calculated threshold temperatures (Samson and Holmes, 1985b).

Based on prevalence and intensity data for the affected muskox population (Gunn and Wobeser, 1993), *U. pallikuukensis* appears to be a very successful parasite in the perhaps harsh arctic environment. The apparent success of this parasite raises a number of questions about transmission of *U. pallikuukensis* in the Arctic, including factors affecting larval development in the intermediate hosts.

The objectives of the present study were to: (1) describe the morphological and morphometric changes as L1 of *U. pallikuukensis* develop to L3 in *D. laeve*; (2) determine the effect of temperature on the rate of larval development in both *D. laeve* and *D. reticulatum*; and (3) determine the threshold temperature(s) and degree-days required for development to L3 in both gastropod species. It was hypothesized that parameters associated with development of *U. pallikuukensis* (growth rate, temperature thresholds, degree-days (DD)) would be driven primarily by extrinsic environmental factors and secondarily by intrinsic phylogenetic constraints on the parasite.

6.3 Materials and Methods

In total 12 experiments were conducted to study larval development at temperatures ranging from 8.5 C to 25.0 C (Table 6.1). All experiments were used

Table 6.1: Experimental infections of *Deroceras laeve* and *Deroceras reticulatum* with *Umingmakstrongylus pallikuukensis*.

Expt. no.	Temp (C)	Gastropod species	L1/ slug	Slug weight (mg) mean \pm 1 SD	Total no. of slugs	Days post-infection slugs examined	No. of slugs examined each day	No. of larvae examined/slug	Median intensity (range)
1	10	<i>D. reticulatum</i>	225	—	15	15,25,47,49,63, 66,81	1 - 3	up to 5 [†]	<10
2	12	<i>D. reticulatum</i>	225	—	5	31,47,66,69	1 - 2	all	5 (1-21)
3	15	<i>D. reticulatum</i>	1000	780 \pm 180	35	every 3 days from 4-28, then 32,35, 37	3	up to 5	<10
4	20	<i>D. reticulatum</i>	1000	800 \pm 210	21	4, eod [*] from 11-21	3	up to 5	<10
5	20	<i>D. reticulatum</i>	1000	770 \pm 190	9	7,11,12	2 - 3	up to 5	<10
6	25	<i>D. reticulatum</i>	1000	710 \pm 160	16	eod from 3-11, then 12	2 - 3	up to 5	<10

* Every other day.

† Some slugs contained fewer than 5 larvae.

Table 6.1 (continued): Experimental infections of *Deroceras laeve* and *Deroceras reticulatum* with *Umingmakstrongylus pallikuukensis*.

Expt. no.	Temp. (C)	Gastropod species	I./I/ slug	Slug weight (mg) mean \pm 1 SD	Total no. of slugs	Days post-infection slugs examined	No. of slugs examined each day	No. of larvae examined/slug	Median intensity (range)
7	8.5	<i>D. laeve</i>	225	114 \pm 39	35	every 7 days from 49 - 90	3	all	6 (0-18)
8	11.5	<i>D. laeve</i>	225	138 \pm 41	35	every 7 days from 49 - 77	3, 5	all	9 (3-21)
9	11.5	<i>D. laeve</i>	225	107 \pm 35	24	25, 35, 42, 49	3	all	8 (2-25)
10	15	<i>D. laeve</i>	225	40 \pm 20	17	8,10,12,18,21,24	3	all	6 (2-29)
11	20	<i>D. laeve</i>	225	40 \pm 20	26	cod from 4-12, then 13, 14,16	3	all	5 (1-18)
12	23.4	<i>D. laeve</i>	300	180 \pm 70	40	daily from 3-13, then 15,36,38	3	up to 5	23 (3-72)

to assess the effect of temperature on larval development and the pattern of transition over time from 1 larval stage to the next. Experiment 12 was also used as the basis for morphological and morphometric descriptions of the larval stages.

6.3.1 Sources of larvae and gastropods

First-stage larvae were obtained from the feces of a muskox which had been experimentally infected with *U. pallikuukensis* (Kutz et al., 1999b). Feces had been frozen for up to 20 mo at $-13\text{ C} \pm 2\text{ C}$. Larvae for gastropod infections were isolated from feces using a funnel (Kutz et al., 1999b) or a beaker Baermann technique (Forrester and Lankester, 1997) and concentrated to 2-4 ml in tap water.

Specimens of *D. reticulatum*, a slug of temperate climates, were obtained from captive-bred protostrongylid-free colonies originating from the Center for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Canada, and from a compost box in Ottawa, Canada. These slugs survived and reproduced well in captivity. They were used for experiments 1-6. Specimens of *D. laeve*, the only slug species native to the North American Arctic (Pilsbry, 1946, 1948), were from 2 sources. Wild *D. laeve*, collected from an area along the Coppermine River near the townsite of Kugluktuk, Nunavut, were used for experiments 10 and 11. This collection area was not used by muskoxen and sightings of caribou were infrequent. Because it was difficult to maintain breeding colonies of the wild-caught specimens, a laboratory colony of *D. laeve* which originated from a Saskatoon greenhouse, and which survived and reproduced well in captivity, was used for experiments 7-9 and 12.

6.3.2 Infection of gastropods

Prior to experimental infections the feet of the wild-caught *D. laeve* were examined for lesions indicative of pre-existing protostrongylid infections (Kutz et al., 2000b). During 2 years of related field studies no lesions were seen in slugs collected from the Coppermine River site and more than 100 *D. laeve* from this site were digested and no larvae were recovered.

Slugs were infected with L1 of *U. pallikuukensis* as described by Hoberg et al. (1995). The numbers of L1 to which the slugs were exposed for each experiment are given in Table 6.1. In preliminary experiments a greater proportion of larvae established in *D. laeve* than in *D. reticulatum* and, therefore, lower numbers of L1 were used to infect the *D. laeve*. Also, to minimize potential slug mortality, relatively low numbers of L1 were used in the long duration, low temperature experiments (10 C and 12 C) with

D. reticulatum. After infection, all slugs were maintained in Rubbermaid® containers, fed once weekly (experiments at 15-25 C) or every 2 wk (experiments at <15 C) (Kutz et al., 1999a) and kept in temperature controlled refrigerated incubators in the dark.

6.3.3 Digestion of gastropods and examination of larvae

Subsets of slugs were examined for larvae at various days post-infection (DPI). The sampling schedules were determined based on the temperature (see Table 6.1). Slugs were weighed and then digested in an artificial pepsin/hydrochloric acid solution (Hoberg et al., 1995). Larvae recovered were heat-killed on glass slides and examined at 400X. They were identified as L1, second-stage larvae (L2), early L3 (aL3), intermediate L3 (bL3), late L3 (cL3) or emerged L3 (eL3) on the basis of morphological characteristics (described below), the age of infection (DPI), and behaviour. The larvae were preserved in 70% ethanol/ 5% glycerine.

In experiment 12, a subset of 15 live L1 were removed from the petri dishes used for slug infection, heat killed on glass slides and measured at 400X using an ocular micrometer. Fifteen larvae were examined and measured on each sampling day thereafter with the exception of the emerged L3, of which only 9 were recovered.

6.3.4 Data analysis

The computer program Statview SE™ SE + Graphics (1988 Abacus Concepts Inc., San Francisco, CA) was used for data analysis. A one-way analysis of variance was used to compare morphometrics of larvae at different stages of development. A regression analysis of temperature on larval development rates (1/days to intermediate L3) was used to determine the theoretical threshold temperatures (T_0) and the degree-days required (K) for development of *U. pallikuukensis* to intermediate L3 in *D. laeve* and *D. reticulatum* ($y = a + bx$, $T_0 = -a/b$ and $K = 1/b$) (Campbell et al., 1974; Samson and Holmes, 1985). The time to the first intermediate L3 in any of the slugs examined on a given day was used as the end point to determine development rates. This stage of development was felt to be the most comparable to that defined by Rose (1957), Halvorsen and Skorping (1982) and Samson and Holmes (1985) as “infective L3”. To compare the T_0 and K values of related protostrongylids, regression analyses were performed using data for the development of *Muellerius capillaris* to “infective L3” in *D. reticulatum* or *D. agrestis* (from Table I in Rose, 1957) and for the development of *E. rangiferi* to “infective L3” in *Arianta arbustorum* and *Euconulus fulvus*. (Figures 1 and 2 in Halvorsen and Skorping, 1982).

6.4 Results

6.4.1 Larval development: morphology

Morphological characteristics of L1 and L3 of *U. pallikuukensis* have been described in detail (Hoberg et al., 1995), and the following descriptions of these stages are limited to observations that facilitate their differentiation from L2. Within the continuum of larval development in *D. laeve* at 23 C (experiment 12), on the bases of morphology, age of infection, and behavior, 6 categories of larvae were identified: L1 on 0, 3, and 4 DPI; L2 on 4-9 DPI; early L3 on 9-11 DPI; intermediate L3 on 12, 13 and 15 DPI; late L3 on 36 and 38 DPI; and emerged L3 from 22-38 DPI. The morphological characteristics useful for defining the stages of development are summarized in Table 6.2.

First-stage larvae (0 DPI). Larvae consistent with those described by Hoberg et al. (1995) with limited variability among larvae.

First-stage larvae (3 DPI): Cuticle and cuticular sheaths: homogenous refractile granules, numerous, small, uniformly ovoid, situated immediately beneath cuticle in single line extending from base of esophageal-intestinal junction (EIJ) to near anus (Fig. 6.1); no separation of the first cuticle. Intestine: prominent walls, large round nuclei present, lumen variable size. Genital primordium (GP): 2 cells. Digestion: larvae motile following digestion (and considered to be alive); assume "C" to comma shape when heat killed. General: anus and excretory pores swollen, prominent.

First to second stage transition (4 DPI): L2. Cuticle and cuticular sheaths: homogenous refractile granules present under first cuticular sheath (CS1); CS1 usually separated at tail and separated or thickened at cephalic extremity (Fig. 6.2). Intestine: lumen usually wide; intestinal cells with large round nuclei. GP: 2 cells. Tail: short ventral point, consistent with first section of L1 tail, visibly separating from CS1. Digestion: larvae nonmotile following digestion (and considered to be dead); assume comma form. General: both L1 and L2 present (8 L1, 7 L2), L1 as described at 3 DPI.

Second-stage larvae (5-9 DPI): Cuticle and cuticular sheaths: separation of CS1 complete, cuticle remaining intact as sheath; cuticular lining of esophagus, excretory duct, rectum molted (Fig. 6.3); homogenous refractile granules absent under CS1 by 5 DPI but increase in number under cuticle of L2 from 6-9 DPI. Intestine: cells very

Table 6.2: Morphological characteristics of developing larvae of *Umingmakstrongylus pallikuukensis*.

Character ¹	L1 [†]	L1-L2	L2	L2-L3	aL3	bL3	c/eL3
Live [‡]	+	-	-	-	-	+	+
Visibility/condition of characters [§]	**/**	*	•	*	**	***	***
CS1 separated [‡]	-	+	+	+	+	+	+
CS2 separated [‡]	na [¶]	na	-	+	+	+	+
Fine granules underlying cuticle [‡]	-	+	-	+	na	na	na
CS1 thickened at cephalic end [‡]	-	+	-	na	na	na	na
Anal pore and excretory pore bulge [‡]	-	+	-	-	-	-	-
GP number of cells visible	2	2	2-4	2-4	6-8	6-8	6-8
Intestine dark/granular [‡]	-	-	-	+	+	+/-	-
Larval form (heat killed/digested) [¶]	a, c	a	a, b	a, b, c	a, c	c	c

[‡]CS1 - first cuticular sheath, CS2 - second cuticular sheath, GP - genital primordium.

[†]L1 - first-stage larvae (in slugs), L1-L2 - transition from L1 to L2, L2 - second-stage larvae, L2-L3 - transition from L2 to aL3, aL3 - early third-stage larvae, bL3 - intermediate L3, c/eL3 - late and emerged L3.

[‡]+ yes, - no, na - not applicable.

[§]* - poor; ** - good, *** - excellent.

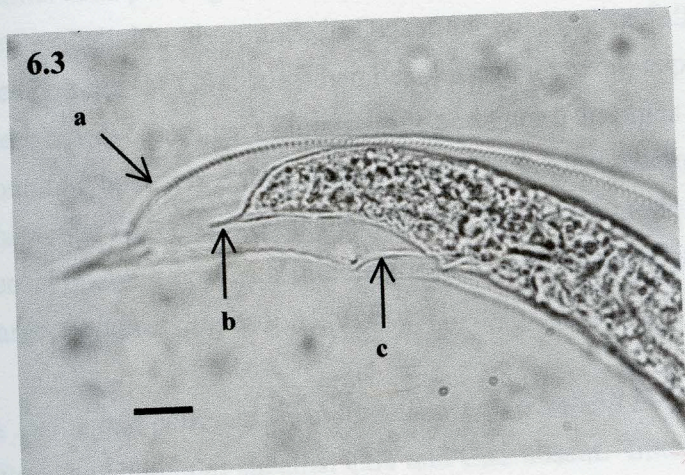
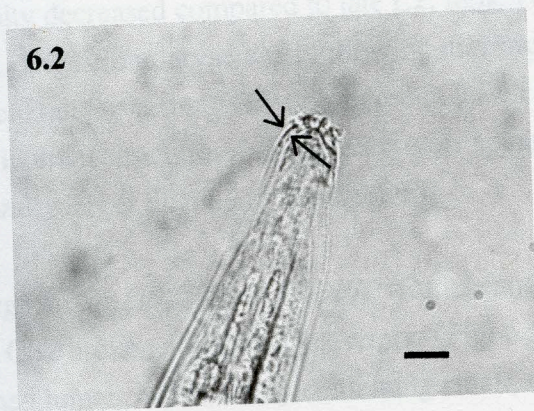
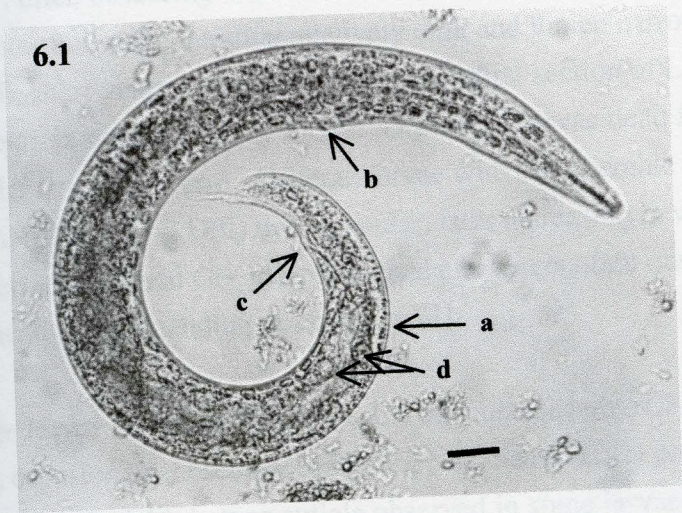
[¶]a - comma form, b - circle form, c - "c" shape.

Figures 6.1-6.3. Larvae of *Umingmakstrongylus pallikuukensis* from *Deroceras laeve* at various days post-infection (DPI).

Figure 6.1. First-stage larva at 3 DPI. Small refractile granules are visible under the cuticle (a). Excretory pore (b) and anus (c) are prominent. Nuclei of intestinal cells (d). (Bar = 20 μ m).

Figure 6.2. First to second-stage larval transition at 4 DPI. The cuticle is thickened at the cephalic extremity (arrows). (Bar = 20 μ m).

Figure 6.3. Second-stage larva at 5 DPI. The first cuticular sheath (CS1) (a) is completely separated from the larva. The tail has a short, ventral point (b). The lining of the rectum has molted (c) (Bar = 20 μ m).



granular from EIJ to GP, often obscuring cellular structure; large round nuclei occasionally visible in intestinal cells; intestine relatively dark and lumen narrow at 6-9 DPI. GP: 2-4 cells. Tail: short ventral point, consistent with first section of L1 tail (Fig. 6.4); point disappears in some larvae by 8 DPI. Digestion: larvae dead following digestion; assume circular or comma form. General: larvae with considerable morphological variation, initially (5-7 DPI) thin, granular, later increasing in width and often a marked bulge between EIJ and GP; morphological characters often obscure; excretory gland prominent, often extending posterior to EIJ.

Early third-stage larvae (9-11 DPI): Cuticle and cuticular sheaths: CS1 often broken at cephalic end, occasionally absent; second cuticular sheath (CS2) present but separated (Fig. 6.4); distortion of third-stage cuticle observed in some larvae. Intestine: walls thick and dark, but density decreased compared to late L2; lumen narrow. GP: 4-6 cells. Tail: bluntly rounded. Digestion: larvae dead following digestion; assume a loose comma or "C" form. General: larvae at L3 first observed at 9 DPI (11 of 15 larvae examined); differentiation of internal structures and organ systems apparent, features more distinct and larvae generally larger than L2 at 8 and 9 DPI.

Intermediate third-stage larvae (12, 13 and 15 DPI): Cuticle and cuticular sheaths: CS1 usually absent; CS2 broken at cephalic extremity (Fig. 6.5); third cuticle thick. Intestine: clearly defined lumen, occasionally containing small, heterogenous, refractile granules; intestinal cells pale, agranular and clearly defined. GP: increased in size, composed of 4-8 cells. Tail: bluntly rounded. Digestion: larvae alive following digestion; assume relaxed comma to "C" shape when heat killed. General: cephalic and buccal structures well developed, 6 papillae of inner circle and lateral amphids prominent, visible surrounding buccal opening; buccal capsule containing cuticularized, stylet-like organ with thickened wall and prominent barb-like points in anterior, extending from near buccal opening posterior into insertion in upper esophagus (Fig. 6.6); excretory gland large, compressing esophagus dorsally.

Late third-stage larvae and emerged third-stage larvae (36 and 38 DPI or 22-38 DPI, respectively): Cuticle and cuticular sheaths: CS1 and CS2 absent. Intestine: fully differentiated, containing few small, heterogenous refractile granules in lumen. Digestion: larvae alive following digestion or emergence; assume "C" shape when heat killed. General: other characters of larvae obtained from digestion and those spontaneously emerged from slugs were consistent with intermediate L3 stages.

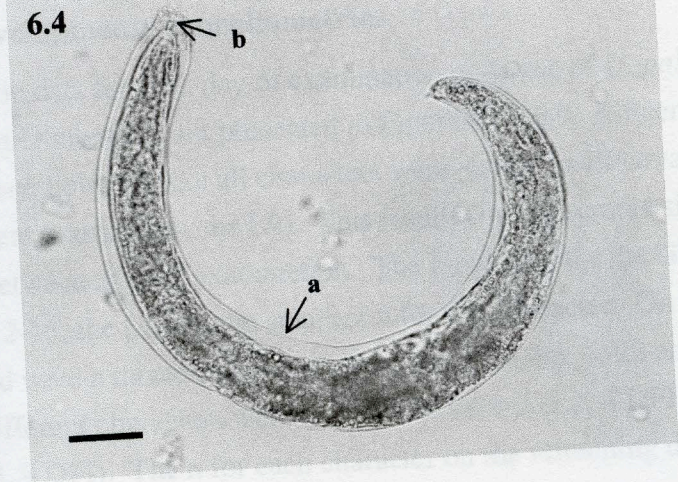
Figures 6.4-6.6. Larvae of *Umingmakstrongylus pallikuukensis* from *Deroceras laeve* at various days post-infection (DPI).

Figure 6.4. Early third-stage larva at 10 DPI. CS1 is absent, the second cuticular sheath (CS2) is present (a) but separated from the larva. The lining of the esophagus is being shed (b) (Bar = 50µm).

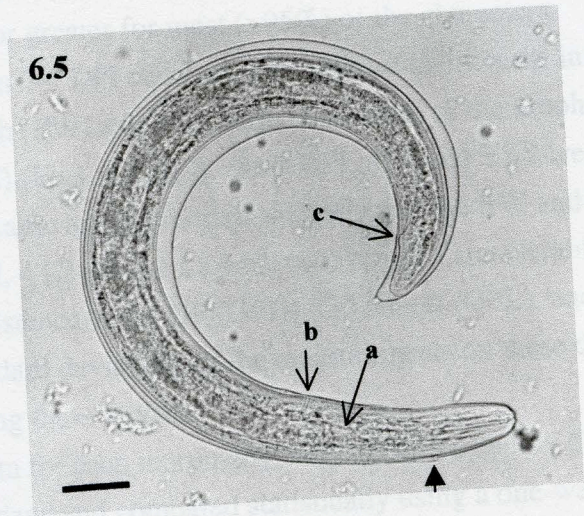
Figure 6.5. Intermediate third-stage larva (L3) at 12 DPI. The CS1 is absent and CS2 is broken at cephalic extremity (arrow). Features more distinct than in earlier larval stages (ie. nerve ring (a), excretory pore (b), and anus (c) are clearly visible). (Bar = 40 µm).

Figure 6.6. Late L3. Cuticularized stylet-like organ with barb-like points in buccal capsule (arrows pointing at either end of structure). (Bar = 10 µm).

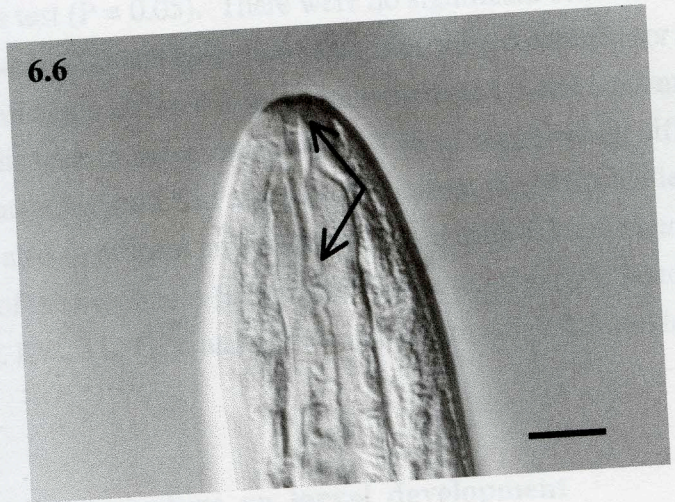
6.4



6.5



6.6



6.4.2 Larval development: morphometrics

Morphometric data for each day of examination of larvae of *U. pallikuukensis* in *D. laeve* at 23 C (experiment 12) are presented in Figures 6.7-6.9. Fifteen larvae were examined on each day, however, not all characters were visible in all larvae at some stages of development (particularly the L2). This resulted in the sample size (n) for each character varying between days of examination. The range of n for the GP measurements was 2-15; the L2 had the smallest n for this character. On 4 and 9 DPI, the larvae recovered were a mixed population (L1 and L2 on day 4, L2 and L3 on day 9) and the n for the different characters were 8 (L1, 4 DPI), 4-7 (L2, 4 DPI), 0-2 (L2, 9 DPI), and 6-11 (L3, 9 DPI). The n for each character on the remaining days ranged from 8 to 15, and was 10 or greater for most (>95%) of the characters.

Larval growth from 0-4 DPI consisted primarily of an increase in body width (BW) and esophagus width (EW) and a decrease in the ratio of the esophagus length to the total body length (%E) (Figs. 6.8, 6.9). At 4 DPI, 8 L1 and 7 L2 were recovered. From 4-9 DPI, the GP became located more posteriorly and the BW and %E were highly variable. At 9 DPI, 4 L2 and 11 L3 were recovered. Considerable changes in total body length (BL), distance to the GP (GPD), GP length (GPL), and %E occurred from 8-10 DPI. The standard deviations of the measurements on these days reflect the variability observed among these larvae.

Morphometric data for each morphologically defined category (Table 6.3; Figs. 6.10, 6.11) were summarized and compared statistically using a one-way analysis of variance and Scheffe's test ($P = 0.05$). There were no significant differences between the late L3 and the emerged L3 and these two categories were combined for further analysis. The L1 significantly differed from the intermediate L3 and late/emerged L3 for all characters except distance to nerve ring (NRD). The L2 significantly differed from intermediate L3 and late/emerged L3 in all characters except the NRD, tail length (TL) and %E. There were also significant differences between early L3 and intermediate L3 (distance to the base of the esophagus (ED) and EW), between early L3 and late/emerged L3 (ED, EW, TL, GPL and GPW), and intermediate L3 and late/emerged L3 (EW and GPL).

6.4.3 The effect of temperature on larval development

In both slug species, larval development of *U. pallikuukensis* occurred more rapidly at warmer temperatures (Figs. 6.12, 6.13). Figures 6.14 - 6.17 show the

Figures 6.7-6.9. Mean measurements of larvae of *Umingmastrongylus pallikuukensis* from *Deroceras laeve* at 23 C at various days post-infection (DPI). L1 - first-stage larvae, L2 - second-stage larvae, aL3 - early third-stage larvae (L3), bL3 - intermediate L3, cL3 - late L3, eL3 - emerged L3. The 2 sets of data for 4 DPI represent measurements from L1 and L2, and at 9 DPI the data represent measurements from L2 and aL3. All measurements in micrometers except for esophagus as a percent of total body length (% E). (Error bars = 1 SD).

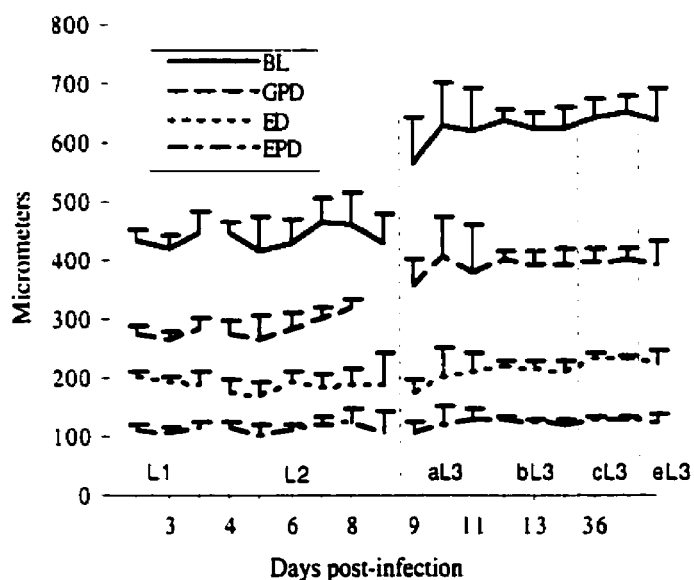


Figure 6.7. Total body length (BL)(not including separated cuticular sheaths) and distances from the cephalic extremity to the genital primordium (GPD), to the base of the esophagus (ED), and to the excretory pore (EPD).

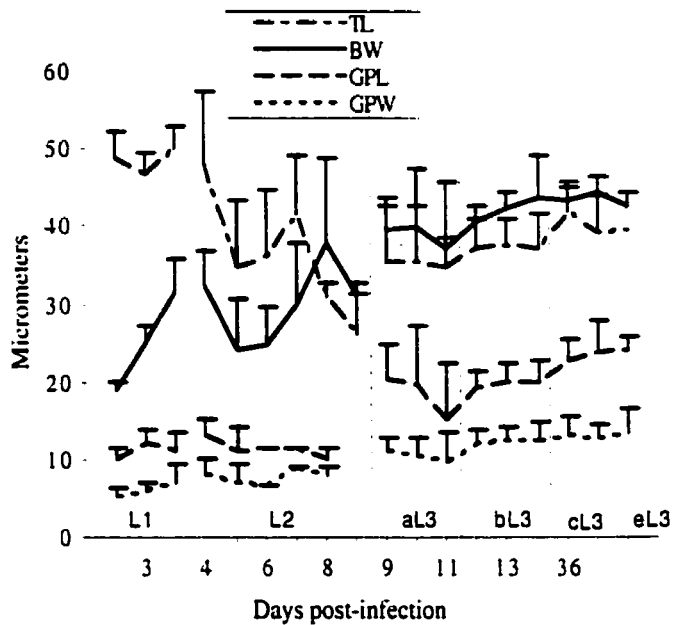


Figure 6.8. Tail length measured from the caudal extremity (TL), body width measured at the base of the esophagus (BW), GP length (GPL) and width (GPW).

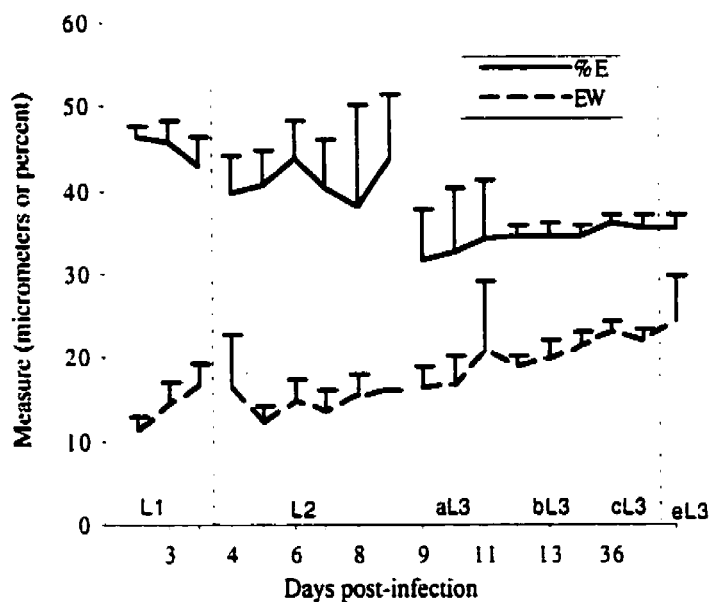


Figure 6.9. Esophagus as a percent of total body length (% E) and width of the esophagus at its base (EW).

Table 6.3: Measurements[†] of the different larval stages of *Umingmakstrongylus pallikuukensis*.

Character	This study					Hoberg et al. 1995
	L1	L2	aL3	bL3	c/eL3	cL3
Nerve ring [‡]	n=22	n=47	n=31	n=46	n=29	n=10
	109 ± 9	110 ± 19.7	101 ± 19	104 ± 6.6	107 ± 6.7	99 ± 4.2
	(83 - 120) [‡]	(69 - 164)	(69 - 157)	(90 - 118)	(83 - 118)	(93 - 106)
Excretory pore [‡]	n=23	n=57	n=38	n=46	n=29	n=10
	113 ± 10	117 ± 19.2	119 ± 23	127 ± 7.2	130 ± 7.9	118 ± 5
	(83 - 134)	(53-173)	(74 - 182)	(111 - 150)	(104 - 146)	(109 - 127)
Esophagus [‡]	n=23	n=57	n=38	n=46	n=29	n=10
	194 ± 15	184 ± 25.6	197 ± 31	219 ± 12	233 ± 13	200 ± 12
	(169 - 238)	(120 - 238)	(141 - 298)	(194 - 249)	(201 - 263)	(181 - 214)
Genital primordium [‡]	n=23	n=28	n=32	n=46	n=29	n=10
	270 ± 18	287 ± 32	380 ± 66	399 ± 20	402 ± 26	361 ± 23
	(247 - 326)	(178 - 342)	(182 - 547)	(344 - 469)	(316 - 432)	(318 - 388)
Body length	n=23	n=63	n=38	n=46	n=29	n=10
	431 ± 30	446 ± 50	612 ± 72	630 ± 29	648 ± 35	560 ± 34
	(400 - 508)	(291 - 552)	(448 - 769)	(547 - 723)	(545 - 691)	(514 - 600)
Tail [‡]	n=23	n=56	n=38	n=46	n=29	n=10
	48 ± 4	37 ± 9.6	35 ± 5.8	37 ± 3.7	40 ± 4.5	31 ± 2.9
	(42 - 55)	(14 - 55)	(23 - 44)	(32 - 49)	(32 - 49)	(26 - 34)

[†]In micrometers except % Esophagus which is esophageal length as percentage of the total body length.

[‡]Measurements are from the cephalic extremity.

[‡]Mean ±1SD, (range).

[‡]Anus to caudal extremity.

Table 6.3 (continued): Measurements^a of the different larval stages of *Umingmakstrongylus pallikuukensis*.

Character	This study					Hoberg et al. 1995
	L1	L2	aL3	bL3	c/eL3	cL3
Esophagus	n=23	n=41	n=35	n=46	n=29	n=10
width	15 ± 2.9 (12 - 23)	15 ± 3.4 (9.2 - 28)	17 ± 3.4 (12 - 23)	20 ± 2.2 (16 - 25)	23 ± 3.1 (18 - 35)	23 ± 3.3 (18 - 26)
Body	n=23	n=48	n=37	n=46	n=29	n=10
width	28 ± 4 (21 - 42)	30 ± 8.9 (16 - 58)	40 ± 6.2 (23 - 55)	43 ± 3.8 (37 - 58)	44 ± 1.8 (42 - 46)	47 ± 7 (39 - 60)
GP ^I	n=23	n=17	n=26	n=44	n=29	
length	12 ± 2 (9 - 14)	12 ± 2.5 (6.9 - 16)	19 ± 5.8 (12 - 35)	20 ± 2.4 (14 - 23)	24 ± 2.9 (16 - 30)	
GP ^I width	n=23	n=17	n=26	n=44	n=29	
	7 ± 2 (5 - 12)	8 ± 1.8 (4.6 - 12)	11 ± 1.9 (7 - 14)	13 ± 1.8 (9 - 18)	13 ± 2.3 (12 - 21)	
%	n=23	n=57	n=38	n=46	n=29	n=10
Esophagus	45 ± 3 (38 - 49)	41 ± 4.9 (33 - 55)	32 ± 5.8 (22 - 47)	35 ± 1.7 (31 - 39)	38 ± 1.4 (35 - 41)	(33 - 39)

^IGenital primordium.

Figures 6.10, 6.11. Mean measurements for morphometric characters of larvae of *Umingmakstrongylus pallikuukensis*. L1 - first-stage larvae, L2 - second-stage larvae, aL3 - early third-stage larvae (L3), bL3 - intermediate L3, cL3 - late L3, eL3 - emerged L3. All measurements are in micrometers except for % E. (Error bars = 1 SD).

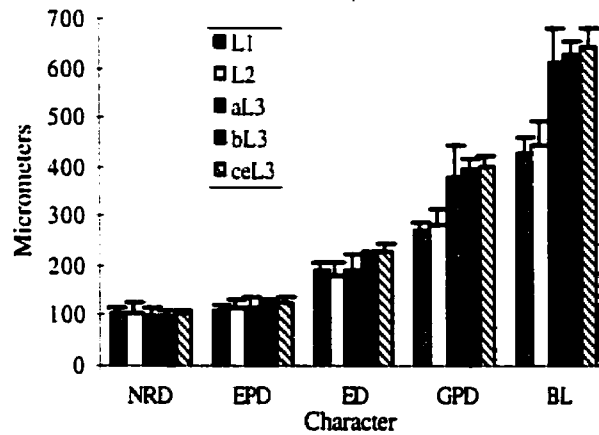


Figure 6.10. Distance from the cephalic extremity to the nerve ring (NRD), excretory pore (EPD), base of the esophagus (ED), and genital primordium (GPD); total body length (BL).

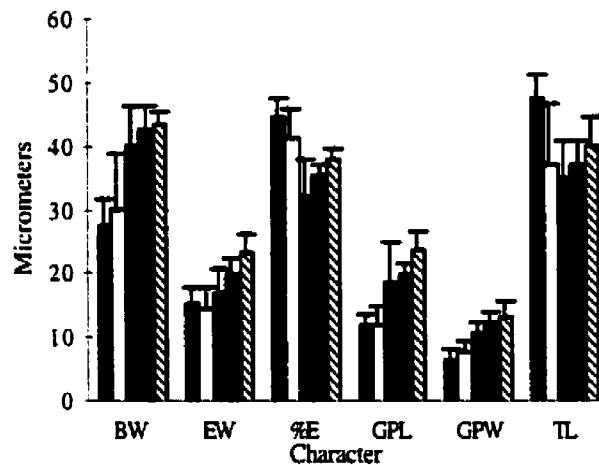


Figure 6.11. Width, measured at the base of the esophagus, of the body (BW) and the esophagus (EW); esophagus length as a percent of total body length (%E); genital primordium width (GPW) and length (GPL); tail length (TL).

proportion of the different larval stages recovered from individual *D. laeve* at different temperatures. A similar pattern was observed in *D. reticulatum* although the transition periods were longer.

Using data from the development of *U. pallikuukensis* to the first intermediate L3 in *D. laeve*, a regression analysis of temperature on development rate was calculated. Based on this regression, $y = -0.051 + 0.006x$, $r^2 = 0.97$, $P = 0.015$, $T_0 = 8.5$ C and $K = 167$ DD (Fig. 6.18). For development in *D. reticulatum* the regression equation was $y = -0.057 + 0.006x$, $r^2 = 0.99$, $P = 0.003$, $T_0 = 9.5$ C and $K = 167$ DD (Fig. 6.19). For development of *M. capillaris* in *D. reticulatum* or *D. agrestis* (data from Rose, 1957) the regression equation was $y = -0.025 + 0.006x$, $r^2 = 0.97$, $P = 0.003$, $T_0 = 4.2$ C and $K = 167$ DD (Fig. 6.20). The regression for development of *E. rangiferi* in *A. arbustorum* was $y = -0.041 + 0.004x$, $r^2 = 1$, $P = 0.002$, $T_0 = 10.25$ C and $K = 250$ DD (Fig. 6.21). For *E. rangiferi* in *E. fulvus* the regression equation was $y = -0.033 + 0.004x$, $r^2 = 1$, $P < 0.001$, $T_0 = 8.25$ C and $K = 250$ DD (Fig. 6.22). The data for *E. rangiferi* at 28 C in *E. fulvus* were not used because rate of development at this temperature did not differ from that at 24 C and was in the non-linear range of the curve (Range III as described by Li, 1998).

6.5 Discussion

6.5.1 Larval development: morphology and morphometrics

The characters most useful for identifying the stage of larval development of *U. pallikuukensis* in *D. laeve* were the separation of the cuticular sheaths, tail structure and viability of the larvae following pepsin/HCl digestion. The larval categories established on the basis of morphological characteristics and independent of morphometric data, corresponded with statistically significant morphometric differences.

Relative to L1 recovered from the petri dishes used for the experimental infections (those that had not penetrated into slugs), by 3-4 DPI the L1 in slugs were more granular and had increased slightly in body and esophageal width. The small, uniform refractile granules under the CS1 forecast an impending molt, which we defined as the first visual evidence of the separation of the CS1. The L2 had an overall granular appearance and were the most morphologically variable stage, particularly with respect to total length and body and esophagus width. These larvae were initially relatively long and narrow (4-6 DPI) then became short and wide (7-9 DPI); during this stage they seemed to have first distended the CS1 and then retracted from it. The greatest changes in the morphometric data, as well as the greatest standard deviations for the

Figures 6.12, 6.13. Number of days post-infection to the first second-stage larva (L2) and the first intermediate third-stage larva (L3) at different temperatures.

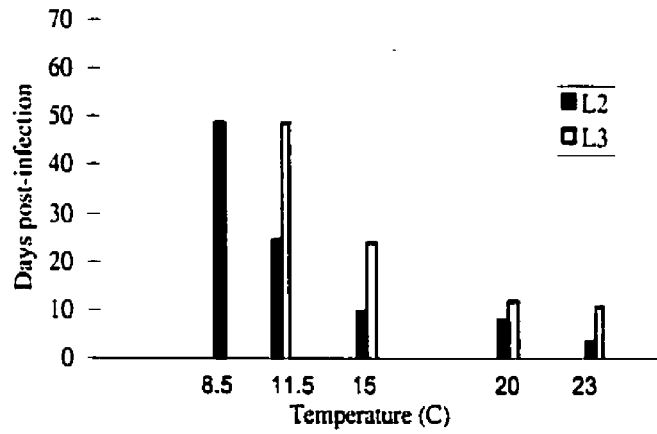


Figure 6.12. In *Deroceras laeve*.

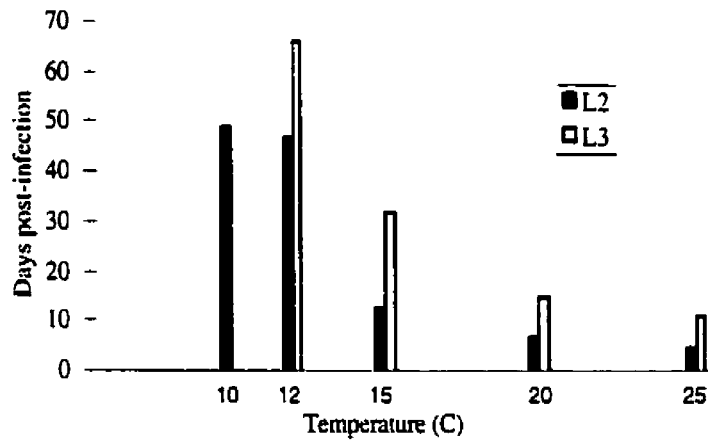


Figure 6.13. In *D. reticulatum*.

Figures 6.14-6.17. Distribution of the larval stages (as a percent of total larvae recovered) of *Umingmakstrongylus pallikuukensis* in *Deroceras laeve* at different temperatures. Each bar represents the larvae recovered from an individual slug. Numbers below the bars are the number of days post-infection. L1 - first-stage larvae, L2 - second-stage larvae, aL3 - early third-stage larvae (L3), bL3 - intermediate or late L3.

Figure 6.14. 23.4 C.

Figure 6.15. 20 C.

Figure 6.16. 15 C.

Figure 6.17. 11.5 C, days 25, 35, 42, and 49 are based on data from experiment 9, remaining days are based on data from experiment 8.

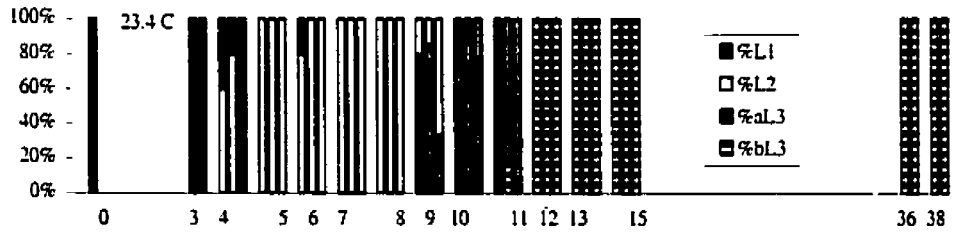


Figure 6.14

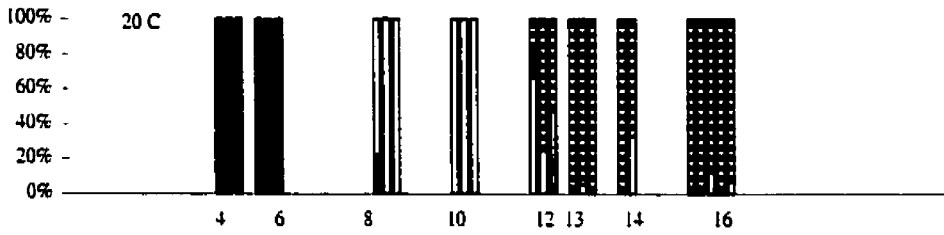


Figure 6.15

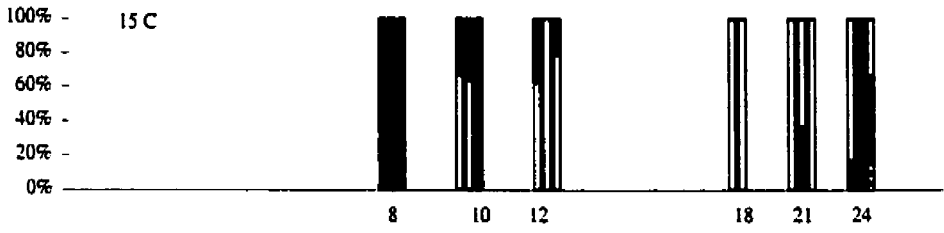


Figure 6.16

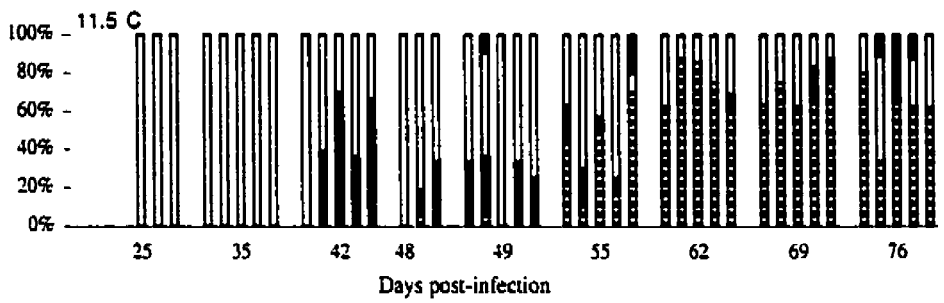


Figure 6.17

Figures 6.18, 6.19. Development rates of *Umingmakstrongylus pallikuukensis* from first to third-stage larvae relative to temperatures.

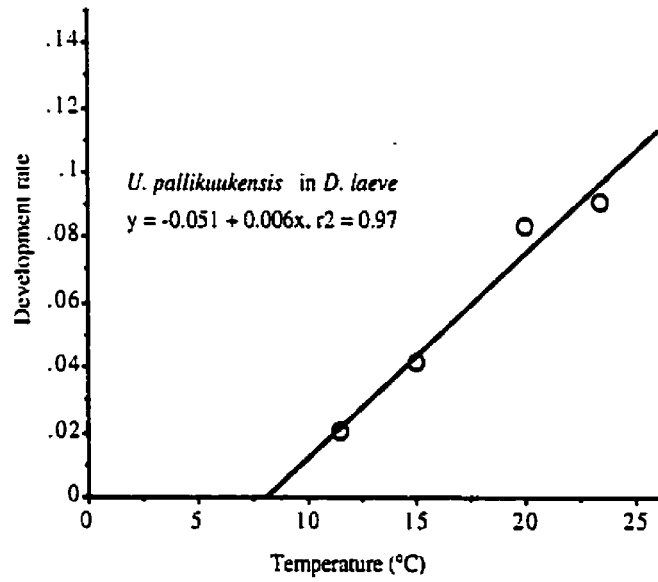


Figure 6.18. In *Deroceras laeve*.

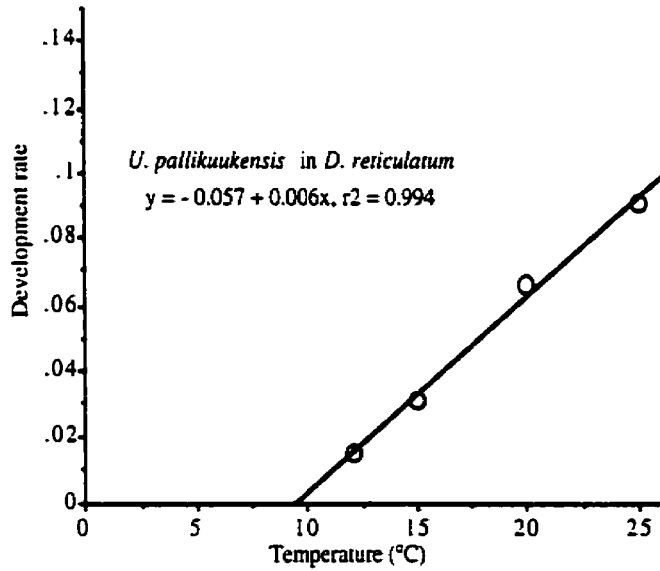


Figure 6.19. In *D. reticulatum*.

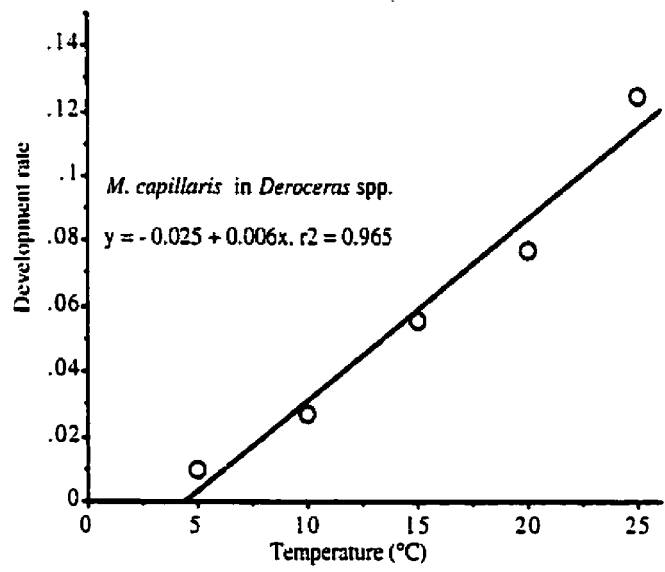


Figure 6.20. Development rates of *Muellerius capillaris* from first to third-stage larvae in *D. reticulatum* or *D. agrestis* (from Rose, 1957) relative to temperatures.

Figures 6.21, 6.22. Development rates of *Elaphostrongylus rangiferi* from first to third-stage larvae relative to temperatures (from Halvorsen and Skorping, 1982).

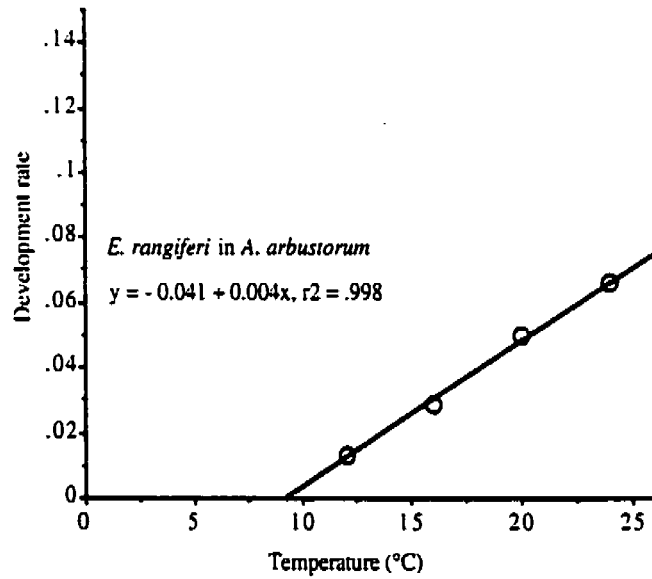


Figure 6.21. In *Arianta arbustorum*.

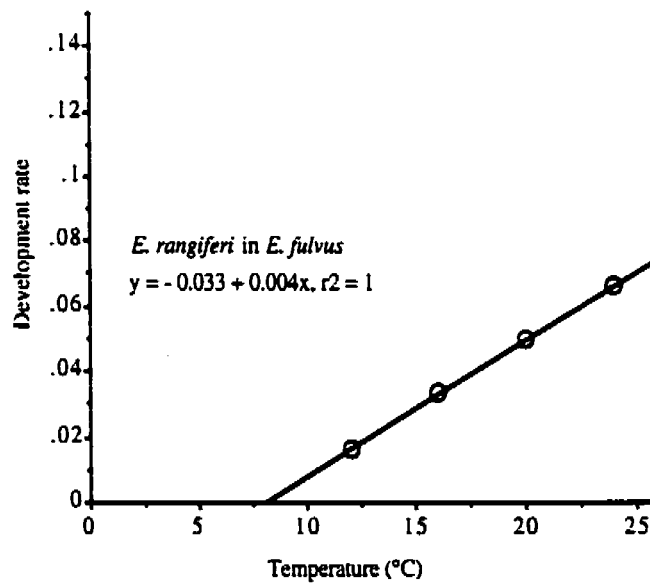


Figure 6.22. In *Euconulus fulvus*.

measurements, were observed from the late L2 through the early L3 stages (8-11 DPI). Although the digestion in all the experiments may have resulted in some distortion of the larvae, similar variability of the L2 stage was seen in preliminary studies where larvae were recovered by dissection from the slug tissue instead of digestion. We believe, therefore, that the observed variability is a real characteristic of the L2 and early L3 stages. Kralka and Samuel (1984b) also reported increasing variability of the L2 of *Protostrongylus boughtoni* as development progressed. Once larvae of *U. pallikuukensis* had become intermediate L3 and late/emerged L3, variability in the structure and morphometrics decreased. The small but statistically significant increase in esophagus width and GP length which occurred from intermediate L3 to late/emerged L3, suggests that the larvae continue to grow after reaching the intermediate L3 stage.

The morphologically defined early L3 in this study were consistent with what has been described for other protostrongylids as "pre-infective L3" (Gerichter, 1948; Rose, 1957; Halvorsen and Skorping, 1982). Rose (1957) concluded that the "pre-infective L3" of *M. capillaris*, which did not survive a trypsin or pepsin digest in the laboratory, would not survive digestion in the abomasum of sheep. Beresford-Jones (1966) reached a similar conclusion for the early L3 of *M. capillaris*.

The patterns of larval growth among the Protostrongylidae deserve attention. With *U. pallikuukensis* there was little growth during the L1 stage, no growth during the L2 stage, and considerable growth immediately following the second molt. This may be a uniform pattern among species of the Muelleriinae. Gerichter (1948), who defined the larval stages in a manner similar to the present study (the molting of the cuticular sheaths), reported considerable growth in the late L1 stage and little growth during the L2 stage for *Cystocaulus ocreatus* and *M. capillaris*. He also reported that the "pre-infective L3" were longer and thinner than the L2, suggesting that growth occurred during this phase of development. Beresford-Jones (1966) observed considerable growth of *M. capillaris* in the L1 stage, little growth of the L2, and then, following the second molt, a progressive increase in length. Third-stage larvae which had exsheathed in the intermediate host were on average longer than those which had not.

Considering the other sub-families of the Protostrongylidae within a comparative context, among the Elaphostrongyliinae, the putative sister group of the Muelleriinae (Carreno and Hoberg, 1999), the greatest growth of larvae of *E. rangiferi*, based on total length, occurred during the late L1 and in the L2 (Halvorsen and Skorping, 1982). In their study, however, the "pre-infective L3" were not distinguished from the L2 and it is not possible, therefore, to determine whether the growth occurred during the L2 or at the second molt. An additional confounding factor, making direct comparisons to the

present study difficult, is that Halvorsen and Skorping (1982) did not morphologically define L1 versus L2 stages. Within the Protostrongylinae, Kralka and Samuel (1984b) defined the larval stages of *P. boughtoni* by the molting of the cuticular sheaths. They provided data on larval length and width which demonstrated that “Growth of larval *P. boughtoni* was difficult to detect prior to the first molt...” and “The period between the first and second molt was characterized by rapid growth, greater variance in length at each period ...”. Such is consistent with maximum growth during the L2, a generality that should be explored among other Protostrongylinae.

Absence or ambiguity of descriptions and lack of standardized format for description of larval stages in the protostrongylids, coupled with great variation in experimental design and gastropod intermediate host species, makes it difficult to accurately compare patterns of larval development among these nematodes. Limited published data and observations from the current study suggest, however, that larvae of different genera and species in the Muellerinae attain maximum growth rates during the L1 and then immediately following the L2 molt. In contrast, among the Elaphostrongylinae, growth occurs during the L1 and L2 stages. In the Protostrongylinae development is apparently minimal during the L1 stage, but maximized during the subsequent L2 phase. The potential linkage of larval growth and differentiation, life history patterns, and phylogeny for the protostrongylids deserves further investigation.

6.5.2 Larval feeding and stomal morphology

Coupled with a paucity of information on development and differentiation of larval forms across the protostrongylids are the somewhat vague reports and observations of food particles in the intestine and implied feeding behavior of the L1, L2 and L3 stages. Svarc and Zmoray (1974), Svarc (1978), and Skorping (1984) implied that larvae feed while developing in gastropods. Gerichter (1948, 1951) reported food granules of varying size in the intestinal cells of the late L1 and attributed the dark tint of the intestine of the L2 to food granules in the cells. Rose (1957) described “food granules in the intestinal region” in L1 and L2 of *M. capillaris*, and Beresford-Jones (1966) described considerable development of the intestinal cells of this species during the first larval stage within the gastropod intermediate host. Lankester et al. (1998) described numerous small droplets in the intestinal cells of the L2 of *Elaphostrongylus alces* and *E. cervi*. Kralka and Samuel (1984b) described thick walled intestines containing refractile granules in L2 of *P. boughtoni*. All these authors reported that the

granules associated with the intestine in L1 and/or L2, decreased in number and/or size by the L3 and, as a result, the later L3 often had a transparent appearance. In addition, they reported that the L2 were enclosed in the CS1. These findings suggest that while the L1 and perhaps L3 may feed within the gastropods the L2 do not feed. In the present study, the increase in body and esophageal width and the increased granularity of the intestinal region of the late L1 of *U. pallikuukensis* support the contention that L1 feed within the gastropods. The L2 of *U. pallikuukensis* were always enclosed in the CS1 which would prevent feeding. As a generality among the protostrongylids, feeding during the L1 stage may provide the necessary energy for continued larval development to the L3 within the gastropods (i.e. growth during the L2 stage (*P. boughtoni*) and during the L2 to L3 moult (*U. pallikuukensis*)). Studies on larval development of *Ancylostoma tubaeforme* have demonstrated the importance of feeding of first-stage larvae in growth and development to the infective stage (Croll, 1972).

The cuticularized stylet-like organ observed in L3 of *U. pallikuukensis* may be important for feeding both within the gastropod and in the external environment subsequent to emergence. It may also play a role in emergence from the gastropod and penetration of the gastro-intestinal mucosa of the definitive host. A similar, protrusible stylet-like organ has been observed in live L3 of *Elaphostrongylus cervi* (A. Gajadhar, pers. comm.). Observations of a functional and protrusible stylet are in contrast to what is known for the Strongylida or Protostrongylidae (e.g., Carreno and Hoberg, 1999). True functioning stylets (protrusible elements of the stoma) are typical of the parasitic nematodes of plants, including the Tylenchida, but apparently are unknown among the Strongylida, although the L2 and L3 may occasionally have a structure that is non-protrusible and simulates a stomatostyl (Chitwood and Chitwood, 1974; Maggenti, 1981; Poinar, 1983). Likewise, taxa considered close to the Strongylida, including the Diplogasterida and the Rhabditida (Blaxter et al., 1998), both groups including entomopathogenic and free-living forms, possess non-protrusible stylets that may be well developed in larval forms but degenerate in adult nematodes (Chitwood and Chitwood, 1974). Further and detailed study is warranted in defining and confirming the homology in structure and function and phylogenetic context for stylet-like organs in the stoma of protostrongyles, strongyles and related nematodes.

The significance of a stylet in *U. pallikuukensis* must also be assessed in relation to larval feeding and emergence from the intermediate host (Kutz et al., 2000b). Although initially the early L3 were enclosed within the CS2 and occasionally the CS1, they lost these sheaths either by the intermediate or late L3 stages or by the time they had emerged. The loss of sheaths, the presence of a stylet within the buccal cavity, and

granules in the intestinal lumen suggest that the L3 may feed. Whether feeding occurs both within and outside of the IH could not be determined in this study. The current literature on emergence of L3 of protostrongylids (Boev, 1975; Kralka and Samuel, 1984a; Kutz et al., 2000b) and larval feeding is sparse, so a context for the observations from the current study is minimal. Larval emergence, however, appears to be a common feature of *U. pallikuukensis* and may be epidemiologically important (Kutz et al., 2000b). It might be expected, therefore, that the emerged L3 are capable of feeding, and that such foraging behavior may contribute to the long term survival of larvae in the environment and ultimately could have an influence on patterns of transmission.

The late L3 in this study were larger than those reported by Hoberg et al. (1995). These differences may be accounted for by differences in intermediate host species (*D. laeve* in present study, *D. reticulatum* in Hoberg et al. (1995)), intensities of infection in gastropods (low to medium vs. very high), methods of preservation (heat killed over a flame and examined immediately vs. heat killed in ethanol and examined cleared in glycerine) or due to natural variation in the larval populations. Ash (1970) postulated that differences in intensities as well as differences in ages of larvae within the gastropods may affect the size of the L3 of *Angiostrongylus cantonensis*.

6.5.3 The effect of temperature on larval development

Rates of larval development for *U. pallikuukensis* were highly temperature dependent in both *D. laeve* and *D. reticulatum*. Detailed comparisons of development between the 2 species were not possible, however, because the experiments were sampled on different DPI.

A high mortality rate of *D. reticulatum* at low temperatures (10 C and 12 C) and the length of these experiments resulted in the examination of few slugs many days apart. It is possible, therefore, that L3 were present before they were detected. For example, at 12 C, L2 were first observed at 47 DPI; by the next sampling period at 66 DPI, live L3 were present. If these L3 were present earlier, for example by 50 DPI, the calculated threshold temperature would have been lower (8.3 C), but the K would have remained the same.

It is interesting to compare the T_0 and K determined for *U. pallikuukensis* with those for related species. Within the Muelleriinae, using the same genus of intermediate host, *Deroceras*, the same K values (167 DD) were calculated for *U. pallikuukensis* and *M. capillaris* although the T_0 values differed considerably (8.5 C in *D. laeve* or 9.5 C in *D. reticulatum* for *U. pallikuukensis*, 4.2 C in *Deroceras* spp. for *M. capillaris*). In

contrast, the T_0 values for *U. pallikuukensis* were similar to that of the more distantly related Elaphostrongyline, *E. rangiferi* (10.25 C in *A. arbustorum* or 8.25 C in *E. fulvus*), while the K values differed (250 DD for *E. rangiferi* in both *A. arbustorum* and *E. fulvus*). Finally, while the K values in different intermediate hosts were the same for *E. rangiferi*, the T_0 values differed between intermediate host species. It is apparent that the larval development parameters are a result of complex interactions between parasite and intermediate host phylogeny and environment. Based on these results, it appears that T_0 may be related to environment, while K is a function of parasite phylogeny. Gastropod phylogeny may influence both parameters.

Schjetlein and Skorping (1995) proposed that protostrongylids from higher latitudes have higher development thresholds. The data in the present study together with those drawn from work on *M. capillaris*, are consistent with this theory. In order to make meaningful comparisons of development patterns among the Protostrongylidae, however, more detailed studies, at the level of the species, genera and subfamilies, are required.

The development parameters, obtained in the laboratory for *U. pallikuukensis* in the natural intermediate host, *D. laeve*, coupled with appropriate microhabitat temperature measurements, may permit us to predict the time required for larvae to develop in the Arctic. This information, verified by field experiments, is being used as the foundation for a model system to predict effects of climate, including global climate change, on geographic distribution and the dynamics of transmission in a complex host-parasite system in the Arctic.

7. DEVELOPMENT OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS* IN *DEROCERAS LAEVE* UNDER ARCTIC FIELD CONDITIONS

7.1 Abstract

The development of *Umingmakstrongylus pallikuukensis* in *Deroceras laeve* near Kugluktuk, Nunavut, Canada was investigated. Groups of 10 laboratory infected slugs were placed in enclosures in the tundra and sampled at biweekly (1997) or weekly (1998) intervals. In 1997, 6 experiments were conducted, established biweekly. Third-stage larvae (L3) were recovered by weeks 4 to 6 post-infection (PI) from slugs of the experiments established on 19 June, 3 and 17 July. The intensity of L3 in slugs peaked at weeks 6 or 8 PI, then significantly decreased; L3 were most abundant in slugs from 31 July to 28 August. Some slugs from these experiments survived over winter and L3 were recovered from them the following June. In the 1997 experiments, third-stage larvae were found on the vegetation in the enclosures from experiments 1 and 2 in the fall of that year; and, in the summer of 1998, from vegetation in enclosures of experiments 2 and 3. Slugs from experiments established on 31 July and 14 August contained second-stage larvae in September, 1997. Other slugs from these experiments survived in enclosures in the tundra over winter and larvae completed development to L3 in 1998. Third-stage larvae in slugs from the experiment established on 28 August, 1997 did not develop past the first stage by 25 September, 1997. In 1998, 3 experiments were conducted starting on 10, 17 and 25 June; L3 were present in slugs from all experiments by weeks 4 or 6 PI. The observed development in both years corresponded with development rates predicted on the basis of surface temperatures and laboratory data for threshold temperatures (8.5 C) and degree days required for development (167).

7.2 Introduction

The inventory and biology of parasites of wild ruminants in the North American Arctic and Sub-arctic has received little attention. The recent discovery of *Umingmakstrongylus pallikuukensis*, a large and potentially pathogenic protostrongylid lungworm of muskoxen exemplifies this (Hoberg et al., 1995, Hoberg, 1997; Brooks and Hoberg, 2000). In the wild, the prevalence of *U. pallikuukensis* in an affected muskox population approaches 100% in adult animals (Gunn and Wobeser, 1993). The geographic distribution of *U. pallikuukensis* appears, however, to be restricted to the central arctic mainland extending west of Kugluktuk, Nunavut (NU) probably as far as the Mackenzie River, Northwest Territories (NWT), and south to Great Bear Lake, NWT, Canada (S. J. Kutz and J. S. Nishi unpubl. obs.). Biotic and abiotic constraints on development and transmission may explain the limited geographic distribution of *U. pallikuukensis* (Hoberg, 1997; Hoberg, 2000).

Umingmakstrongylus pallikuukensis has an indirect life cycle, requiring a gastropod intermediate host (IH) for development from the first-stage larva (L1) to the infective third-stage larva (L3) (Kutz et al., 1999b). Protostrongylid larval development in the IH is temperature dependent (see Chapter 6; Rose, 1957, Halvorsen and Skorping, 1982; Samson and Holmes, 1984b), thus the high prevalence of *U. pallikuukensis* is surprising considering the short, cool Arctic summers.

Climate plays a critical role in development of invertebrates in the environment and it is predicted that a changing global climate may influence their abundance and distribution (Danks, 1992; Heliövaara, 1993). To better understand the current abundance and distribution of *U. pallikuukensis* and the potential effects of climate change on parasite population dynamics, it is important to understand patterns of larval development and L3 availability in the Arctic. Other than a single report on development of *Neostongylus linearis* in snails in Spain (Morrondo-Pelayo et al., 1987), there are no published experimental data for development of protostrongylids under field conditions. Epidemiological observations and laboratory studies on *Elaphostrongylus rangiferi*, a protostrongylid of northern Norway, suggest that under average climatic conditions larval development in gastropods requires more than 1 summer (Halvorsen and Skorping, 1982). In abnormally warm years, however, outbreaks of severe clinical disease may be the result of mass development to L3 in a single summer (Handeland and Slettbakk, 1994). In contrast, laboratory research coupled with field temperature data, suggest that larvae of the more temperate species of *Protostrongylus* in bighorn sheep

from west-central North America routinely develop to L3 within 1 summer (Samson and Holmes, 1985b).

In the present study field experiments were used to better understand natural patterns of *U. pallikuukensis* larval development and availability. A model system for investigating the effects of climate on parasite development in the Arctic was also developed. The specific objectives of this research were: (1) to determine whether *U. pallikuukensis* can develop to L3 under arctic conditions within a single summer; (2) to determine the temporal pattern of L3 availability in experimentally infected gastropods in the Arctic; (3) to determine, using development parameters (threshold temperature and thermal constant) established in the laboratory (see Chapter 6), which temperature measurements (soil, surface, air or some combination) are useful for predicting development of L1 to L3 in the field; and (4) to determine if larvae can overwinter in gastropods and resume development the following summer.

7.3 Materials and Methods

7.3.1 Study site

The study site was located at 67° 45.60' N, 115° 15.00' W near the hamlet of Kugluktuk, NU, Canada. Kugluktuk is located on the mainland of Nunavut in the "Arctic climate region" as defined by Environment Canada. Based on data recorded from 1977 to 1992 (Environment Canada, Atmospheric Environment Service, Climate Research Branch), the mean daily temperatures exceeded 0 C only from June through September (June 4.3 C, July 10 C, August 8.3 C, and September 2.8 C.). Mean daily maximums for any of these months did not exceed 15 C and the extreme daily maximum in July was 26 C. Total rainfall during these months was: June 13.4 mm; July 34.5 mm; August 44.5 mm; and September 31.8 mm. The study site was a 30 m by 50 m mesic sedge meadow dominated by sedges, grasses, and mosses with a few willows and forbs. The area had a 5-15% standing water coverage, depending on the time of year and recent precipitation, and was surrounded by a solar powered electric fence to deter wildlife.

7.3.2 General experimental design

Six experiments on larval development of *U. pallikuukensis* in slugs were performed in the summer of 1997 and 3 in the summer of 1998. Slugs of the locally abundant species *Deroceras laeve* were used. The experiments were established at

biweekly intervals throughout the summer (Table 7.1). At the start of each experiment 10 experimentally infected slugs were placed in each of a series of enclosures and 10 uninfected slugs were placed in a series of separate enclosures as controls. The total number of enclosures used depended on the start date of the experiment. Enclosures with infected slugs from each experiment were collected approximately biweekly (1997), or weekly (1998), and slugs were recovered and examined for larvae of *U. pallikuukensis*. Control enclosures in 1997 were collected every 4 wk and in 1998 every 1 to 3 wk (Table 7.1). In 1997, enclosures from experiments 1-5 were left in place in the tundra over winter and were examined for slugs and larvae in either June or July of 1998.

7.3.3 Slug source and infection

Deroceras laeve were collected from mesic sedge meadows along the Coppermine River near Kugluktuk, NU. Slugs were collected once or twice daily during the week prior to the start of each experiment. Wet masonite boards (30 cm by 30 cm) were placed arbitrarily, rough side down, and the undersurface of the boards and underlying vegetation were examined for slugs after 2 to 12 hr. Slugs were classified as small (<15 mg), medium (15-44 mg) or large (>44 mg) and maintained at 4 C in Rubbermaid® containers with small pieces of turf and moist paper towel until they were used for experiments.

First-stage larvae for infections were obtained from the feces of an experimentally infected muskox (Kutz et al., 1999b) and had been frozen 2-4 mo (1997 experiments) or 14-15 mo (1998 experiments). Larvae were recovered either by a funnel (Kutz et al., 1999b) or beaker Baermann technique. In the latter, approximately 5 grams of lightly crushed feces were placed in a single layer of cheesecloth and suspended in 80 ml of tap water in a 100 ml glass jar at room temperature (21-27 C) in the light for 16 to 20 hr. The supernatant was drawn off and the sediment containing L1 was held at 4 C for a maximum of 48 hr before being used for infection of slugs.

Slugs were infected on filter paper in petri dishes (Hoberg et al., 1995). Twelve slugs were placed in each petri dish and exposed to a total of approximately 2700 L1 for 3 hr. Control slugs were handled in the same manner except no larvae were placed in the petri dishes. After the infection procedure slugs were distributed among the enclosures in the field. In an attempt to approximate the population of slugs that would be exposed to natural infection at the different points in time represented by the experiments, slugs were assigned to each enclosure in the same size ratios

Table 7.1: Design of the 1997 and 1998 larval development experiments.

Expt. #	Slug size (%)		1997 No. of enclosures examined (infected/control)												1998 No. of enclosures examined (infected/control)											
	Small	Medium	19 Jun	3 Jul	17 Jul	31 Jul	14 Aug	28 Aug	11 Sep	25 Sep	10 Jun	15 Jun	17 Jun	25 Jun	1 Jul	8 Jul	15 Jul	22 Jul	28 Jul							
1	47	20	33	S*	3/0	3/3	3/0	3/3	3/0	3/3	—	—	6/0	—	—	—	—	—	—							
2	44	34	22	S	3/0	3/3	3/0	3/3	3/0	—	—	—	4/3	—	—	—	—	—	—							
3	17	59	24	S	3/0	S	3/0	3/3	3/0	3/3	3/0	—	6/0	—	—	—	—	—	—							
4	8	30	62	S	S	3/0	S	3/0	3/3	3/0	3/3	—	—	—	—	3/0	3/0	—	—							
5	25	16	59	S	S	3/0	3/3	3/0	3/0	3/0	3/0	—	—	—	—	—	3/3	3/0	—							
6	35	13	52	S	S	3/0	S	3/0	3/3	3/0	3/3	—	—	—	—	—	—	—	—							
7	45	28	27	S	—	—	—	—	—	—	S	—	—	—	3/0	3/3	3/0	3/0	3/3							
8	42	42	16	S	—	—	—	—	—	—	—	S	—	—	3/0	3/0	3/3	3/0	3/3							
9	48	43	9	S	—	—	—	—	—	—	—	—	S	—	3/0	3/0	3/0	3/3	3/0							

* Experiment started.

(small:medium:large) as they were collected during the week preceding the experiment (Table 7.1). In addition, when each experiment was established, 10 infected and 10 control slugs were kept in separate Rubbermaid® containers in the laboratory (20 - 27 C) and examined after 2 wk to assess consistency in larval development rates between experiments as well as to examine control slugs for natural infections.

7.3.4 Experimental enclosures and temperature measurements

Enclosures were placed randomly within the study site and prepared as follows. For each enclosure a columnar section of turf, approximately 10-11 cm deep and 18-20 cm in diameter, was collected and placed in a white plastic pail. The pail was then filled to the brim with water and left on the tundra 24-48 hr to flood out any resident gastropods. The excess water was then drained and the turf was placed into a specially prepared white plastic pail. The effectiveness of the flooding technique for gastropod removal was tested in 1998 by flooding 3 additional pails at the same time as those used for each experiment and subsequently examining these in the laboratory for gastropods.

For experiments 1-3 in 1997 and all experiments in 1998, pails were 15 cm high with a 20 cm top inside diameter. Due to a shortage of pails, for experiments 4-6 in 1997 pails were 19 cm high, with the same inside diameter. Pails were prepared by drilling 30 holes (1-2 mm diameter) in the base and 20 around the side within 4 cm of the base. The insides of the pails were lined with one layer of white polyester bridal netting to prevent slugs from escaping. In 1997 the pails were covered with two layers of netting which were pulled tight across the top of the pail and fixed in place with the rim of the original lid from which the center had been removed. The lids were securely fastened, and pails were placed back into the hole from which the turf had been removed. Four to 5 cm of the pail remained above ground (Fig. 7.1).

Because of differences in 1997 between temperatures inside and outside the enclosures, the enclosures for 1998 experiments were modified. The turf inside extended to the top of the pails and the pails were placed in the tundra so that the upper rim was level with the soil surface. The netting was not drawn tight over the rim, but extended in a closed column approximately 30 cm tall and 20 cm wide above the pail and suspended by a monofilament fishing line attached to a wooden support (Fig. 7.2). In 1997 enclosures for all experiments were prepared either in late June or early July. In 1998 they were prepared during the week before the start of each experiment

Temperatures were monitored inside and outside enclosures in both years to test for an enclosure effect on surface and soil temperatures. In 1997, 3 enclosures were

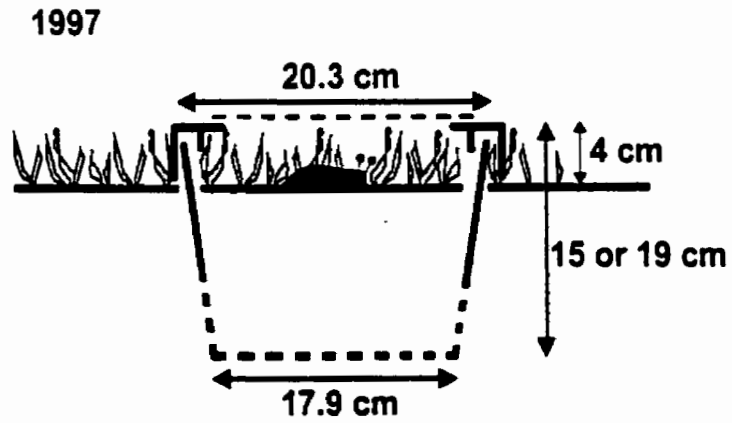


Figure 7.1. Gastropod enclosure used in 1997.

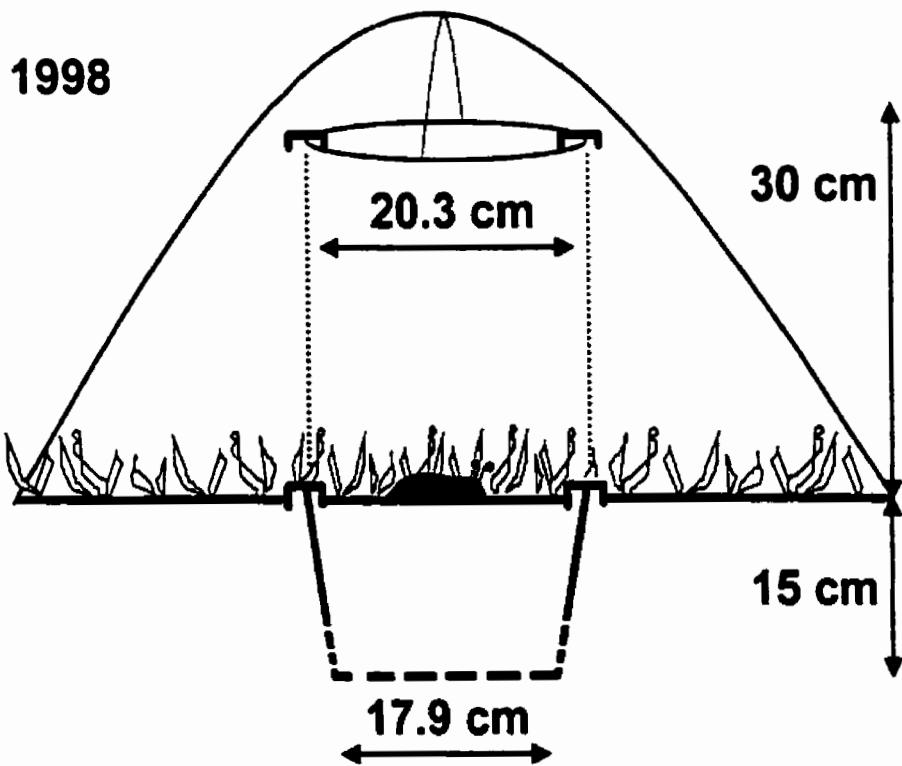


Figure 7.2. Gastropod enclosure used in 1998.

prepared in a manner identical to the experimental enclosures and placed arbitrarily in the site. The leads from 2 temperature monitors (Hobo XT Temperature Logger, onset computer corporation, Pocasset, MA, USA) were placed in each enclosure, 1 at the surface of the soil/leaf litter ("surface"), the other inserted vertically 1 cm into the soil/leaf litter ("soil"). Two additional probes (1 surface and 1 soil) were placed at a central site within the study area but not in an enclosure. The probes were programmed to measure mean temperatures every 30 min, 24 hr each day. Surface and soil temperatures inside enclosures were measured beginning 25 June 1997, while those outside enclosures were measured beginning 8 July 1997. As a result of technical difficulties and destruction of probes by rodents only 1 set of inside enclosure and 1 set of outside enclosure temperatures were consistently measured during 1997. In 1998, however, from 10 June to 28 July temperatures were recorded from 1 soil and 1 surface site inside and outside 3 randomly placed enclosures except from 20-25 June, when only 2 of 3 outside enclosure soil temperatures were recorded. Air temperatures were obtained from the Kugluktuk airport weather station (67°49'N, 115°08'W) approximately 5 km east of the study site.

7.3.5 Slug recovery and examination

The schedule for enclosure collection and examination is presented in Table 7.1. Turf was removed with the netting lining the enclosures and placed in unperforated pails. Cold water was added to the pails over a 3 day period and slugs recovered as they were forced up on the vegetation, sides and lids to avoid drowning (Kralka, 1986). Enclosures and slugs recovered were held at 4 C during the flooding and until examination to prevent further larval development. Slugs were weighed, their feet examined for lesions using a dissecting microscope and over-head light source (Kutz et al., 2000b), and then digested in a pepsin/hydrochloric acid solution (Hoberg et al., 1995) at 37-40 C for 1-2 hr. The digest was examined for larvae which were then classified to stage of development (see Chapter 6) and preserved in steaming ethanol (70%) and glycerine (5%). Control enclosures were processed in the same manner. Slugs weighing less than 10 mg with no foot lesions and recovered from experiments 1-3 6 wk or later after the start of the experiment, or from experiments 4 or 5 in 1998, were considered recently hatched (either from eggs present prior to flooding of the pails during preparation of the enclosures or eggs deposited by experimental slugs) and were not considered as part of the experiments for slug survival or larval recovery. To

confirm this assumption, these slugs were digested, either individually or in groups, and examined for larvae.

To investigate the relationship between larval development and temperatures at different levels in the microhabitat it was necessary to define when L3 were considered present. In Chapter 6 the temperature threshold and degree-days (DD) required for development of L3 was described using the time elapsed to the appearance of the first L3 which was mobile after pepsin/HCl digestion of the slugs. The same criterion was used for the field experiments in which the endpoint was defined as the date at which at least 1 slug from at least 1 enclosure contained an intermediate or late L3 as described in Chapter 6. In the present study these larvae are referred to simply as L3.

The relative abundance of L3 in slugs in 1997 was determined as follows. First, the date at which the mean number of L3/slug was the highest was identified as the Max L3/slug for each experiment, and was assigned a value of 1. The L3/slug on each of the remaining sampling days was divided by the Max L3/slug for the respective experiment. These values were multiplied by the slug survival for each date. The product was the relative number of L3-in-slugs at each sampling day in an experiment. These numbers were summed among experiments for each date to determine the relative abundance of L3-in-slugs over time.

7.3.6 Degree-days calculations

For the microhabitats, the temperatures were first averaged for each measurement type. For example, the mean of all the soil temperatures inside enclosures was calculated (soil inside), the mean of all the soil temperatures outside enclosures was calculated (soil outside), the same was done for surface temperatures inside (surface inside) and outside (surface outside) enclosures. The mean of the soil and surface (mSS) was a calculated value based on the average of the soil and surface temperatures. The soil and surface temperatures from each site were averaged (for inside and for outside the enclosures separately), and then the mean temperature calculated for all inside enclosures and all outside enclosures.

The DD accumulated above the threshold of 8.5 C (as determined in Chapter 6) were calculated for inside and outside enclosures using the mean surface, mean soil and mSS temperatures at the study site, as well as for air temperatures from the Kugluktuk airport. Degree-days for temperatures based on microhabitat measurements were calculated using 30 min mean temperatures. First, temperatures above 21 C were converted to 21 C. This was done because, based on available literature (Dainton, 1989;

Rollo, 1991), it was assumed that *D. laeve* would move to avoid temperatures greater than 21 C. The threshold temperature was then subtracted from each 30 min mean temperature (corrected for temperatures > 21 C) and negative results automatically converted to 0. The DD values calculated for each 30 min period during a single day were averaged to determine the DD accumulated for that day. Cumulative DD in an experiment were calculated by adding the daily DD. Degree-days accumulated in the air were calculated in a similar manner but were based on hourly mean temperatures.

7.3.7 Other analyses

Statistical analyses were performed using the computer program StatView™ SE + Graphics (Abacus Concepts Inc. 1988, San Francisco, California, USA), Sokal and Rohlf (1995) and Siegal and Castellan (1988). Mean L3/slugs within an experiment but at different times PI, were compared using a Kruskal-Wallis test followed by a multiple comparisons test. Probability of significance was set at $P \leq 0.05$.

7.4 Results

7.4.1 Slug recovery and examination

Based on the 1998 assessments, flooding turf to eliminate resident gastropods before starting an experiment appeared to be effective for removing *D. laeve*. Although some pails contained the small snails, *Vertigo cf modesta*, no slugs were recovered.

Recovery rates of infected slugs from the experiments in 1997 and in 1998 are presented in Table 7.2. In 1997 survival differed between early and late experiments. In experiments 1, 2 and 3 there was high mortality during the first 2-8 wk (17 July to 14 August) then the mortality rate decreased. A similar mortality rate was not observed during the first 2-8 wk (14 August to 25 September) in experiments 4, 5 or 6. Slug recovery from the modified enclosures in 1998 was extremely poor (Experiments 7, 8, and 9 in Table 7.2). Several slugs were found dead in the netting, suggesting that others may have escaped from the enclosures.

The weekly (June to September) or monthly (October to May) mean air temperatures from the Kugluktuk airport weather station and the surface temperatures inside and outside enclosures during the 2 yr study period are presented in Figure 7.3. In 1997, L3 were first recovered from experiments 1, 2 and 3, at weeks 6, 4 and 4 PI, respectively. Larvae in experiments 4 and 5 had developed only to the second stage (L2) at weeks 8 and 6 PI, respectively, when examination of these experiments ended

Table 7.2: Recovery of slugs from enclosures in 1997 and 1998, reported as the mean percentage (± 1 SD) of the original number of slugs (10) placed in each enclosure. Numbers in parentheses represent percentage recovery (± 1 SD) from control enclosures.

Expt. #	Weeks post-infection									
	2	3	4	5	6	7	8	10	12	48 - 52
1	80 \pm 10	—	70 \pm 10 (63 \pm 31)	—	30 \pm 10	—	30 \pm 17 (30 \pm 27)	30 \pm 14	13 \pm 12 (5 \pm 7)	0
2	80 \pm 10	—	50 \pm 20 (47 \pm 29)	—	37 \pm 21	—	33 \pm 25 (30 \pm 42)	33 \pm 32	—	3 \pm 5 (3 \pm 6)
3	60 \pm 20	—	30 \pm 10 (57 \pm 31)	—	37 \pm 21	—	33 \pm 25 (33 \pm 15)	—	—	3 \pm 5
4	73 \pm 6	—	77 \pm 6 (70 \pm 14)	—	60 \pm 27	—	45 \pm 7 (47 \pm 6)	—	—	7 \pm 8
5	90 \pm 10	—	67 \pm 12 (60 \pm 20)	—	77 \pm 6	—	—	—	—	2 \pm 4 (7 \pm 6)
6	60 \pm 20	—	83 \pm 15 (50 \pm 20)	—	—	—	—	—	—	—
7	—	17 \pm 6	7 \pm 12 (3 \pm 6)	0	2 \pm 4	7 \pm 12 (17 \pm 12)	—	—	—	—
8	20 \pm 20	25 \pm 21	7 \pm 12 (7 \pm 12)	5 \pm 6	0 (3 \pm 6)	—	—	—	—	—
9	7 \pm 12	0	3 \pm 6 (3 \pm 6)	0	—	—	—	—	—	—

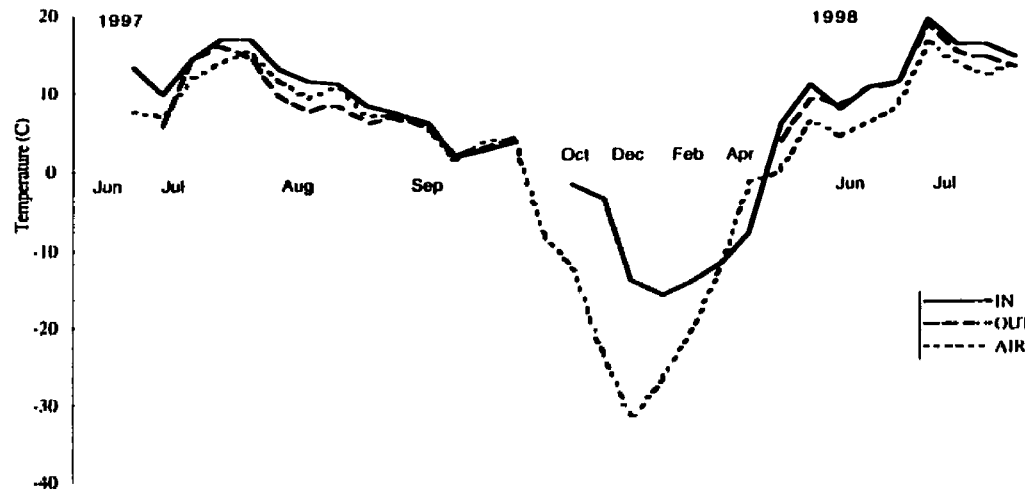


Figure 7.3. Surface temperatures inside and outside of enclosures and air temperatures from the Kugluktuk airport. From June to September 1997 and from June to July 1998 temperatures are reported as the weekly means, from October 1997 to May 1998 they are reported as the monthly means.

for 1997. The larvae in experiment 6 had not developed past L1 by week 4 PI (25 September) when the experiment ended.

The mean proportion of the various larval stages in slugs is summarized for each enclosure in each experiment (Figs. 7.4-7.12). In 1997, the rate of development to L3 in different slugs within and between enclosures examined at each time period was relatively synchronous (as determined within the constraints of the biweekly sampling intervals). In experiment 1 the first L3 were present at week 6 PI at which time all slugs (n = 9) from all 3 enclosures contained at least 1 L3. In experiment 2, the first L3 were present at week 4 PI in 6 of 12 slugs from 2 enclosures and 0 of 3 slugs from the third enclosure, but at week 6 PI all slugs (n = 10) from all 3 enclosures contained at least 1 L3. In experiment 3, L3 were present at week 4 PI in 8 of 9 slugs from all 3 enclosures and, at week 6 PI all slugs (n = 11) in all 3 enclosures contained L3.

Development of larvae in slugs infected and kept in the laboratory was consistent between experiments, with the majority of larvae in all slugs at the L3 stage by week 2 PI. No larvae were recovered and no foot lesions were seen in any of the uninfected control slugs from either the laboratory or field experiments. No larvae were found in slugs which weighed less than 10 mg, had no foot lesions and were recovered from experiments 1-3 at week 6 PI or later, or from experiments 4 and 5 in 1998.

In 1998, L3 were present in experiments 7, 8 and 9 by weeks 6, 5 and 4 PI, respectively (Figs. 7.10-7.12). No slugs were recovered from any of the 3 enclosures examined at week 4 and 5 PI in experiment 7 or week 2 PI in experiment 9. It is possible, therefore, that L3 may have developed earlier in these experiments but because of the poor slug recovery this could not be determined.

7.4.2 Degree-days calculations

The DD accumulated based on a threshold of 8.5 C and using temperatures at the different microhabitat levels and in the air are presented in Table 7.3. In both years, DD were accumulated most rapidly at the surface. In 1997, early in July fewer DD were accumulated in the air compared to the microhabitat. The air DD then paralleled those at the surface from mid to late July, and then accumulated more slowly, leveling off by the end of August. In 1998, air DD lagged behind those at the surface in June, paralleled those at the surface during the first week of July, after which they accumulated at a slower rate. Surface temperature data were not available to compare patterns of DD accumulation later in the summer and autumn of 1998.

Figures 7.4-7.9. The percent of different larval stages recovered from slugs from each enclosure at different times post-infection for experiments established in 1997. Each bar represents the mean percent of each larval stage in all infected slugs from an individual enclosure. Numbers above bars represent the number of slugs recovered from the enclosure on which the data are based. In cases where there are no bars associated with the numbers, either no slugs were recovered from the enclosures (indicated by a 0) or, slugs that were recovered contained no larvae. L1- first-stage larvae, L2 - second-stage larvae, aL3 - early third-stage larvae (L3), bL3 = intermediate or late L3 (as described in Chapter 6).

Figure 7.4. Experiment 1.

Figure 7.5. Experiment 2.

Figure 7.6. Experiment 3.

Figure 7.7. Experiment 4.

Figure 7.8. Experiment 5.

Figure 7.9. Experiment 6.

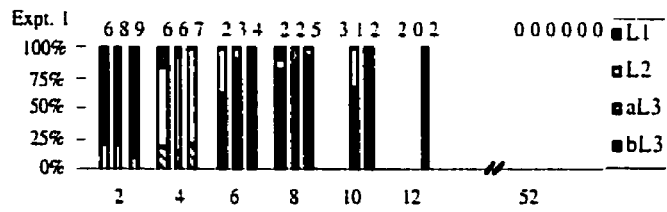


Fig. 7.4

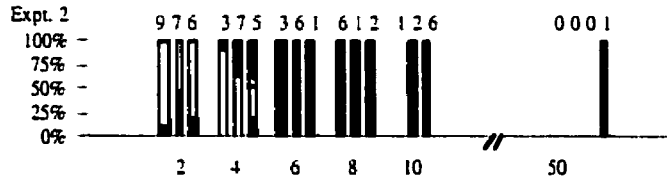


Fig. 7.5



Fig. 7.6

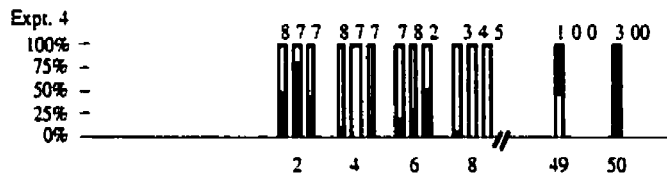


Fig. 7.7

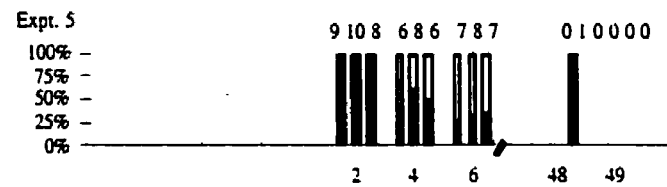


Fig. 7.8

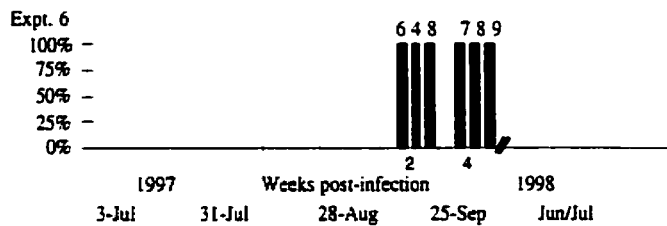


Fig. 7.9

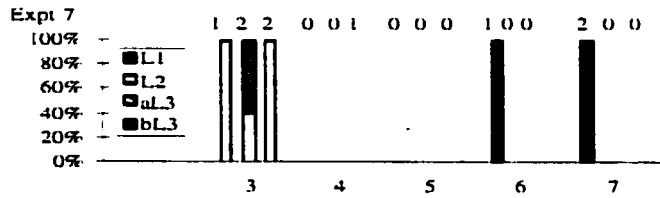


Fig. 7.10

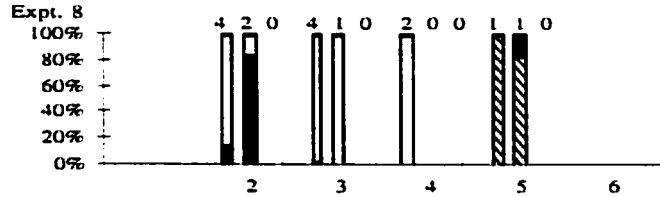


Fig. 7.11

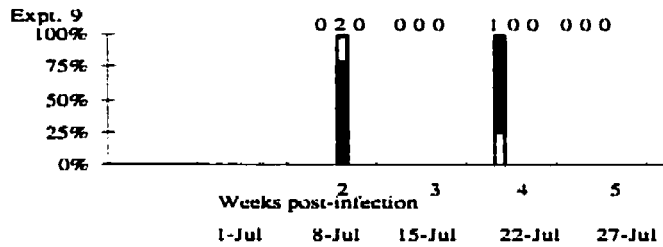


Fig. 7.12

Figures 7.10-7.12. The percent of different larval stages recovered from slugs from each enclosure at different times post-infection for experiments established in 1998. Each bar represents the mean percent of each larval stage in all infected slugs from an individual enclosure. Numbers above bars represent the number of slugs recovered from the enclosure on which the data are based. In cases where there are no bars associated with the numbers, either no slugs were recovered from the enclosures (indicated by a 0) or, slugs that were recovered contained no larvae. L1- first-stage larvae, L2 - second-stage larvae, aL3 - early third-stage larvae (L3), bL3 = intermediate or late L3 (as described in Chapter 6).

Figure 7.10. Experiment 7.

Figure 7.11. Experiment 8.

Figure 7.12. Experiment 9.

There was a difference between 1997 (the fifth warmest year on record) and 1998 (the warmest year on record) with respect to the rate at which DD were accumulated. Air and surface temperatures in enclosures were available in both years from 25 June to 27 July; during this time fewer DD were accumulated in 1997 (air 122 DD, surface 187 DD) than in 1998 (air 158 DD, surface 211 DD) (Table 7.3).

Based on laboratory experiments (see Chapter 6), it was assumed that 167 DD were required for development of L1 to L3. In 1997, in experiments 1-3, only DD at the surface were sufficient to account for the observed larval development (i.e., 167 DD had accumulated by the time the first L3 was present) (Fig. 7.13). In experiments 4-6, however, sufficient DD for development to L3 were not accumulated at the surface by 25 September, 1997 (Fig. 7.13). On this date, only L1 or L2 were recovered from the slugs in these experiments (Figs. 7.7-7.9). In all 3 experiments in 1998, the DD accumulated at the surface and mSS inside the enclosures were consistent with the observed development to L3 (Figs. 7.10-7.13).

In 1997, temperatures inside enclosures were 1-2 C warmer than outside enclosures during early and mid-July, and up to 5 C warmer from the end of July and throughout August (Fig. 7.3). In 1998, the enclosures were modified and temperatures were recorded from an increased number of sites (3 inside and 2-3 outside the enclosures). This resulted in reduced temperature differences between inside and outside (1-2 C on warmer days) relative to 1997 (Fig. 7.3). The effect of these temperature differences on accumulated DD is presented in Table 7.3. In 1997 there was a large difference in the DD accumulated inside and outside of enclosures from mid to late July through to the end of August. In 1998, DD accumulated inside and outside the enclosures were very similar. The 1998 study period did not extend beyond the end of July and, therefore, cannot be compared to 1997.

7.4.3 Overwintering of slugs and larvae

The results for experiments where slugs were overwintered from 1997 to 1998 are shown in Figures 7.4-7.8 and Table 7.2 and 7.4. In general, slug recovery was poor and no slugs were found in any of the 6 enclosures of experiment 1 examined on 15 June 1998. Slugs containing L3 were recovered from experiments 2 and 3 in June 1998 and from experiments 4 and 5 in July 1998. Degree-days for these overwintering experiments were calculated from surface temperatures in the 1997 enclosures up until 25 September 1997 and, starting 10 June 1998, from surface temperatures in the modified 1998 enclosures (Figs. 7.3, 7.13).

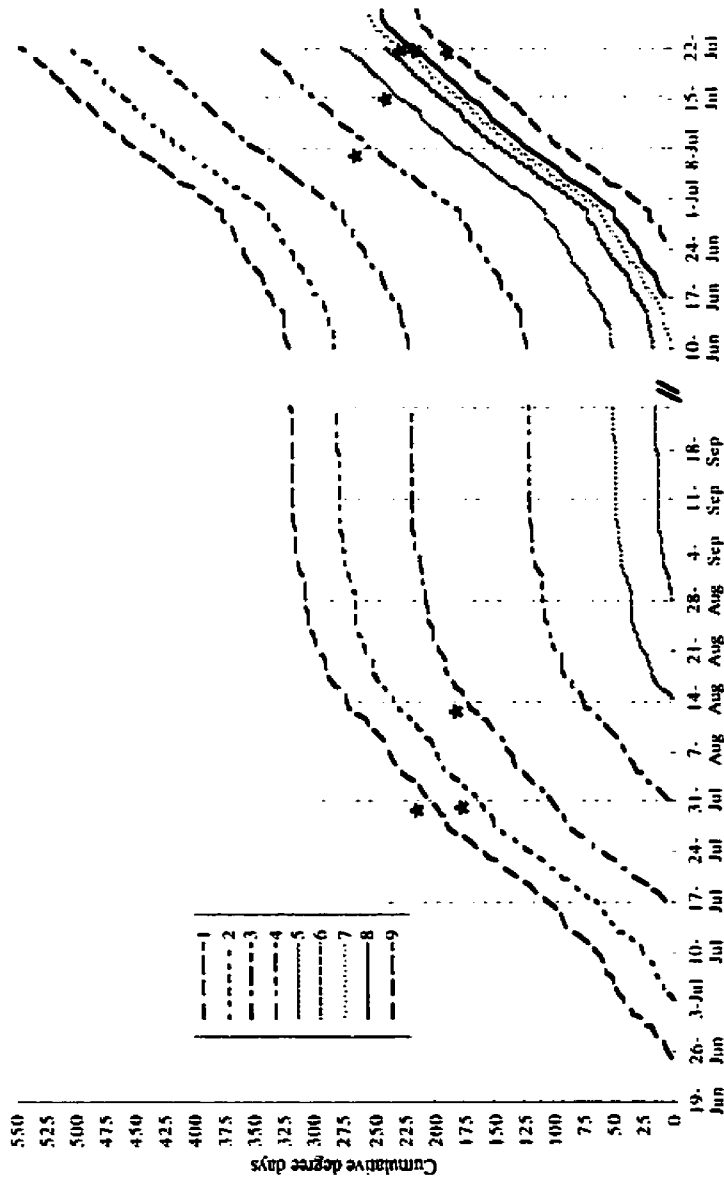


Figure 7.13. Degree-days (DD) accumulated based on surface temperatures for experiments 1-9 in 1997 and 1998. Horizontal line at 167 DD represents the accumulated DD required for development to L3 based on laboratory data (see Chapter 6). Vertical dashed lines represent sampling days. Degree-days reported in 1998 for experiments 1-6 are calculated based on temperature recordings from 1998 enclosures. Asterix* represent the date that the first L3 were observed in each experiment.

Table 7.3: Degree-days accumulated in 1997 and 1998 based on temperatures from inside and outside enclosures at the surface, soil and the mean of the surface and soil (mSS), as well as in the air at the Kugluktuk airport.

1997	1997 In			1997 Out				1998	1998 In			1998 out			
	Surface	Soil	mSS	Surface	Soil	mSS	Air		Surface	Soil	mSS	Surface	Soil	mSS	Air
Jun 25	S*	S	S	—	S	—	S	Jun 10	S	S	S	S	S	S	S
Jul 3	41	9	10	—	6	—	15	Jun 17	15	7	7	17	6	6	2
Jul 8	57	12	19	S	6	S	19	Jun 24	39	26	26	43	18	18	3
Jul 17	107	37	59	49	23	36	47	Jul 1	71	48	54	74	35	41	19
Jul 31	203	104	145	131	67	103	127	Jul 8	131	102	112	130	80	94	76
Aug 14	272	134	199	153	73	116	181	Jul 15	176	135	152	170	113	132	105
Aug 28	306	142	220	160	73	119	202	Jul 22	225	171	196	212	149	171	135
Sept 11	317	142	223	170	74	123	212	Jul 27	250	191	219	234	169	192	161
Sept 25	318	142	224	176	74	124	213								

* Date that temperature recording was started.

7.4.4 Patterns of L3 intensity in slugs over time

The mean intensity of L3 in slugs on different sampling days (including after overwintering) was investigated for experiments 1, 2 and 3. The mean intensity within an experiment on a given sampling day did not significantly differ between enclosures ($P > 0.05$ for all experiments). Data from the enclosures on a single day were grouped, therefore, and the mean intensity of L3 in slugs was then compared to other sampling days within an experiment. In experiment 1, the number of L3/slug significantly decreased from week 6 to weeks 10 and 12 PI, and from week 8 to weeks 10 and 12 PI (KW = 16.259, df = 3, $P = 0.001$) (Table 7.4). In experiment 2, in 1997 there were significantly fewer L3 at week 4 compared to week 6 PI, but more L3/slug at weeks 6 and 8 than at week 10 PI. Also in experiment 2, there were significantly more L3/slug at weeks 4, 6, 8 and 10 PI in 1997 than at week 50 PI in 1998 (KW = 19.009, df = 4, $P = 0.0008$). In experiment 3 there were fewer L3/slug at week 4 than at weeks 6 and 8 PI, but no differences between weeks 6 and 8 PI. There were, however, significantly more L3/slug at weeks 6 and 8 PI in 1997 than at week 48 PI in 1998 (KW 11.659, df = 3, $P = 0.0086$). The mean number of L3/slug peaked at 6 wk PI in all 3 experiments.

The relative numbers of L3-in-slugs contributed by each of experiments 1, 2 and 3 on different dates in 1997 and 1998 is presented in Figure 7.14. The highest overall proportion of L3 were available in slugs on 14 and 28 August 1997.

7.5 Discussion

7.5.1 General

An enclosure-based system was designed and successfully used to investigate the development of a protostrongylid parasite in its intermediate host under arctic field conditions. Only one similar field study for protostrongylids has been published, for *Neostrongylus linearis* in *Cernuella cespitum arigonis* in Spain (Morrondo-Pelayo, 1987). Under climatic conditions that differed considerably from the present study, larvae were able to develop at all times during the year although development rates slowed during the winter.

Slug recovery from enclosures in 1997 was generally good. The different survival patterns between early and later experiments may reflect an annual cycle of *D. laeve*, with older slugs dying in the middle of the summer as has been observed at more temperate latitudes (Lankester and Anderson, 1968; Samuel et al., 1985). Because of

Table 7.4: Mean number of third-stage larvae per slug (\pm 1SD) from experiments 1-3 established in 1997. Numbers in parentheses indicate the total number of slugs examined.

Expt. # and date started	Weeks post-infection						
	2	4	6	8	10	12	48-52
1. 19 Jun 1997	0 (23)	0 (19)	7.8 \pm 6.5 (9)	6.3 \pm 4.5 (9)	1.0 \pm 1.5 (7)	0.25 \pm 0.5 (4)	—
2. 3 July 1997	0 (22)	1.9 \pm 2.9 (15)	23.7 \pm 28.8 (10)	22.3 \pm 28.3 (9)	2.2 \pm 2.9 (9)	—	0.5 \pm 0.7 (2)
3. 17 July 1997	0 (18)	3.1 \pm 3.4 (9)	15.3 \pm 12.1 (11)	11.9 \pm 6.0 (10)	—	—	4 \pm 4.2 (2)

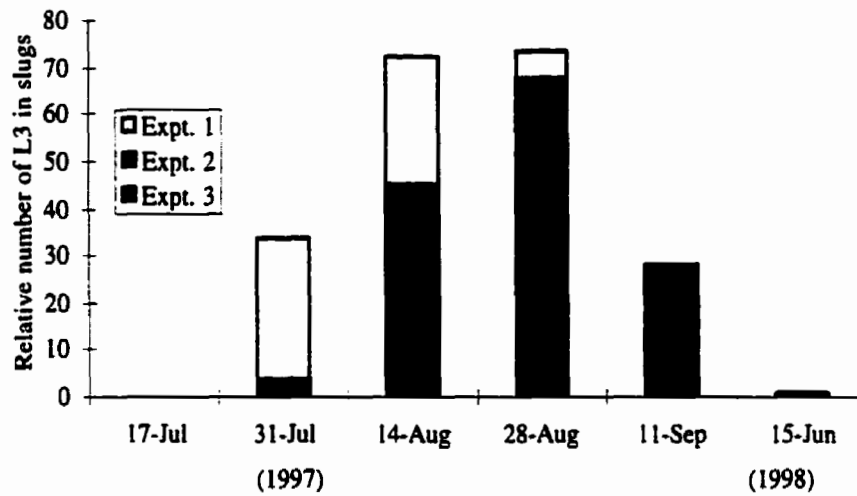


Figure 7.14. The relative number of L3 in slugs from each experiment in 1997 as determined from the mean intensity of L3 in slugs on each sampling date and slug survival.

possible emigration and enclosure induced mortalities, slug survival in 1998 could not be assessed.

In 1997, development rates of larvae in slugs within and among enclosures in an individual experiment were relatively uniform. Thus, although slug recovery in 1998 was poor, the consistency between slugs within experiments in 1997 suggests that results from the few slugs in 1998 were representative of the patterns of larval development. It is possible, due to the small sample sizes and days where no slugs were recovered, that results in 1998 over-estimated the time required for development to L3.

7.5.2 Degree-days model

Degree-days were calculated based on temperatures in the air as well as at the surface, soil, and mSS inside enclosures in an attempt to determine which best matched the observed larval development. The DD accumulated in the air did not correspond with development to L3 in either 1997 or 1998. The DD accumulated based on surface temperatures, however, corresponded very well with field observations for development to L3 in both 1997 and 1998. This heating at the surface early in the season may allow for more larval development earlier than would be predicted from air temperatures. A similar pattern may be expected later in the season. In 1998 the DD accumulated based on the mSS temperatures were very close to those accumulated at the surface, and also corresponded with the observed development to L3. 1998 was an exceptionally warm year, which, because of the 21 C limit used in our DD calculations, may explain why DD from the mSS approached those at the surface.

Although the DD determined at the surface inside enclosures corresponded to the observed larval development, the complexity of this system and the influence physiological condition and behaviour of the intermediate hosts may have on larval development should be emphasized. Starvation, aestivation and differences in age may influence larval development (Skorping, 1984; Solomon et al., 1996). Through their own activity, slugs may influence their ambient temperatures (Dainton, 1989; Rollo, 1991) and, therefore, those of the larvae. There is substantial variation in temperatures within an area of a few square meters at the level of the tundra microhabitat. These temperatures are influenced by numerous factors including vegetation, moisture, and slope direction. In addition, within a 10 to 20 cm vertical range the temperatures may differ considerably (Hansen, 1973; Coulson et al., 1993; Young et al., 1997). The synchronicity in larval development within and between enclosures of an experiment

support the suggestion that the slugs, perhaps through thermal preferences, have a significant influence on the ambient temperature of the larvae. In contrast to parasites which have direct life cycles, those using intermediate hosts, such as the protostrongylids, may be less influenced by short term climate variations and may be at an advantage in terms of survival, development and transmission because of the relatively constant environment sought by their intermediate hosts.

For our DD calculations the behaviour of *D. reticulatum*, which at temperatures greater than 21 C, moves to cooler shelters, was considered (Dainton, 1989; Rollo, 1991). These behavioural characteristics were extrapolated to *D. laeve* by considering 21 C as the maximum temperature to which larvae within slugs were exposed. Using this as a starting point, it is possible that a more comprehensive model for larval development could be developed by incorporating the preferred temperatures and the circadian rhythms of the intermediate hosts into the DD calculations.

Temperature differences were observed between the inside and outside of the enclosures in 1997. These may have been an artifact of insufficient temperature probes (1-3) coupled with downloading and repositioning of probes in late July and considerable microclimate variability; alternatively the differences may have been real, demonstrating a warming effect of the enclosures. If the enclosures artificially elevated the temperatures it is still reasonable to assume that these temperatures were within the natural variation seen between habitats, microhabitats and years and, therefore, that the experiments are representative of the natural pattern of development. In addition, temperatures were similar until the end of July, by which time L3 were already present in experiments 1 and 2. Larval development in these experiments theoretically may have been hastened near the end of July because of elevated enclosure temperatures. Nevertheless, even without this heating, it is likely that larval development could have been completed. Even when DD accumulations are based on the cooler outside enclosure surface temperatures, larvae infecting slugs on or before 10 July 1997 would have developed to L3 by the end of September 1997; larvae infecting slugs on or before 30 June 1998 would have harboured infective L3 by 27 July 1998 (Table 7.3).

7.5.3 Over-wintering of slugs and larvae

Slugs that were sampled from experimental enclosures which over-wintered from 1997 to 1998 confirmed that (1) slugs infected with larvae of *U. pallikuukensis* can survive from 1 year to the next, (2) L3 within slugs may be able to over-winter (experiments 2 and 3), and (3) larvae entering winter as L2 can resume development to

L3 the following year (experiments 4 and 5). Developing larvae and L3 within over-wintering gastropods may be important sources of L3 early the following year, because they would be available before those that developed in slugs infected in the spring of that year (Fig. 7.13 see experiments 2-5 vs. 7-9). The survival rates of these over-wintering larvae must be considered in the epidemiology of *U. pallikuukensis*. The survival rates of the various larval stages (L1 vs. L2 vs L3) of *Elaphostrongylus rangiferi* in hibernating gastropods differ, as do the survival rates of the gastropods depending on which larval stages they contain (Schjetlein and Skorpung, 1995). Our experimental design did not allow us to determine the number of slugs in an enclosure entering the winter, nor the prevalence or intensity of infection in these slugs and, therefore, the overwinter survival rates of slugs and larvae could not be determined. Further studies designed to quantify the survival of over-wintering larvae and infected gastropods are necessary to better address the significance of such larvae in the epidemiology of *U. pallikuukensis*.

7.5.4 Patterns of L3 intensity in slugs over time

Within an experiment the number of L3/slug changed over time. In experiment 1 the mean intensity peaked on the same day as the first L3 was observed. In experiments 2 and 3 the mean intensity peaked on the sampling day following (2 wk later) the appearance of the first L3. In all 3 experiments the peak mean intensity occurred at week 6 PI, remained high until week 8 PI, and then declined. The initial low mean intensities of L3 in experiments 2 and 3 at week 4 PI probably represent a time of transition from L2 to L3. In experiment 1 this transition was not observed, but probably occurred between weeks 4 and 6 PI.

The decline of L3/slug in experiments 1-3 at weeks 10, 10 and 48 PI, respectively, suggest that larvae either leave the slugs or die after reaching L3. Third-stage larvae were recovered from the vegetation of experiments 1 and 2 in the late summer of 1997 and experiments 2 and 3 the following June, 1998 (Kutz et al., 2000b). These findings, coupled with extensive laboratory data demonstrating emergence as a consistent trait of *U. pallikuukensis* (Kutz et al., 2000b), suggest that the change in numbers of L3/slug over time may have been a result of L3 emergence.

Our findings on the temporal patterns of L3 in slugs suggest that under natural conditions, assuming a constant rate of L1 acquisition by slugs throughout the summer, L3 in slugs would be most abundant in August. The numbers of L3 in slugs would subsequently decline, perhaps as a result of L3 emergence, and thus become available

free in the environment. The epidemiological implications of such emergence, including the possible availability of L3 to muskoxen during the winter, have been discussed by Kutz et al. (2000b). A similar pattern of mean L3 intensities in gastropods was observed in some of the field experiments on *N. linearis* development (Morrondo-Pelayo, 1987). This pattern and its significance were not discussed by the authors, however, nor has the phenomenon of L3 emergence been investigated for this species.

Umingmakstrongylus pallikuukensis may use several strategies to maintain L3 in the environment throughout the year. Rapid development within a single summer, emergence of L3, overwinter survival of L3 in gastropods, with or without subsequent emergence, and overwinter survival of L1 and L2 in gastropods with subsequent development to L3, theoretically result in L3 being available in the environment (either in gastropods or on vegetation), throughout the year. Although the relative significance of each of these strategies in the survival and transmission of *U. pallikuukensis* requires further investigation, we believe that these developmental characteristics of the parasite contribute greatly to its ecological success.

Equipped with this information on larval development of *U. pallikuukensis* in the Arctic environment, the effect of global climate change on the parasite population dynamics in gastropods and the environment can be addressed. In the form of earlier springs and later autumns, global warming in the Arctic may extend the temporal window in which the gastropod intermediate hosts are active and larvae can develop. In the form of increased summer daily temperatures, global warming may accelerate the rate of larval development. These 2 factors combined could result in L3 being available earlier in the season, the peak intensity of L3 in slugs extending over a longer time period, and larval development occurring later into the fall. Also, the influence of the IH on its ambient temperature must be considered in a climate change scenario. A linear relationship between increased environmental temperatures and larval development rates is unlikely. For example, a mean 2 C increase in air temperature may not translate directly to a 2 C increase in larval ambient temperature because of gastropod behaviour. Such behaviour, as well as other life history traits of the gastropods which could be affected by climate change, are intimately linked to larval development rates and accentuate the complexity of this system.

The enclosure-based model system established in the present study may be useful for examining development rates of other protostrongylids, as well as *U. pallikuukensis*, under natural conditions in a variety of intermediate host species, habitat types and geographic locations. As well, using degree-days calculations based on surface temperatures recorded from different geographic regions or those predicted with

global warming, the potential for larval development (and, therefore, risk of parasite establishment and transmission) in other geographic regions could be assessed.

**8. SURVEYS IN THE CANADIAN ARCTIC FOR GASTROPOD
INTERMEDIATE HOSTS OF THE MUSKOX LUNGWORM
UMINGMAKSTRONGYLUS PALLIKUUKENSIS
(METASTRONGYLOIDEA: PROTOSTRONGYLIDAE)**

8.1 Abstract

As part of an investigation of the geographic distribution of the muskox lungworm *Umingmakstrongylus pallikuukensis*, tundra habitats were surveyed for potential terrestrial and freshwater gastropod intermediate hosts. On the central arctic mainland, near the hamlet of Kugluktuk, Nunavut, Canada, wet and mesic sedge meadows, riverbanks and lake shores and the water bodies associated with each of these habitat types were sampled. Near Simpson Bay on Victoria Island, Nunavut, Canada, riverbanks and lake shores were sampled. Terrestrial gastropods were surveyed systematically using a water bath technique, freshwater gastropods were surveyed opportunistically. On the mainland 6 terrestrial species were found: *Deroceras laeve*, *Columella alticola*, *C. edentula*, *Euconulus fulvus*, *Vertigo* sp. and *Catinella* sp.; and 4 freshwater species: *Aplexa hypnorum*, *Physa jennessi jennessi*, *Stagnicola* sp. and *Valvata* sp.. On Victoria Island 1 terrestrial, *D. laeve*, and 3 freshwater species, *A. hypnorum*, *Stagnicola* sp., and *Valvata* sp. were found. In the laboratory, larvae of *U. pallikuukensis* developed from first to third stage in *D. laeve*, *Catinella* sp., *C. alticola*, *A. hypnorum*, and *E. fulvus*, but only to second stage in *P. j. jennessi*, after which all remaining snails died. The presence of 2 potential intermediate host species on Victoria Island, raises the possibility that, if introduced, *U. pallikuukensis* could establish in the muskox population.

8.2 Introduction

Umingmakstrongylus pallikuukensis is a protostrongylid nematode of muskoxen found in cysts up to 4 cm in diameter in the lungs. It was present in 92.5% of muskoxen surveyed from 1989 - 1990 in the region of the Rae and Richardson river

valleys, west of Kugluktuk, Nunavut, Canada (67°49'N, 115°08'W) (Gunn and Wobeser, 1993). The geographic distribution of the parasite appears to be limited to the region west of Kugluktuk, Nunavut (J. S. Nishi, unpubl. obs.), extending west of Paulatuk and southwest to near Norman Wells (65°17'N, 126°50'W), Northwest Territories, Canada (S. J. Kutz, unpubl. obs.) (Fig. 1). *Umingmakstrongylus pallikuukensis* has not been found on Victoria Island (S. J. Kutz, unpubl. obs) or Banks Island (Gunn et al., 1989; Rowell, 1989) despite thorough examinations of several hundred animals during commercial muskox harvests. On the mainland extending east of Kugluktuk to the Adelaide Peninsula, Nunavut, dorsal spined first-stage protostrongylid larvae (L1) have been recovered from the feces of muskoxen in the absence of adult *U. pallikuukensis* in the lungs (S. J. Kutz and J. S. Nishi, unpubl. obs.), suggesting that these L1 may be produced by a species of protostrongylid other than *U. pallikuukensis*.

Muskoxen are an important wildlife species in the Arctic, used for food and commercial purposes (sport hunting, harvests for sale of meat and hides, tourism) (Gunn et al., 1990; 1991). As *U. pallikuukensis* is potentially pathogenic, an understanding of its effect on muskoxen and factors affecting its development, transmission and current geographic range, is important.

The limited geographic distribution of *U. pallikuukensis* may reflect a combination of historical events and contemporary climatic and biophysical conditions. Hoberg et al. (1995) discussed the distribution in the context of historical isolation of the host-parasite system on deep (during the Pleistocene) and more recent scales. Local extirpations of muskoxen, probably associated with European colonization of North America and perhaps climatic conditions, resulted in severely reduced and disjunct populations (Gunn, 1982; Gunn, 1990). Muskox populations have since recovered and have a continuous distribution on the mainland north of the treeline and on the central and western arctic islands across the Northwest Territories and Nunavut (Fig. 1) (Fournier and Gunn, 1998). It appears that the distribution of *U. pallikuukensis* has not, however, followed this expansion of the muskox populations and other factors influencing its geographic distribution must be examined.

Umingmakstrongylus pallikuukensis requires a gastropod intermediate host to develop from the first larval stage (L1, shed in the feces of muskoxen), to the third larval stage (L3, the stage which is infective to muskoxen). Larval development occurs more quickly at warmer temperatures, and there is a lower threshold below which no development is detected (8.5 C for *U. pallikuukensis* in *Deroceras laeve*) (see Chapter 6). Because of the effect of temperature on development, the geographic distribution of

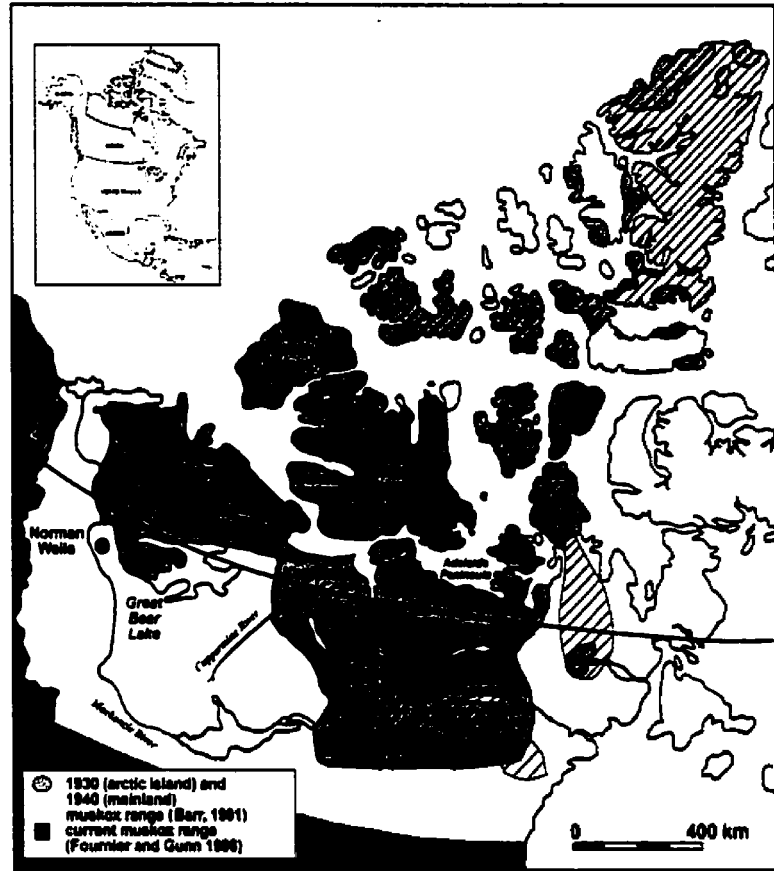


Figure 8.1. Map of the Northwest Territories and Nunavut Territory, Canada, showing historic and current muskox range and gastropod survey areas (see text for details).

U. pallikuukensis is intimately linked to climatic conditions. Hoberg (1997), suggested that the recent “appearance” of *U. pallikuukensis* may represent an emerging parasite, responding to changing climatic conditions. Warming trends of 1.6 C and 0.8 C over the last 51 yr in the Mackenzie and Arctic regions, respectively (Environment Canada, Atmospheric Environment Service, Climate Research Branch), do not refute this contention.

Another critical factor determining the geographic distribution of *U. pallikuukensis* is the availability of suitable gastropod intermediate host species. Unfortunately, the literature on gastropod abundance and distribution in the North American Arctic is sparse, particularly for terrestrial gastropods, and is primarily anecdotal (Mozley, 1937; Pilsbry, 1946, 1948; Clarke, 1973; Holyoak, 1983).

In the present study the gastropod fauna was surveyed in a region of the mainland where the *U. pallikuukensis* is present, (near Kugluktuk, Nunavut), and in a region on Victoria Island, Nunavut where, despite high densities of muskoxen, the parasite is apparently absent. Also, through experimental infections in the laboratory, the ability of larvae of *U. pallikuukensis* to develop in the different gastropod species recovered was determined.

8.3 Materials and Methods

8.3.1 Gastropod surveys

Study sites: On the mainland, surveys were conducted west of Basil Bay and along the Rae and Coppermine rivers; on Victoria Island surveys were conducted near Simpson Bay (Fig. 8.1). On the basis of plant species composition, moisture and topographical location 4 habitat types were defined: wet sedge meadows (WSM); mesic sedge meadows (MSM); riverbanks (RB); and lake shores (LS).

All sites were surveyed in August. On the mainland in 1995, 3 WSM, 3 MSM and 2 LS sites near Basil Bay were surveyed. These sites, 2 more MSM and 1 RB site near the Rae River, and 2 RB sites along the Coppermine River were surveyed in 1996. In 1997, only 1 RB site was surveyed along the Coppermine River (Fig. 8.1). On Victoria Island, 5 RB and 5 LS sites were surveyed near Simpson Bay on August 21, 1997 (Fig. 1).

Wet sedge meadows occurred in low-lying drainage and seepage plains and because these habitats were frequently located close to small, shallow ponds, many sites were inundated with standing water. These meadows were dominated by the sedge *Carex aquatilis*. Mosses provided a consistent ground cover, but cottongrasses

(*Eriophorum angustifolium*, *E. callitrix* and *E. scheuchzeri*) were less abundant. There were few willows (*Salix* spp.) and forbs other than *Pedicularis* spp. and *Equisetum scirpoides* were generally absent.

Mesic sedge meadows occurred in comparatively well-drained areas with small tussocks and contained an increased diversity of graminoids (e.g. *Agrostis* spp., *Festuca* spp., *Poa* spp, and *Hierochloe odorata*) and forbs (*Saxifraga* spp., *Polygonum* spp., *Stellaria* spp., *Melandrium* spp., and *Pedicularis* spp.) growing on small tussocks. Mosses provided a consistent ground cover. *Carex aquatilis* and *E. angustifolium* were dominant, but relative to WSM, there was an increased occurrence of other sedges (*C. misandra*, *C. bigelowii*, *C. membranacea*, *C. rupestris*, and *Carex* spp.), and occasionally rushes (*Juncus* spp.). Willows (*Salix arctica*, and *S. lanata*) and dwarf shrubs (*Dryas integrifolia* and *Arctostaphylos rubra*) were also sporadically found in this habitat type.

The overall moisture regime of LS and RB habitats were similar to MSM. Sampling of these habitats was restricted to within 15 m of the respective shorelines. Both LS and RB habitats were distinguished by the consistent ground coverage with *Salix reticulata* (ca. 10 % cover) and mosses. *Carex aquatilis* and other sedges (*Carex* spp. and *E. angustifolium*) were present in these habitats, but were less abundant than in either WSM or MSM. Abundance of forbs was greater than in MSM. Dwarf shrubs that were characteristic of LS and RB habitats included *A. rubra*, *D. integrifolia*, *S. arctica*, *Rhododendron lapponicum*, *S. lanata*, *Cassiope tetragona*, and *Vaccinium uliginosum*. Terricolous lichens (*Cetraria nivalis*, *C. islandica*, *C. cucullata*) were also conspicuous. Small hummocks (ca. 0.1 – 0.25 m in height) were common in LS but absent from RB. Riverbank sites were associated with slower moving tributaries of the Rae and Coppermine rivers, lake shore habitats with large, deep lakes.

On Victoria Island, the LS habitats were similar to those on the mainland, but had apparently been subject to more grazing pressure (mammalian and avian as evidenced by feces) and the vegetation was not as dense nor as tall. The RB habitats on Victoria Island were similar to those on the mainland, but the associated rivers were smaller.

Gastropod collections: Terrestrial gastropods were surveyed using a water bath technique modified from Kralka (1986). Turf samples, approximately 20 cm in diameter and 10 cm deep, were placed in plastic pails within 4 hr of collection. Water was added to the pails to a depth of 3–4 cm and a lid secured (day 1). The vegetation and the lid and sides of the pails were examined the following day (day 2), gastropods

were removed and more water was added (to a depth of 6-7 cm). On day 3 the vegetation and pails were again examined for gastropods and enough water was added to ensure all the soil was submerged. The pails were examined again on day 4, after which they were discarded.

In 1995 and 1996, MSM and WSM sites were sampled on the mainland at 20 m intervals along 2 100 m straight transects (total of 10 samples for each site). At LS sites in 1995, 4 samples were taken at 2 m intervals on each of 3 lines (approximately 2 m apart) extending perpendicular to the shoreline. This was done in 2 separate areas at each LS site (a total of 24 samples for each site). For LS sites in 1996, 5 samples were taken within 1.5 m of the shoreline from 2 separate areas at each site (a total of 10 samples for each site). Of the 3 RB sites sampled in 1996, 1 was associated with a branch of the Rae River through which water flowed only during spring melt, but a permanent pond remained throughout the summer. For this site, 5 samples were taken at 20 m intervals along 2 straight 100 m transects which ran parallel to and within 15 m of the pond. The other 2 RB sites were sampled on multiple straight lines extending perpendicular from the shoreline for no more than 5 m. On Victoria Island, 5 arbitrarily selected samples were taken within 1.5 m of the shoreline at each LS site and within 5 m of the shoreline at each RB site.

Freshwater gastropods were sampled opportunistically either by hand-picking or by sweeping hand held sieves through the water bodies at all habitat types on the mainland and at all RB sites on Victoria Island. Freshwater gastropods were not quantified. Gastropods were identified with the aid of Pilsbry (1946, 1948) and by J. Van Es, University of Alberta, Edmonton, Alberta, Canada.

Statistical analysis: The Wilcoxon-Mann-Whitney test was used to compare gastropod densities between transects within a site and between habitats in different years. The Wilcoxon-Mann-Whitney test (for LS) or a Kruskal-Wallis one-way analysis of variance, followed by a multiple comparisons test, were used to compare gastropod densities within and between habitat types (Siegel and Castellan, 1988). Most analyses were done using the computer program StatView™ SE + Graphics (Abacus Concepts Inc., 1988). The probability of significance was set at $P \leq 0.05$ for all statistical tests.

8.3.2 Gastropod infections with *U. pallikuukensis*

When possible, within a species (i.e., *Deroceras laeve*, *Vertigo* sp., freshwater species), gastropods of similar size were used for the experimental infections. For some species (i.e. *Euconulus fulvus*, *Columella alticola*, and *Catinella* sp.) few specimens were available, therefore, specimens of different size classes were used. Insufficient specimens of *Columella edentula* and *Valvata sincera helicoidea* were available for experimental infections. Groups of 6 gastropods of each species were exposed to L1 on filter paper in petri dishes as described by Hoberg et al. (1995). First-stage larvae of *U. pallikuukensis* were obtained from the feces of an experimentally infected muskoxen (Kutz et al. 1999b). A drop of larval suspension was placed on the foot of each gastropod and the remaining suspension put on the filter paper (a total of 3000 L1/dish). Every 20-30 min for 2.5-3 hr gastropods were removed from the sides and lids of the dishes and placed on the filter paper. After the exposure procedure terrestrial gastropods were housed overnight at room temperature (20-22 C) in petri dishes containing moistened filter paper and lettuce. Freshwater species were housed in jars of tap water.

The following day terrestrial gastropods were rinsed with distilled water and moved to glass petri dishes (maximum of 6/dish) with moistened filter paper and pieces of lettuce, carrots, chalk and oven dried (70 C X 48 hr) natural vegetation from gastropod survey waterbaths. Freshwater gastropods were rinsed, housed in glass jars containing 500-700 ml of distilled water, and provided with similar food. All gastropods were maintained at room temperature with approximately 8-12 hr of light/day. Food and water were changed every 4-7 days. Gastropods were examined microscopically for foot lesions (Kutz et al., 2000b) and then digested (Hoberg et al., 1995) at various days post-infection (PI). Control gastropods, when available, were treated and examined in the same manner except that they were not exposed to L1.

8.4 Results

8.4.1 Gastropod surveys

The results from the gastropod surveys are presented in Tables 8.1, 8.2, and Figure 8.2. On the mainland 6 terrestrial species were found: *Deroceras laeve*; *Columella alticola*; *Columella edentula*; *Euconulus fulvus*; *Vertigo* cf *modesta*; *Catinella* sp.; and 4 freshwater species: *Aplexa hypnorum*; *Physa jennessi jennessi*; *Valvata sincera helicoidea*; *Stagnicola* sp. (*S. arctica* or *S. kennicotti*). Riverbanks had the highest diversity with all 6 species of terrestrial gastropods found in this habitat. On

Table 8.1: Mean number (range) of terrestrial gastropods recovered from turf samples (gastropods/m²) from four tundra habitat types on the central arctic mainland (ML) and on Victoria Island (VI), Canada.

	ML WSM ¹						ML RB			ML LS			VI RB		VI LS	
	Aug 23-	Aug 7-16	Aug 23-31	Aug 7-16	Aug 7-16	Aug 7-16	Aug 7-16	Aug 7-16	Sept 8-9	Aug 23-31	Aug 7-16	Aug 21	Aug 21	Aug 21	Aug 21	Aug 21
	31 [†] 1995	1996	1995	1996	1996	1996	1996	1997	1997	1995	1996	1997	1997	1997	1997	1997
No. of sites	3	3	3	5	3	3	1	2	2	2	2	5	5	5	5	5
No. of samples	30	30	30	50	42	42	50	48	20	25	25	25	25	25	25	25
Total species	1	0	2	2	6	6	6	2	2	0	0	0	0	0	0	1
<i>D. laevis</i>	0.3 (0.0-0.8)	0 (0.0-0.0)	1.6 (0.0-15.9)	4.5 (0.0-55.7)	3.2 (0.0-32.0)	3.2 (0.0-32.0)	3.8 (0.0-40.0)	1.0 (0.0-8.0)	9.9 (0.0-31.8)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	6.1 (0.0-31.8)
Pupillidae [‡]	0 (0.0-0.0)	0 (0.0-0.0)	0.3 (0.0-8.0)	0.3 (0.0-8.0)	29.4 (0.9-207.0)	29.4 (0.9-207.0)	15.3 (0.0-135.0)	79.1 (0.0-684.0)	165.0 (0.0-1058.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
<i>Catinella</i>	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	5.5 (0.0-39.8)	5.5 (0.0-39.8)	7.2 (0.0-63.7)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
<i>E. fulvus</i>	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	1.7 (0.0-16.0)	1.7 (0.0-16.0)	0.8 (0.0-8.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
Total density	0.3 (0.0-8.0)	0 (0.0-0.0)	1.9 (0.0-23.9)	4.8 (0.0-55.7)	39.8 (0.0-223.0)	39.8 (0.0-223.0)	27.1 (0.0-175.0)	80.1 (0.0-684.0)	175.0 (8.0-1058.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	6.1 (0.0-31.8)	6.1 (0.0-31.8)

¹WSM - wet sedge meadow, MSM - mesic sedge meadow, LS - lake shore, RB - riverbank.

[†]Time periods during which samples were collected.

[‡]Pupillidae in MSM and LS were *Vertigo* sp. only, those in RB were *Vertigo* sp., *Columella edentula*, and *C. alticola*.

Table 8.2: Presence (+) or absence (-) of freshwater gastropods in water bodies associated with the four habitat types on the central arctic mainland (ML) and riverbank habitats on Victoria Island (VI), Canada.

Gastropod species	ML WSM*		ML MSM		ML RB		ML LS		VIRB Aug 21 1997
	Aug 23-31 1995	Aug 7-16 1996	Aug 23-31 1995	Aug 7-16 1996	Aug 7-16 1996	Sept 8-9 1997	Aug 23- 31 1995	Aug 7-16 1996	
<i>A. hypnorum</i>	+	+	+	+	+	+	+	+	+
<i>P. j. jennessi</i>	+	+	+	+	+	+	+	+	-
<i>Stagnicola</i> sp.	-	-	-	-	+	+	+	+	+
<i>Valvata</i> sp.	-	-	+	-	-	-	-	-	+

* WSM - wet sedge meadow, MSM - mesic sedge meadow, RB - riverbank, LS - lake shore.

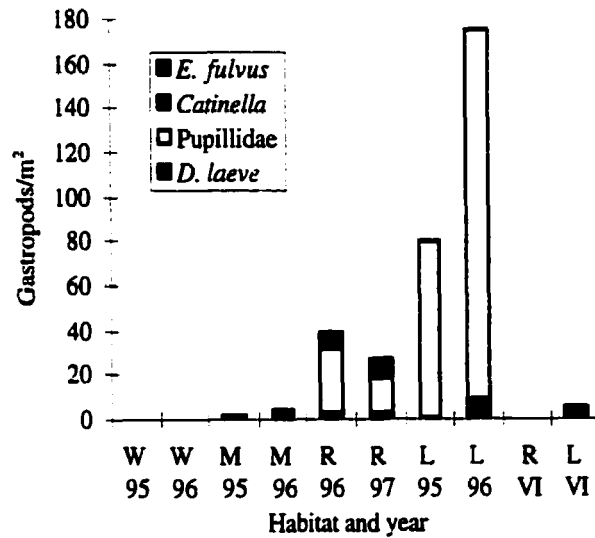


Figure 8.2. Abundance of gastropod species on the mainland in wet sedge meadows (W), mesic sedge meadows (M), riverbanks (R) in 1995 (95), 1996 (96) and 1997 (97), and on Victoria Island in 1997 in lake shores (L VI) and riverbanks (R VI).

Victoria Island, 1 terrestrial species, *D. laeue*, was found in LS habitats only, and 3 freshwater species, *A. hypnorum*, *Valvata* sp., and *Stagnicola* sp., were found in water bodies associated with RB habitats. Gastropods were highly aggregated within habitats.

In 1995 on the mainland, there was no significant difference in gastropod density between sites within habitat types (WSM: $df = 2$, $KW = 2$, $P = 0.3679$; MSM: $df = 2$, $KW = 4.11$, $P = 0.1281$; LS: $W_x = -0.961$, $P = 0.3367$). Lake shores had significantly more gastropods overall ($df = 2$, $KW = 38.738$, $P = 0.0001$), with more *Vertigo* sp. than either the WSM or MSM (WSM: $df = 2$, $KW = 34.03$, $P \leq 0.05$; MSM: $df = 2$, $KW = 32.7$, $P \leq 0.05$). There was no difference in density of *D. laeue* among habitat types ($df = 2$, $KW = 2.191$, $P = 0.3343$).

In 1996 on the mainland, the density of *D. laeue* differed among the MSM sites ($df = 4$, $KW = 15.893$, $P = 0.0032$) and among the RB sites ($df = 2$, $KW = 16.412$, $P = 0.0003$), but not among the LS ($W_x = -0.946$, $P = 0.344$). No gastropods were recovered from any of the WSM sites. Riverbanks had a higher overall density of gastropods compared to WSM and MSM (WSM: $df = 3$, $KW = 57.6$, $P \leq 0.05$; MSM: $df = 3$, $KW = 40.5$, $P \leq 0.05$), with more pupillids (WSM: $df = 3$, $KW = 38$, $P \leq 0.05$; MSM: $df = 3$, $KW = 36$, $P \leq 0.05$). Lake shores also had a higher overall density of gastropods compared to WSM and MSM (WSM: $df = 3$, $KW = 84.55$, $P \leq 0.05$; MSM: $df = 3$, $KW = 67.6$, $P \leq 0.05$), more pupillids than WSM and MSM (WSM: $df = 3$, $KW = 69.8$, $P \leq 0.05$; MSM: $df = 3$, $KW = 67.6$, $P \leq 0.05$), and more *D. laeue* than WSM, MSM and RB (WSM: $df = 3$, $KW = 54.3$, $P \leq 0.05$; MSM: $df = 3$, $KW = 34.2$, $P \leq 0.05$; RB: $df = 3$, $KW = 34.7$, $P \leq 0.05$).

Comparing 1995 to 1996, there were more *D. laeue* and *Vertigo* sp. in LS in 1996 (*D. laeue*: $W_x = -5.315$, $P = 0.0001$; *Vertigo* sp.: $W_x = -2.566$, $P = 0.0103$). There were, however, no differences between years for WSM and MSM (WSM: $W_x = -1$, $P = 0.3173$; MSM: $W_x = -1.819$, $P = 0.0689$). There was no difference in gastropod density between RB from 1996 to 1997. On Victoria Island in 1997, there was no difference in the density of *D. laeue* among LS ($df = 4$, $KW = 8.094$, $P = 0.0882$). Meaningful statistical comparisons to mainland LS are not possible because these habitats were not sampled in 1997, however, densities were similar between the island in 1997 and the mainland in 1995 and 1996.

Abundance of gastropod species differed among habitat types. The slug *D. laeue* was the most common species in MSM (85-93% of the total gastropods recovered), the only terrestrial species in WSM, the only species in all habitat types, and the only terrestrial species on Victoria Island. Members of the Pupillidae were the most common species found in LS (*V. cf modesta*, 94-99%) and RB habitats (*V. cf modesta*,

C. edentula and *C. alticola*, 56-74 %). *Columella* spp., *Euconulus fulvus* and *Catinella* sp. were absent from WSM, MSM and LS.

The behaviour and location within the microhabitat of the various gastropod species differed. *Deroceras laeve* was observed to be a highly mobile slug. During one calm, clear evening in August 1997, numerous slugs were observed climbing on the vegetation to a vertical height up to 30 cm. *Vertigo* cf *modesta* were found near the surface of the soil and in the vegetation. *Catinella* sp. were found on vegetation only after being in a water bath (not during hand searches or under masonite boards used in other studies), and were usually coated in sand or mud. *Euconulus fulvus* was often found on or in the folds of rotting leaves. *Stagnicola* sp. were most common in larger lakes and rivers, usually on the bottom or on rocks near the shoreline. In contrast, *A. hypnorum* and *P. j. jennessi* were usually found in small, shallow ponds or very slow flowing tributaries of rivers. They were often observed floating upside down at the water surface or attached to vegetation near the surface.

8.4.2 Gastropod infections with *U. pallikuukensis*

The results of the experimental infections are presented in Tables 8.3 and 8.4. Development from L1 to L3 occurred in *D. laeve*, *C. alticola*, *E. fulvus*, *Catinella* sp., and *A. hypnorum*. The highest prevalence and intensity (based on detection of all larval stages) and earliest L3 were seen in *D. laeve* and *Catinella* sp.. In *P. j. jennessi* development to L2 occurred by 12 days PI. All remaining *P. j. jennessi* died before the next examination day, thus, further development was not determined. Neither infection nor development occurred in *Vertigo* sp. or *Stagnicola* sp.. No scars were observed in the feet of any of the control gastropods or in slugs collected from Victoria Island and no larvae were recovered on digestion. Survival of all species, with the exception of *P. j. jennessi*, was high and there was no difference in survival between infected and control gastropods (Tables 8.3, 8.4). *Stagnicola* spp. and *A. hypnorum* did not feed much during the course of the experiments. The remaining gastropods (infected and control) from the *D. laeve* and *Catinella* sp. experiments were removed on 16 and 20 days PI, respectively, and used in an experiment investigating L3 emergence (Kutz et al., 2000b). The mortality reported for these 2 species extends to 60 days PI while mortality for the other species is reported only up to the last examination day of each respective experiment.

Table 8.3: Experimental infections of terrestrial gastropods with first-stage larvae of *Umingmakstrongylus pallikuukensis*.

Gastropod species	No. gastropods (infected/control)	No. dying (infected/control)	Length in mm (# whorls)	Days post-infection (PI) examined ^a	First L2 (days PI)	First L3 (days PI)	Prevalence ^b	Intensity ^c
<i>D. laeve</i>	24/24	3/3	10-20 (na) ^d	12, 16	12	12	100	6.25
<i>Vertigo</i> sp.	24/24	4/4	1-2 (4-5)	12, 16, 20, 24, 28	na	na	0	na
<i>Catinella</i> sp.	18/9	1/1	2-8 (2-3)	12(3), 16, 20	12	12	90	6.0
<i>C. alticola</i>	8/0	4/na	3-4 (6)	12(3), 16(3), 20(1), 24(1)	16	24	37.5	1.67
<i>E. fulvus</i>	5/0	1/na	nr ^e (nr)	12(1), 16(1), 24(1), 28(1)	na	24	25	1

^aFour specimens were examined on each day unless specified in parenthesis after each day.

^bPrevalence indicates the percent of gastropods containing any first (L.1), second (L.2) or third (L.3) stage larvae of *U. pallikuukensis*.

^cIntensity indicates the mean number of larvae (L.1+L.2+L.3) in the infected gastropods. ^dna - Not applicable.

^enr - n not recorded.

Table 8.4: Experimental infections of freshwater gastropods with first-stage larvae of *Umingakstrongylus pallikuukensis*.

Gastropod species	No. gastropods (infected/control)	No. dying (infected/control)	Length in mm (# whorls)	Days post-infection (PI) examined [*]	First L2 (days PI)	First L3 (days PI)	Prevalence [†]	Intensity [‡]
<i>Stagnicola</i> sp.	24/24	2/0	15-19 (5)	16, 20, 24	na [§]	na	0	na
<i>A. hypnorum</i>	24/21	6/4	12 (4-5)	12, 16, 20, 24	16	24	31.3	3.4
<i>P. j. jennessi</i>	24/24	19/15	5-11 (3)	12	12	na	50	2.5

^{*}Four specimens were examined on each day.

[†]Prevalence indicates the percent of gastropods containing any first (L1), second (L2) or third (L3) stage larvae of *U. pallikuukensis*.

[‡]Intensity indicates the mean number of larvae (L1+L2+L3) in the infected gastropods.

[§]na - Not applicable.

8.5 Discussion

The present study represents the initial steps in understanding the importance of different gastropod species and habitat types in the transmission of *U. pallikuukensis* among muskoxen. It also facilitates the examination of the current geographic distribution of the parasite with respect to distribution and abundance of suitable gastropod intermediate hosts.

8.5.1 Gastropod surveys

Small variations in gastropod sampling techniques between years and habitat types may have affected some results. For example, differences observed between years for LS may reflect differences in sampling techniques rather than real differences; in 1995, samples were taken up to 10 m from the shoreline, whereas in 1996 they were taken only from within 1.5 m of the shoreline. Wet sedge meadows and MSM, however, were sampled at the same time and with comparable methods in both years, and similar patterns of gastropod abundance were observed within these habitat types and relative to LS and RB. Despite discrepancies in sampling techniques, and although the sampling protocol may not have been sufficiently intense to evaluate subtle differences between years or habitat types, we believe that our results describe the general pattern of gastropod distribution and abundance for August in the regions sampled.

The greater diversity of gastropod species in the RBs may reflect differences in gastropod life history traits and habitat preferences, but also may represent a fauna that has not yet reached a stable distribution. According to Clarke (1973) colonization of the Arctic by freshwater gastropods since the Pleistocene is an ongoing process, with some species not yet in distributional equilibrium. Rivers may be a significant route for gastropod dispersal and colonization and this is reflected by the greater species diversity observed in these habitats. The ubiquitous distribution of *D. laeve* on the mainland may reflect this species' high mobility and adaptability with an enhanced ability to colonize new habitats. *Deroceras laeve* is able to hatch and move underwater (Rollo and Shibata, 1991). This may explain its dominance in WSM and MSM habitats which are subject to seasonal flooding.

The gastropod species reported in this study are known to have an arctic distribution. There are, however, few reports of gastropods from the Canadian arctic

islands, and the finding of *D. laeve* on Victoria Island represents a new geographic record (Pilsbry, 1946, 1948; LaRocque, 1953; Holyoak, 1983). The terrestrial species, *Deroceras laeve* and *Vertigo* sp., have been reported from south Baffin Island, Nunavut, and these genera, along with species of the Succineaidae (of which *Catinella* is a member), are known from Greenland (Pilsbry, 1946, 1948; La Rocque, 1953). *Euconulus fulvus* and *C. edentula* have been reported from the mainland of the North American Arctic, but not from the arctic islands (Pilsbry, 1946, 1948; LaRocque, 1953). No terrestrial species have been found on Banks Island (Holyoak, 1983). Among the freshwater species, *Stagnicola* spp., *P. j. jennessi*, *A. hypnorum*, and *V. sincera helicoidea* have been reported across the arctic mainland as well as on Wollaston Peninsula, Victoria Island, but not on Baffin Island (Clarke, 1973). Although there is fossil evidence of *S. arctica* and *V.s. helicoidea* on Banks Island, only *A. hypnorum* has been reported (Holyoak, 1983).

8.5.2 Gastropod infections with *U. pallikuukensis*

The relatively rapid development of *U. pallikuukensis* in *D. laeve* and *Catinella* sp., together with a high prevalence and moderate intensity of infection, suggest that these species are good natural intermediate hosts. Development in *E. fulvus*, *C. alticola*, *P. j. jennessi* and *A. hypnorum* was slower and prevalence and intensity much lower. The terrestrial snails, however, were often found aestivating during these experiments, and the freshwater snails may not have been infected and maintained under ideal conditions (as suggested by the high mortality of *P. j. jennessi* and diminished feeding observed by *Stagnicola* sp. and *A. hypnorum*). Development of protostrongylids in aestivating or starved gastropods may be retarded (Skorping, 1984; Solomon et al., 1996) and this may have influenced the results in these species.

Any consideration of the roles of the various gastropod species in transmission of *U. pallikuukensis* must incorporate aspects of the life history of the gastropods and of the muskoxen. Although terrestrial and/or freshwater gastropods were found in habitat types commonly used by muskoxen (Wilkinson et al., 1976), gastropod behaviour, distribution and abundance relative to muskox grazing strategies and behaviour will influence the contribution of any given gastropod species to parasite transmission.

Although not the most abundant gastropod overall, *Deroceras laeve* was ubiquitous among habitats in this study, was very mobile and is a highly suitable intermediate host both in the laboratory (this study and Kutz et al., 2000b) and in field experiments (see Chapter 7). *Catinella* sp. and *E. fulvus*, also suitable laboratory

intermediate hosts, were found only at RB, and appeared to spend much time in the soil (*Catinella*) or in the leaf litter (*E. fulvus*). The importance of these 2 species in transmission of *U. pallikuukensis*, therefore, may be limited. *Vertigo* sp., found at a high density at LS and RB, was unable to support larval development. The suitability of the remaining terrestrial and freshwater species as laboratory intermediate hosts of *U. pallikuukensis* could not be determined in the present study. Further controlled experiments, with larger sample sizes and in which gastropods are reared under conditions in which they remain active and healthy, are required before the importance of these species in the field can be addressed.

The significance of freshwater species in the epidemiology of protostrongylids deserves attention. Experimentally, it has been established that the freshwater snails, *Lymnaea* spp., support development of *Parelaphostrongylus tenuis*, *Muellerius capillaris*, *Neostrongylus linearis* and *Elaphostrongylus rangiferi* to L3 (Anderson, 1963; Hourdin et al., 1990; Skorping, 1985). Temperatures in small tundra ponds can at times exceed air temperatures (Holyoak, 1983) and may be sufficient for larval development in freshwater gastropods. Also, survival and subsequent infectivity of L1 is diminished by desiccation and fluctuating temperatures, particularly freezing and thawing (Shostak and Samuel, 1984; Lorentzen and Halvorsen, 1986; Morrondo-Pelayo et al., 1992). It might be expected, therefore, that L1 in aquatic habitats, where moisture is constant and temperature fluctuations dampened, have a greater chance of successfully completing their development in intermediate hosts than if they remained in terrestrial habitats, exposed to desiccation, widely fluctuating surface temperatures, and increased UV light. For the same reasons, L3 of *U. pallikuukensis* that emerge from gastropod intermediate hosts and can survive in water at 0–4 C for more than 1 yr (Kutz et al., 2000b), may have a similar pattern of survival in aquatic versus terrestrial habitats. For L3 that remain in the gastropods, the behaviour of *A. hypnorum* and *P. j. jennessi*, frequently found floating at the water surface or climbing on vegetation near the surface, would make the L3 readily accessible to muskoxen feeding or drinking at that site. The significance of development in freshwater gastropods, with or without subsequent emergence of L3, as an important life history strategy for *U. pallikuukensis* and protostrongylids in general, should be investigated further.

The contemporary geographic range of *U. pallikuukensis* may not be limited by gastropod distribution. Two potential intermediate host species were found on Victoria Island and it is reasonable to assume that suitable terrestrial and freshwater gastropods are present on the mainland east of Kugluktuk (Mozley, 1937; Pilsbry, 1946, 1948; La Rocque, 1953; Clarke, 1973). The presence of these intermediate hosts, however, does

not ensure parasite transmission. The abundance and distribution of potential intermediate host species relative to muskox densities and habitat use are important for successful parasite transmission. Examination of the gastropod-muskox interactions may provide insight into the reasons for the limited geographic distribution of the parasite.

Assuming gastropod abundance and distribution are adequate for parasite development and transmission, the limited distribution of *U. pallikuukensis* on the mainland is puzzling. If *U. pallikuukensis* was restricted to the remnant mainland muskox population north of Great Bear Lake during the first half of this century, it is possible that it is slowly expanding its range with this host population. If this is so, and climatic conditions and gastropod abundance are sufficient, it is expected that the parasite will further expand its geographic distribution across the mainland. The contemporary absence of *U. pallikuukensis* from the eastern mainland and Victoria Island suggests that either the remnant muskox populations in the first half of this century in these areas were free of the parasite or alternatively, recent climatic conditions may have been unsuitable for its development and transmission. It is also possible that gastropod abundance on Victoria Island is insufficient to maintain the life cycle; this explanation is less likely for the mainland.

There is potential for *U. pallikuukensis* to expand its range on the mainland and to the arctic islands. *Umingmakstrongylus pallikuukensis* is well adapted to its arctic environment. Near Kugluktuk, it developed from L1 to L3 within 4 weeks in July, overwintered as L3 in gastropods and as L2 in gastropods. These L2 resumed development the following year (see Chapter 7). These efficient development traits, coupled with the long period of patency (at least 2 yr in an experimentally infected muskox, Kutz et al. 1999b), large numbers of L1 produced by adult parasites (Kutz et al., 1999b), general resistance of protostrongylid L1 to environment conditions (Boev. 1975), and ability of L3 to leave the intermediate hosts (Kutz et al., 2000b) and perhaps survive on vegetation, may enable *U. pallikuukensis* to persist in a muskox population over 1 or more consecutive years when climatic conditions are insufficient for development to occur within a single season. Additionally, a warming global climate, predicted (and already observed) to be most extreme in the Arctic, may, by improving gastropod survival, extending the seasonal window when gastropods are active (and larvae may be developing), and accelerating the rate of larval development, facilitate development and transmission of *U. pallikuukensis* at higher latitudes.

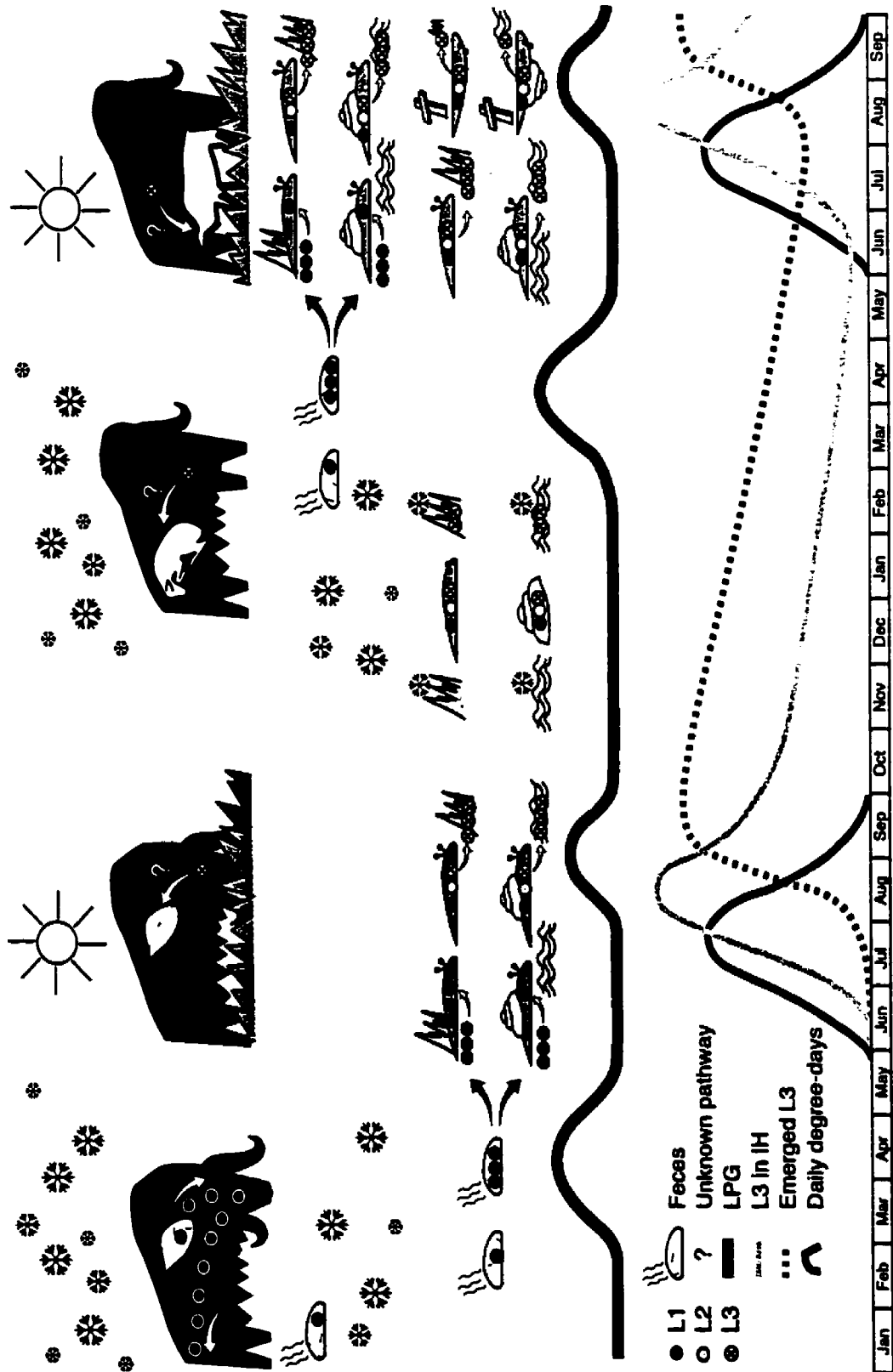
9. DISCUSSION: THE EPIDEMIOLOGY OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS*

9.1 Introduction

Since its discovery in 1988 and description in 1995 *U. pallikuukensis* has been the focus of intensive laboratory and field experiments that have resulted in tremendous progress in understanding the biology of this parasite. Research reported in this thesis has addressed critical aspects of the biology of *U. pallikuukensis* within both a comparative context among the Protostrongylidae and an epidemiological context. In some instances conclusions drawn from these studies have challenged traditional views of protostrongylid biology and transmission (e.g., emergence of L3), and in others have supported what is known (e.g., the typical life cycle). In yet other ways, this work has provided novel information specifically on *U. pallikuukensis* (lung pathology, larval development rates and L3 availability in the field) that should be examined among the Protostrongylidae, as well as insights (e.g., stylet in the L3) that may have an impact on understanding patterns of development and phylogeny among strongylates and related free-living nematodes.

Integrating this new information for the biology and development of *U. pallikuukensis* with general features of the Protostrongylidae, I now propose a descriptive model for the transmission of *U. pallikuukensis* in the Arctic which considers critical biotic and abiotic factors (Fig. 8.1). This model considers patterns of parasite development in the definitive hosts, production and survival of L1, infection of gastropods and subsequent larval development rates, larval emergence, and availability of L3 to muskoxen in the Arctic. Transmission patterns of *U. pallikuukensis* are interpreted in relation to the ecology of the definitive and intermediate hosts as well as with respect to climate change. The effects of the parasite on the host population and factors determining its current geographic distribution are discussed. Finally, the use of this parasite and the novel enclosure system to monitor or predict the effects of climate change on parasite distribution and abundance in the Arctic are outlined.

Figure 9.1. An illustrated model for the annual pattern of development and transmission of *Umingmakstrongylus pallikuukensis* in the Arctic. L1 - first-stage larvae, L2 - second-stage larvae, L3 - third-stage larvae, LPG - larvae per gram, IH - intermediate host.



9.2 A proposed annual pattern for transmission of *U. pallikuukensis* in the Arctic

9.2.1 *Umingakstrongylus pallikuukensis* in the definitive host

Umingakstrongylus pallikuukensis follows the typical protostrongylid life cycle, requiring a gastropod intermediate host for larval development outside the definitive host. Third-stage larvae in gastropods or free on the vegetation are ingested by muskoxen and migrate to the lungs. The route of migration could not be determined in these studies and, unless a suitable laboratory host is identified, it is likely that this aspect of the biology of *U. pallikuukensis* will remain a mystery.

The prepatent period of *U. pallikuukensis* is approximately 90 days and the duration of patency extended at least 2 yr in 1 experimentally infected muskox. Although the pattern of larval acquisition by wild muskoxen may differ from the experimental exposure (smaller number of L3 over a more prolonged period), and the cyst populations of wild animals are considerably more variable, it seems likely that adults of *U. pallikuukensis* are long-lived in nature.

Longevity of adult *U. pallikuukensis* may ensure the long term survival of the parasite in a muskox population. For example, if in 1 year climatic conditions were unsuitable for larval development in the intermediate hosts, long lived adult parasites could provide L1 the following year. The cyst architecture, which appears to maintain the parasites in an environment suitable for continued reproduction and somewhat protected from the host immune system, may facilitate this longevity.

9.2.2 Production and survival of L1

Patterns of L1 production and survival influence the availability of L1 to gastropods and, therefore, the subsequent larval development and availability of L3 to muskoxen. Seasonal variations in larval shedding are known for most protostrongylids, typically with peaks of L1 production at times of stress, for example around rut or parturition (Uhazy et al., 1973; Halvorsen et al., 1985). In this model we assume similar seasonal patterns of larval production for *U. pallikuukensis*, with a primary periparturient peak and a lower peak associated with the rut (Fig. 9.1).

The quantity of L1 shed in the environment by a single muskox is potentially considerable. For example, at 26 mo post-infection an experimentally infected muskox produced a total of 1.6 million L1/day (range of 0.6 - 3.4 million L1/day). The intensity of infection in this animal, based on cyst counts at post mortem, was within the limits

observed in naturally infected animals. Exactly how this environmental contamination is translated to infection of gastropods is not known, but depends on larval survival, dispersal, availability of suitable intermediate hosts and suitable climatic conditions for infection of gastropods.

Seasonally defined environmental effects, including ambient temperature, snow cover and moisture, may be critical as parameters limiting larval survival and availability to intermediate hosts. Larvae shed in the Arctic during the winter and spring (March to mid-May) would be deposited in feces on the snow. Frozen almost immediately and perhaps covered with snow, L1 would probably survive well until the spring thaw. Larval survival rates may subsequently decrease with the varying weather conditions in May and June (repeated thawing and freezing). These same weather conditions, however, may be of value in dispersing L1 in the environment. Repeated freezing and thawing may break down fecal pellets, and the run-off associated with the melting snow and ice would result in efficient dispersal of larvae in a moist environment, with an increased potential to infect gastropods. During the summer, larvae shed on to the tundra would be subject to varying temperatures, ultra-violet light and desiccation, resulting in decreased survival. Additionally, because of limited precipitation, larvae would be less likely to be dispersed from the feces into the environment. Larvae shed during the summer but deposited in water may have better survival rates.

Survival of L1 shed around the time of rut (August and September) may vary. In August, desiccation and variable temperatures may reduce survival rates of L1. In contrast, during September, L1 frozen immediately and subsequently covered by snow and sheltered from the sunlight, desiccation and fluctuating temperatures, may have relatively good survival rates. During spring thaw these L1 may also become widely dispersed in the environment with those shed during winter and spring.

The seasonal patterns of L1 production and survival should be further investigated to quantify patterns of environmental contamination in the Arctic. This may best be done by examining the feces from different age and sex classes from naturally infected populations at various times throughout the year. In addition, studies of larval survival should be done both in the laboratory as well as under field conditions to determine the longevity of larvae in the environment, for example, whether viable larvae can remain in the environment over several years.

9.2.3 Infection of gastropods

Once in the environment L1 require suitable gastropod intermediate host species for development from L1 to L3. The gastropod surveys demonstrated that in the vicinity

of Kugluktuk, Nunavut on the Arctic mainland, several potential terrestrial and freshwater intermediate hosts are present and active from early June to mid-September. *Deroceras laeve*, the most widespread and mobile of the terrestrial species, is an excellent intermediate host for *U. pallikuukensis*. Other terrestrial species, though more abundant in some habitat types, are less widespread (e.g., *Catinella* sp., *Euconulus fulvus*, *Columella* spp.), or are unsuitable as intermediate hosts (e.g., *V. cf. modesta*). Freshwater snails, *A. hypnorum* and probably *P. j. jennessi*, also support larval development, suggesting that an aquatic cycle of larval development may parallel the terrestrial cycle and play an important role in the epidemiology of *U. pallikuukensis*.

Infection rates of gastropods may be affected by environmental parameters such as moisture and microhabitat temperatures. Larvae depend on a thin film of moisture for movement and to actively infect gastropods. Ambient temperatures are also important, with infection rates of gastropods increasing as temperatures increase (Skorping, 1982). The proposed patterns of *U. pallikuukensis* larval production suggest that the most L1 would be available and best dispersed in the environment during the spring. It is likely, therefore, that infection of terrestrial gastropods may occur most commonly in the spring and early summer when microhabitats are moist, temperatures, particularly at the soil surface, are warming, and many L1 are available.

9.2.4 Larval development and availability

Development from L1 to L3 depends on suitable intermediate hosts and environmental conditions. Subsequent availability of the L3 to the definitive host depends on rates of larval development, patterns of retention or emergence of L3 from the gastropods, the life history of the gastropods, and environmental conditions. The following discussion of larval development is based on field and laboratory data for development of *U. pallikuukensis* in *D. laeve*. It is acknowledged that these patterns may vary among intermediate host species and that the dynamics may differ in an aquatic system.

Infection of gastropods and subsequent larval development may begin shortly after the gastropods emerge from hibernation in June. At the soil surface, temperatures exceed the calculated threshold for larval development (8.5 C) as early as the beginning of June. The daily degree-days peak during July and then taper off to the middle of September (Fig. 9.1).

Relatively rapid larval development of *U. pallikuukensis* occurs during July and early August. Gastropods infected before the middle of July may develop to L3 in the

same year. The maximum number of L3 in gastropods occurs during August and rapidly declines in September and over winter (Fig. 9.1).

Larvae infecting gastropods towards the end of July or later are not likely to develop to L3 before winter. Sufficient degree-days may be accumulated to allow larvae to develop from L1 to L2 but not to L3; based on laboratory experiments at 23 C larvae develop to L2 by day 4 PI, but not to L3 until day 11 PI. Gastropods may, therefore, contain all 3 larval stages during the winter. These over-wintering gastropods may provide L3 in the environment the following year earlier than those that develop from L1 infecting gastropods in the spring of that year (Fig. 9.1). The contribution of over-wintering larvae to the total L3 available for transmission is not well understood. Carefully designed experiments are necessary to further investigate this aspect of the life cycle.

The decline in the numbers of L3 in gastropods in late August and September may reflect L3 emergence (Fig. 9.1). Emerged L3 in the environment may survive on vegetation or in water for prolonged periods, and perhaps have increased over-winter survival rates relative to L3 which remain in gastropods during hibernation. In addition, L3 which emerge from moribund or dead gastropods and remain on the vegetation or in the water may have an increased availability for transmission over those which remain in the gastropods.

The stylet-like structure in the buccal cavity of the L3 may function to facilitate emergence from gastropods and for feeding in the environment. The ability to feed may increase the longevity of L3 in the environment and, therefore, subsequent transmission to muskoxen.

Emergence of L3 may represent an important life history strategy for *U. pallikuukensis*, providing L3 in the environment when gastropods are not available. Factors inducing emergence are not known, nor has the pattern of emergence in the field been characterized or quantified in detail. Studies under natural conditions, describing L3 emergence and subsequent mobility and survival, are important for understanding the significance of this phenomenon.

9.2.5 Spatial and temporal transmission patterns of *U. pallikuukensis*

Transmission of *U. pallikuukensis* may occur either when L3 in gastropods are accidentally ingested by muskoxen, or when L3, which have emerged from gastropods into the environment, are ingested with vegetation or water. The first mode of transmission requires both temporal and spatial overlap of muskoxen and gastropods,

whereas the second mode requires only spatial overlap of these hosts. A third mode of transmission may be vertical, with L3 transmitted either across the placenta to the fetus or in the milk to the calf. First-stage larvae of *U. pallikuukensis* have been reported in the feces of 3 month old wild muskox calves, indicating the possibility of vertical transmission (Gunn and Wobeser, 1993; Hoberg et al., 1995). This route of transmission could not, however, be investigated in these studies.

The question remains as to where transmission of *U. pallikuukensis* occurs. Specifically, are there habitat types or particular locations that are important for transmission? Based on the presence of excellent intermediate hosts (*D. laeve*), and on information from the available literature on muskox habitat use (Wilkinson et al., 1976), mesic sedge meadows are probably good terrestrial habitat types for transmission of *U. pallikuukensis*. In wetter habitats with freshwater gastropods, however, the dynamics of larval development, emergence and transmission are not well understood and, therefore, the potential for these habitats as foci for transmission cannot be assessed.

Specific sites in which muskoxen concentrate (e.g., high quality vegetation, mineral licks), and in which suitable intermediate hosts are present, could also represent potential foci for transmission. Foci of this type may vary among muskox populations and across geographic regions. Further theoretical and experimental investigations of the life history traits, abundance, and distribution of the various gastropod species in relation to distribution and habitat use of muskoxen, on both broad and more localized scales, are recommended to determine if there are spatial foci for transmission of *U. pallikuukensis* in the Arctic.

The temporal pattern for larval transmission to muskoxen may vary. Third-stage larva are theoretically available in varying quantities in gastropods or on the vegetation and water at all times during the year. Even in winter, muskoxen may ingest emerged L3 while grazing. Considering both emerged L3 and those remaining in gastropods, most L3 would be available in August and September, the fewest probably in June. In theory, low level transmission could occur year round, while a peak may occur during late summer.

If the greatest exposure of muskoxen to L3 occurs during August, the prepatent period and subsequent pattern of larval production appear to maximize the survival of L1 in the environment and subsequent infection of gastropods. Animals infected in August would start producing L1 by November. Small numbers of L1 would be produced during the first 3 mo of patency (until February), and subsequently larval production would increase considerably, depositing numerous L1 in feces on the snow surface. Survival of L1 may be relatively high because larvae would be frozen almost

immediately and remain frozen until the spring thaw. Numerous L1 in the environment during the spring would result in early infection of gastropods, thereby maximizing the potential time (degree-days) available for development to L3.

It is obvious that transmission dynamics are closely linked to ambient environmental settings, vegetation, parasite development and survival, and that ameliorating conditions may have an effect on parasite populations over time. This is of immediate concern in the Arctic where the global warming trend is most pronounced (Environment Canada Atmospheric Environmental Service, Climate Research Branch). A warming climate may result in a longer season for parasite development in the environment and more rapid larval development on a daily basis. In areas where *U. pallikuukensis* is currently established this could mean a wider window for development and transmission, possibly resulting in parasite intensity increasing in the muskox population. In addition, warming conditions may provide suitable environmental temperatures in regions which currently cannot support parasite development, and the parasite may extend its geographic range. The gastropods and vegetation are also intimately linked to the life cycle of *U. pallikuukensis* and, therefore, the effects of climate change at these levels should also be investigated.

9.3 Effects of *U. pallikuukensis* on the host population

The effect of *U. pallikuukensis* on the muskox, at individual and population levels, remains speculative. Although the pulmonary pathology appears limited in mild infections, the infection seems to be cumulative (Gunn et al., 1991; Hoberg et al., 1995) and, as the size and number of cysts increase, pulmonary function may be compromised. In nature, clinical signs of exercise intolerance, direct mortality, and indirect mortality associated with grizzly bear predation, have been attributed to these lungworms. The best strategy for examining the effect of *U. pallikuukensis* on infected populations may be through treatment trials of wild muskoxen, coupled with extensive investigation of causes of mortality in these populations. Computed tomography may prove to be a useful tool for characterizing and quantifying pulmonary pathology and cysts of naturally infected animals. Concurrent investigation of other pathogens is also necessary. The recent discovery of a moderate prevalence of antibody titres to *Toxoplasma gondii* in this muskox population is a reminder that other disease processes may affect the host population dynamics and that such processes may be additive or synergistic (Kutz et al., 2000a). This also suggests that it is important to determine in a

more comprehensive manner the host and geographic distributions of pathogens and parasites across the Arctic.

9.4 The geographic distribution of *U. pallikuukensis*

Hoberg et al. (1995) suggested that the current geographic distribution of *U. pallikuukensis* may be a result of isolation of the host and/or parasite on both deep and more recent historical scales. Our findings also suggest that recent host isolation may be a factor in the restricted distribution of *U. pallikuukensis*. Local extirpations in the first half of this century resulted in few, small, disjunct muskox populations across the mainland and arctic islands (see Fournier and Gunn, 1998). In some of these populations *U. pallikuukensis* may not have been able to maintain itself because of insufficient host densities, climatic conditions, or availability of gastropod intermediate hosts. It appears, however, that the parasite was able to survive in the remnant muskox population north of Great Bear Lake. The current distribution of *U. pallikuukensis* may be following this muskox population as it slowly expands. Unlike caribou, muskoxen are not highly mobile, migratory animals and, therefore, although L1 production is high, the geographic extent of environmental contamination has been limited. Climatic conditions and gastropod availability on the eastern mainland are likely suitable for transmission of *U. pallikuukensis* and we should expect, therefore, the gradual expansion of the parasite's range across the mainland. The presence of an as yet unidentified protostrongylid (based on larvae in the feces) of caribou and muskoxen east of the current known distribution for *U. pallikuukensis* supports that climatic conditions and gastropod species are suitable for transmission of these parasites.

In the absence of human interference, the establishment of *U. pallikuukensis* on the arctic islands is less likely. Muskoxen appear to be the only suitable definitive host of *U. pallikuukensis* and, because movement of muskoxen between the mainland and arctic islands is rare, introduction of the parasite is unlikely. If caribou were suitable hosts a widespread distribution of *U. pallikuukensis* across the mainland and, given suitable climatic conditions and intermediate hosts, on the arctic islands would be expected. An example of the contribution of caribou to the epidemiology of a muskox parasite is apparent with the abomasal trichostrongylid *Teladorsagia boreoarcticus*. The absence of genetic variability between mainland and island populations of this parasite has been attributed to the historical gene flow mediated by movement of the parasite between these locations coincident with annual caribou migrations (Hoberg et al., 1999). Thus, although *T. boreoarcticus* is less common in caribou than muskoxen, it is

the former host species that provides for a wider geographic distribution. Because *U. pallikuukensis* appears to be host specific, translocation of the parasite with the movement of caribou or other species is unlikely.

9.5 *Umingmakstrongylus pallikuukensis* as a model system for investigation of parasite dynamics and climate change

This research has provided the foundation for a descriptive model to help understand current limitations for transmission and distribution of *U. pallikuukensis* (Fig. 9.1). Evolving from a combination of laboratory and field research, including novel studies on larval development in gastropods in an artificial enclosure system in the tundra, this model provides a basis for developing predictions about biotic responses to climate change in the Arctic. Using degree-days calculations incorporating microhabitat temperatures and features of gastropod behaviour, this model may be useful for predicting whether a parasite, if introduced, could establish in a geographic location. Also, by incorporating predicted global warming temperatures it may be used to anticipate the effects of climate change. Tundra-based experiments could employ the enclosure system to examine current development of *U. pallikuukensis* or other protostrongylids in other intermediate hosts, habitat types or geographic locations. This enclosure system could be used to monitor the effects of a changing climate on parasite development rates, and could be modified to simulate global warming and thus used to predict the effects of warming on parasite development.

9.6 Conclusion

In the last 5 years we have made great progress in understanding the biology of an Arctic protostrongylid in its intermediate and definitive hosts. These studies have demonstrated that *U. pallikuukensis* may use several strategies to successfully maintain itself in the muskox population, for example: localized pulmonary pathology; high levels of L1 production; extended patency; use of a variety of terrestrial and freshwater intermediate hosts; rapid development in the environment; overwinter survival; and larval emergence. These findings are important not only with respect to *U. pallikuukensis*, but have increased our knowledge of the Protostrongylidae in general. Perhaps more importantly, this research is the foundation for a model which may be applied to other parasites, geographic locations or climatic conditions to investigate or predict rates of parasite development and availability in the intermediate hosts.

Through this doctoral research and related projects it has become apparent that our knowledge of the parasite fauna of arctic ruminants is sparse. Our understanding of the biotic and abiotic influences on the epidemiology of this parasite fauna is less. The Arctic is recognized as a very fragile ecosystem, where small perturbations at 1 level may have far-reaching consequences at another. Parasites are an example of 1 level in this ecosystem. They can be dramatically influenced by climate and have the potential to be detrimental at another level, the host populations. Further research on biotic and abiotic influences on the parasites of arctic mammals should be pursued with an awareness of how the changing global climate will influence the parasites, their hosts and the arctic ecosystem as a whole.

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