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Experimental Infections of Muskoxen (*Ovibos moschatus*) and Domestic Sheep with *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae): Parasite Development, Population Structure, and Pathology

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Experimental infections of muskoxen (*Ovibos moschatus*) and domestic sheep with *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae): parasite development, population structure, and pathology

S.J. Kutz, E.P. Hoberg, and L. Polley

Abstract: Three captive muskoxen (*Ovibos moschatus*) were successfully infected with third-stage larvae of *Umingmakstrongylus pallikuukensis* digested or emerged from the slugs *Deroceras reticulatum* and *D. laeve*, for the first time completing the life cycle of this parasite under experimental conditions. The course of parasite development and patency was followed for 26 months post infection (p.i.) using fecal examinations and radiography. The prepatent periods in two of the muskoxen were 91 and 95 days and the patent period in one extended for 23 months. Larval production peaked 13–14 months p.i. On postmortem of two of the muskoxen at months 14 and 26 p.i., adult parasites were found only within pulmonary cysts and cysts were randomly distributed between left and right lungs. Cyst dimensions were positively correlated with the number of adult parasites they contained. On postmortem of the third muskox at day 97 p.i., not all adult parasites were within typical cysts; two were found free in interlobular septa. First-stage larvae were recovered from lung cysts of this animal but not from feces. Lung pathology in all three muskoxen appeared localized and associated with the adult nematodes. Infection of two sheep with third-stage larvae of *U. pallikuukensis* did not result in parasite establishment.

Résumé : Nous avons réussi à infecter trois Boeufs-musqués (*Ovibos moschatus*) en captivité au moyen de larves de troisième stade d'*Umingmakstrongylus pallikuukensis* digérées ou émergées de limaces *Deroceras reticulatum* et *D. laeve*, et c'est la première fois que tout le cycle de ce parasite se déroule avec succès dans des conditions expérimentales. Le développement du parasite et l'apparition des symptômes de l'infection ont été observés pendant 26 mois après le début de l'infection par examen des fèces et par radiographie. La période précédant l'apparition des symptômes a été évaluée à 91 et 95 jours chez deux boeufs-musqués et la période symptomatique a duré 23 mois chez l'un d'eux. La production de larves a atteint son maximum 13–14 mois après l'infection. L'examen postmortem de deux des animaux aux mois 14 et 26 de l'infection a révélé la présence de parasites adultes, mais seulement dans des kystes pulmonaires répartis au hasard dans les deux poumons. La taille de ces kystes était directement reliée au nombre de parasites adultes qu'ils contenaient. L'examen postmortem du troisième animal, au jour 97 de l'infection, a révélé que les parasites adultes n'étaient pas tous dans des kystes typiquees, puisque deux d'entre eux ont été trouvés libres dans les septums interlobulaires. Des larves de premier stade ont été repérées dans des kystes pulmonaires, mais pas dans les fèces. La pathologie de ces trois boeufs-musqués semblait bien localisée et associée à la présence des nématodes adultes. Les tentatives d'infection de trois ovidés au moyen de larves de troisième stade d'*U. pallikuukensis* ont échoué.

[Traduit par la Rédaction]

Introduction

Umingmakstrongylus pallikuukensis (Protostrongylidae: Muelleriinae) is a large protostrongylid lungworm first detected in muskoxen (*Ovibos moschatus*) west of Kugluktuk, Nunavut (67°50′N, 115°05′W) by Gunn and Wobeser (1993) and subsequently described and named by Hoberg et al. (1995). Adult female parasites are up to 47 cm in length and both sexes occur coiled together in large cysts (up to 40 mm diameter) in the lung parenchyma (Hoberg et al. 1995).

Umingmakstrongylus pallikuukensis is common in muskoxen west of Kugluktuk, and some animals are heavily infected. Gunn and Wobeser (1993) reported a 92% prevalence of dorsal-spined first-stage larvae (L1) in muskox fecal samples

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Table 1. Experimental infections of muskoxen and sheep with *U. pallikuukensis*.

Animal ID No.	Age	Sex[a]	Date infected	Source of L1	Intermediate host (IH) Species[b]	IH No.[c]	Source of L3[d] (days p.i. of IH)	Dose of L3	Euthanasia (days p.i.)[f]
Muskox 1	4 years	M (c)	1 Mar. 1995	Feces (wild)	*D.r.*	5	D (24–29)	850	791
					D.r.	6	E (26–38)	220[e]	
Muskox 2	13 years	F (h)	4 Apr. 1995	Feces (wild)	*D.r.*	2 (47)	D (25–27)	97	441
					D.l.	2 (50)			
Muskox 3	14 years	M (c)	21 Feb. 1996	Feces (muskox 1)	*D.r.*	13	E (20–38)	175	97
Lamb A	3 months	M	22 Dec. 1994	Lungs (wild)	*D.r.*	1	D (22–26)	200	78
Lamb B	4 months	M	28 July 1995	Feces (muskox 1)	*D.r.*	5	D (31–38)	175	187

Note: "Wild" denotes samples from muskoxen killed by hunters near Kugluktuk in which adult parasites were identified as *U. pallikuukensis*. Numbers in parentheses are numbers of L3 used from each IH species.

[a]c, castrated; h, hysterectomized.

[b]*D.r.*, *D. reticulatum*; *D.l.*, *D. laeve*.

[c]Number of gastropods used for L1 to L3 development. Numbers in parentheses indicate the number of L3 used from each slug species.

[d]"D" denotes L3 recovered by artificial digestion of slugs and "E" denotes L3 recovered after emerging from slugs.

[e]Second dose of emerged L3 given 96 days after first dose.

[f]Days p.i. in table and text refer to days after first infection.

collected from the tundra and found up to 258 lung cysts in a single animal. Between 1988 and 1994, the infected muskox population declined by approximately 50% (Gunn 1994; J. Nishi, unpublished data). Although the parasite's role in this decline is unknown, its high prevalence and intensity and possibly deleterious effects led to further study.

The objectives of the research reported in this paper were (*i*) to complete and describe the life cycle of *U. pallikuukensis* in captive muskoxen; (*ii*) to describe the pathology and population structure of adult parasites in the lungs in infections of different ages and intensities; (*iii*) to determine whether third-stage larvae (L3) spontaneously emerged from gastropods are infective to muskoxen; and (*iv*) to determine whether *U. pallikuukensis* will develop to maturity in domestic sheep, a possible experimental host.

Materials and methods

Infection of gastropods

Slugs of the species *Deroceras reticulatum* and *D. laeve* served as intermediate hosts and were the source of L3. The *D. reticulatum* were from protostrongylid-free laboratory cultures at the Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Sask., and from a culture that originated from a composting box in Ottawa. The *D. laeve* were collected from a Saskatoon greenhouse. *Deroceras laeve* was used because it occurs in the Kugluktuk region (J. Nishi and G. Wobeser, personal communication). L1 of *U. pallikuukensis* were either from hunter-killed muskoxen from west of Kugluktuk, from which adult parasites were recovered, or from the feces of muskox 1. Details of the slugs and L1 are given in Table 1.

Gastropods were infected as described by Hoberg et al. (1995). Each gastropod was exposed to L1 daily for 1–5 days; the maximum total was approximately 2000 L1. Between successive exposures and after the last exposure, gastropods were housed overnight in clean plastic containers with water, lettuce, and carrots. On the day following the last exposure, slugs were transferred to an autoclaved, moistened 50:50 soil/vermiculite mixture in Rubbermaid® containers, maintained at room temperature (20 ± 2°C) and fed washed lettuce, carrots, potatoes, and chalk weekly.

Recovery of third-stage larvae

Two methods were used: (1) digestion of infected slugs in a pepsin – hydrochloric acid solution at 24–38 days post infection (p.i.) as described by Hoberg et al. (1995); and (2) recovery of L3 that had emerged from live slugs. To recover the emerged L3, beginning approximately 7–14 days p.i., gastropods were transferred from the Rubbermaid® containers to autoclaved, moistened petri dishes containing carrots and lettuce. Every 2–5 days, using a dissecting microscope, the internal surfaces of each dish were examined for larvae. At the same time, the remaining food material from each dish was suspended in warm tap water (30 ± 5°C) in a glass funnel at room temperature in the light. After 16–24 h the sediment from the funnel was examined for larvae. None of the slugs in the petri dishes died, indicating that the L3 emerged from living gastropods. Digested larvae were held for up to 24 h, and emerged larvae for up to 18 days, in tap water at 4°C before being given to muskoxen or sheep. L3 still within the second-stage sheath and those that had shed the sheath were used for the experimental infections.

Experimental infections of muskoxen and sheep

Animals

Three muskoxen from the Western College of Veterinary Medicine Muskox Research Herd and two Dorset-cross lambs from a farm near Saskatoon were used (Table 1). Funnel Baermann examinations (Gajadhar et al. 1994) and flotations using Sheather's saturated sugar solution (Foreyt 1994) were carried out on fecal samples from all animals. All Baermann examinations were negative. Eggs of *Trichuris* sp., *Nematodirus* sp., and other trichostrongylids were detected in the feces of muskox 2, which was treated with a single dose of oral fenbendazole (Panacur Suspension®, Hoechst Roussel Vet) at 10 mg/kg body mass 11 days before infection. The animals were housed indoors according to Canadian Council on Animal Care guidelines (Olfert et al. 1993).

Infection with U. pallikuukensis

Animals were given L3 suspended in 20 mL of tap water by gastric tube, followed immediately by a minimum of 120 mL of water and 60 mL of air. In the absence of any information on experimental infections of mammalian hosts with *U. pallikuukensis*, the numbers of L3 given to muskoxen (97–1070) were chosen in an attempt to provide a range of infection intensities (Table 1). Muskox 1 was given a second dose of L3 because of concern that the initial infection had not established. The numbers of L3 given to the

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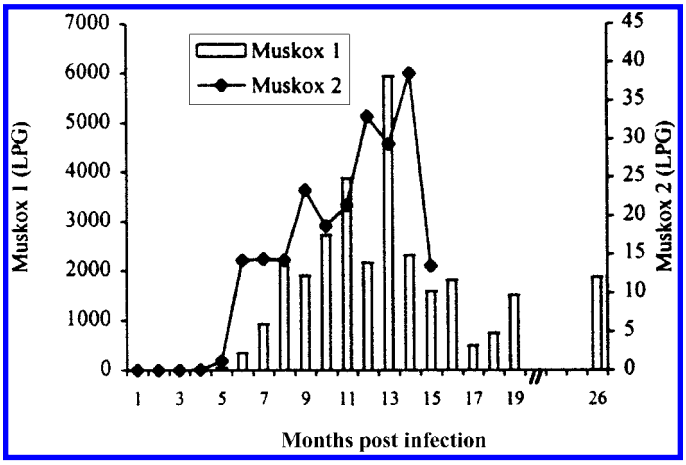
Fig. 1. Mean monthly production of *U. pallikuukensis* L1 by muskoxen 1 and 2. LPG counts (larvae per gram) are based on wet mass of feces (error bars show 1 standard deviation).



lambs were chosen arbitrarily, based in part on numbers of larvae available. Clinical monitoring consisted of thoracic radiographs and hematological and blood biochemistry evaluations every 2–3 months as well as daily observations for signs of respiratory distress and for altered feed consumption or behaviour.

Monitoring parasite development

Fecal examinations were scheduled as follows: muskox 1 first on day 16 p.i. and then every 1–2 days to patency, then weekly or biweekly to month 19 p.i., then irregularly to month 26 p.i.; muskox 2 first on day 45 p.i., then weekly to day 75 p.i., then daily to patency then weekly or biweekly to month 14 p.i.; muskox 3 first on day 63 p.i., then every 7–10 days from days 63 to 89 p.i., then daily to euthanasia; lamb A daily from day 9 p.i. to euthanasia; and lamb B every 1–2 weeks from day 60 p.i. to euthanasia. Once the prepatent period was established in muskox 1 these schedules were determined by logistical considerations.

During the prepatent periods all feces voided by an animal over a 24-h period were pooled and a minimum of three, but usually six to nine, samples were examined. A funnel Baermann technique was used: 20–30 g of feces were lightly crushed (squeezed once between thumb and forefinger), suspended in a plastic strainer (110 mm internal diameter, 1 mm mesh size) containing two layers of cheesecloth, and placed in a glass funnel with an attached tube and clamp. The funnel was filled to the brim with warm tap water (30 ± 5°C) and left at room temperature in the light. After 16–24 h, 40–50 mL of water was collected and centrifuged at 1500 rpm for 10 min and the sediment was examined in a scored petri dish.

Following patency, all feces voided by each animal during a 24-h period were collected and mixed. Three 10-g samples were examined using the funnel Baermann technique described above. At first, all larvae in the sediment from each of the 3 samples from each animal were counted. As larval numbers increased, larvae in three 0.01-mL (muskox 1) or 0.1-mL (muskox 2) aliquots from each sediment were counted and the mean number of larvae per gram (LPG) of wet feces on each day of sampling was calculated. The monthly LPG counts reported (Fig. 1) are the means of the daily counts performed during that month.

For muskox 1, LPG counts of dry feces were determined from months 16–26 p.i. by drying two 10-g samples from a day's pooled feces at 70°C for 48 h. To avoid variation in dry mass due to differences in pellet size, the samples that were dried contained the same number of pellets as the wet samples used for larval counts. Total daily fecal output by muskox 1 was measured over five 24-h periods during month 26 p.i.

Postmortem examinations

Dates of euthanasia were selected to allow examination of infections at different stages of development. Animals were sedated with intramuscular xylazine hydrochloride (Rompun® 20 mg/mL Injectable, Bayer) and euthanized using intravenous sodium pentobarbital (Euthanyl-Forte®, Bimeda MTC). Standard postmortem examinations were carried out on all animals, with emphasis on the lungs. In addition, following death a gastric tube was tied into the tracheas of muskoxen 1 and 2, the lungs were inflated, and the tube was closed with a clamp. Within 3 h of death the lungs of muskox 1 were examined by computed tomography (CT) and those of muskox 2 by standard radiography.

The fresh lungs of all three muskoxen were dissected along the larger bronchi and the surrounding pulmonary tissue was removed in slices 3–5 mm thick. Each slice was meticulously examined visually and by palpation. All lung tissue from each animal was examined in this way within 48 h of euthanasia. Cyst location was noted for muskoxen 1 and 2 and the cysts were removed and classified by palpation as soft or mineralized. In muskox 1, because of initial difficulty in accurately defining the anatomical separation between the right apical and middle lung lobes, data for cyst location in these lobes were combined. Cyst locations for muskox 3 were not recorded. Cyst length and width was recorded and the mean of these two measurements was considered the diameter of the cyst. Cyst volume was then calculated using the formula for the volume of a sphere. To estimate the mean proportional volume contributed by each of the five lung lobes to total lung volume, the lungs of five hunter-killed adult muskoxen were dissected and the lobes weighed individually.

All cysts from muskox 2 were measured and dissected, whereas only some cysts from muskoxen 1 and 3 were examined. Cysts were dissected immediately or the walls were carefully incised and the cysts placed in normal saline at 37°C for a few minutes to several hours to encourage movement of the adult parasites from the cysts. An attempt was made to recover the adult parasites intact and to quantify and characterize them within the cysts. Undissected cysts from muskox 1 and lung tissue containing nematodes from muskox 3 were fixed in 10% buffered formalin, embedded in paraffin blocks, sectioned, 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 fresh cysts from muskox 1 were submitted for bacteriological examination. Complete postmortem examinations were carried out on both lambs. Lung and liver lesions detected macroscopically were fixed and processed in the same manner as the lung tissue from the muskoxen.

Statistical analyses were performed using StatView™ SE+ Graphics (Abacus Concepts Inc., San Francisco, Calif., U.S.A., 1988), *Biostatistical Analysis* (Zar 1984), and *Nonparametric Statistics for the Behavioural Sciences* (Siegel and Castellan 1988).

Results

Monitoring parasite development: muskoxen

L1 were first recovered from the feces of muskoxen 1 and 2 on days 95 and 91 p.i., respectively, and were shed until euthanasia on days 791 and 441 p.i., respectively (Table 2). No larvae were found in the feces of muskox 3, but gravid adult female parasites, eggs, and L1 were recovered by dissection of cysts at postmortem on day 97 p.i.

L1 were recovered from all fecal samples examined from muskoxen 1 and 2 following patency (Fig. 1). Larval output from both animals began to increase at 6–7 months p.i. and peaked at 13–14 months p.i. Changes in larval output associated with the second infection of muskox 1 were not appar-

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Table 2. Population characteristics of *U. pallikuukensis* in experimentally infected muskoxen.

Muskox ID No.	Prepatent period (days)	Patent period (days)	Total no. of cysts	Cyst vol. (cm^3)	Sex ratio (M:F)	No. of adults per cyst		Total no. of adults recovered	Recovery of adults per L3 (%)
						Median	Range		
1	95	696	183	1.40±0.99[a] (n = 22)	0.86 (n = 22)	3 (n = 24)	0–6	595[b]	56
2	91	350	16	0.99±0.54[a] (n = 13)	1.3 (n = 16)	2 (n = 16)	2–5	41[c]	42
3	>97	n/a	13	0.09 (n = 1)	1.2 (n = 5)	2 (n = 6)	1–5	34[b]	19[d]

Note: n is the number of cysts on which the data are based.
[a]Mean ± 1 SD.
[b]Extrapolated from the mean of subsets of 24 (muskox 1) and 6 (muskox 3) dissected nodules.
[c]Actual number recovered based on dissection of all 16 nodules detected.
[d]Probably underestimated, owing to early stage of infection.

ent. The maximum daily and maximum mean monthly LPG counts from wet feces were as follows: muskox 1, 8953 ± 3268 (mean ± SD) on day 391 p.i. (month 13 p.i.) and 5942 ± 2419 during month 13 p.i.; muskox 2, 71 ± 12 on day 414 p.i. (month 14 p.i.) and 39 ± 28 during month 14 p.i. LPG counts in dried feces were approximately double those in wet feces. Over the five 24-h measurement periods during month 26 p.i., muskox 1 produced an average of 880 ± 164 g feces/day (wet mass). Based on a mean daily LPG count of 1872 (range 700–3898) recorded during that month, a mean of 1 647 360 L1 (range 616 000 – 3 430 240) were shed in the feces of this animal each day.

Standard radiography demonstrated lesions attributable to *U. pallikuukensis* in muskoxen 1 and 2 on days 191 and 178 p.i., respectively (Fig. 2). Muskox 3 showed no radiographic changes consistent with lungworm infection immediately prior to euthanasia on day 97 p.i. No abnormalities specific to the lungworm infection were detected in the hematological or blood biochemistry evaluations or by clinical observation.

Postmortem examinations: muskoxen

Findings of parasitological significance were restricted to the lungs.

Cyst distribution

The majority of the cysts were found deep in the lung parenchyma, with only 24 of 183 and 1 of 16 visible on the lung surface in muskoxen 1 and 2, respectively (Figs. 3, 4, and 5). None of the 13 cysts detected in muskox 3 were visible on the lung surface. On gross dissection, most of the cysts in muskox 1 were located centrally in the lungs adjacent to the larger bronchi; this distribution was confirmed by CT examination (Fig. 6). Cysts were found in all lung lobes of muskox 1 but only in the diaphragmatic lobes of muskox 2 (Table 3). In both animals cysts were distributed randomly between left and right lungs (muskox 1: $\chi^2 = 0.911$, df = 1, $P = 0.3398$; muskox 2: $\chi^2 = 0.206$, df = 1, $P = 0.6496$). In muskox 1 cysts were randomly distributed among the lobes of the left lung ($\chi^2 = 0.59$, df = 1, $P = 0.4424$) but in the right lung there were more cysts in the combined apical/middle lobes and fewer in the diaphragmatic lobe than would be expected on the basis of estimated lung volumes ($\chi^2 = 11.403$, df = 2, $P = 0.0033$).

Cyst characteristics

All cysts in muskoxen 1 and 2 had tough, grey, well-defined walls and on palpation showed no evidence of mineralization. In muskox 3 not all parasites were in discrete cysts; one male and one female were found non-encysted in different locations within interlobular septa. Cysts and individual parasites were extremely difficult to locate in this animal and the cysts were small with poorly defined walls. With the exception of a single empty cyst in muskox 1, all cysts examined in muskoxen 1 and 2 contained at least one male, one gravid female, free eggs, and L1. In muskox 3, two of three cysts dissected contained gravid female *U. pallikuukensis* and other cysts examined contained free eggs and L1. The ratio of female to male parasites was different in the three muskoxen (Table 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. Five contained more males than females and 11 equal numbers. In muskox 3, of three cysts examined, one contained more males than females and two equal numbers. The number of adult parasites per cyst was significantly greater in muskox 1 than in muskox 2 (Table 2) (Wilcoxon–Mann–Whitney test, $P = 0.0148$). Cyst size and number of parasites per cyst were positively correlated in muskoxen 1 and 2 (muskox 1: $n = 22$, $r^2 = 0.650$, $P < 0.001$; muskox 2: $n = 13$, $r^2 = 0.636$, $P = < 0.002$) (Fig. 7). When mean cyst sizes were compared for cysts containing two 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 three to six parasites were insufficient for statistical comparison.

Histopathology

In muskox 3 on day 97 p.i., non-encysted adult parasites were detected in two separate sections. In the first there were two groups of cross sections through one or more adult nematodes (Fig. 8). One of these groups was near an interlobular septum and the other near a bronchiole. There was mild hemorrhage into the alveoli containing the parasite(s) and the alveoli were distended. The surrounding alveolar septa

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Fig. 2. Lateral caudal thoracic radiograph of muskox 1 at day 317 p.i., showing several *U. pallikuukensis* cysts (arrows) in the dorsal part of the diaphragmatic lobes. Scale bar = 60 mm. **Figs. 3–5.** Cysts of *U. pallikuukensis* from muskox 1 at day 791 p.i. Fig. 3. Lateral view of the left lung showing six cysts (arrows) beneath the pleural surface. Fig. 4. Close-up of three subpleural cysts (arrows) from Fig. 3. Scale bar = 10 mm. Fig. 5. Cross section of the lung, showing a typical subpleural cyst of *U. pallikuukensis* containing adult parasites (arrow) surrounded by the cyst wall (arrowhead) (modified from Kutz et al. 1999). Scale bar = 5 mm.

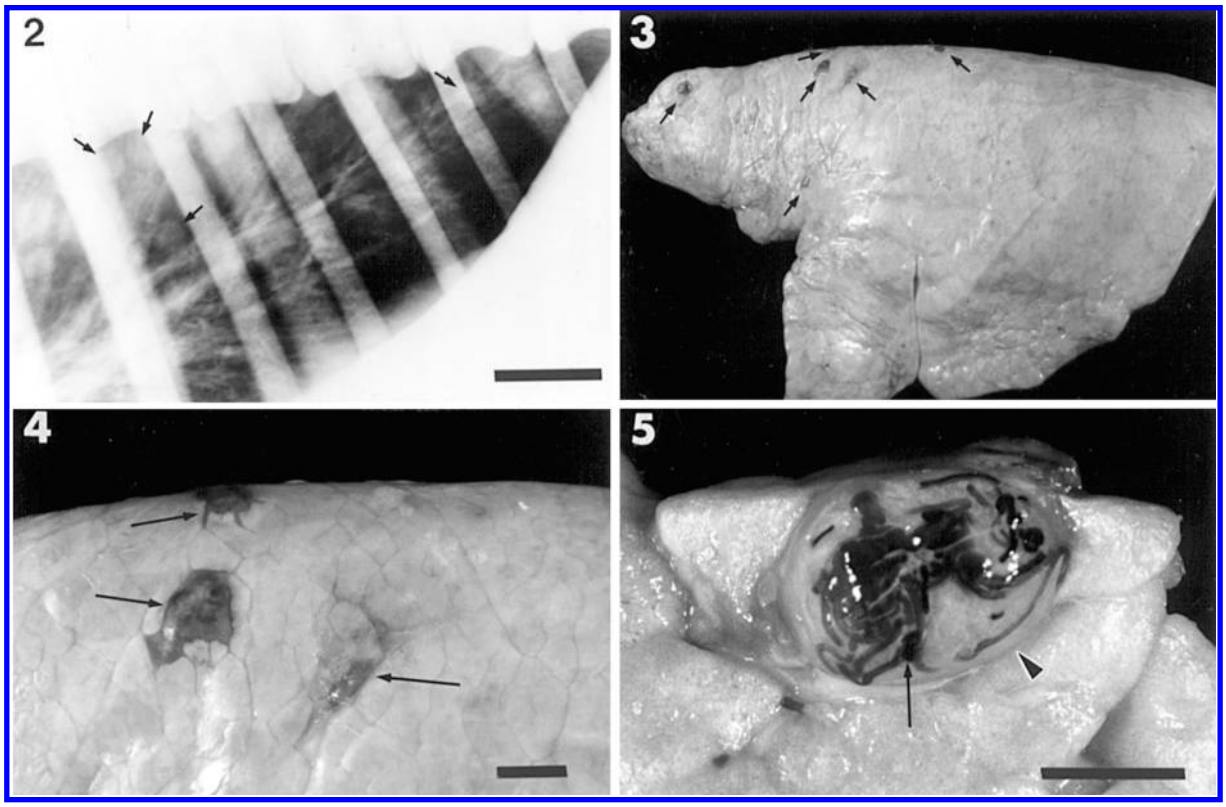
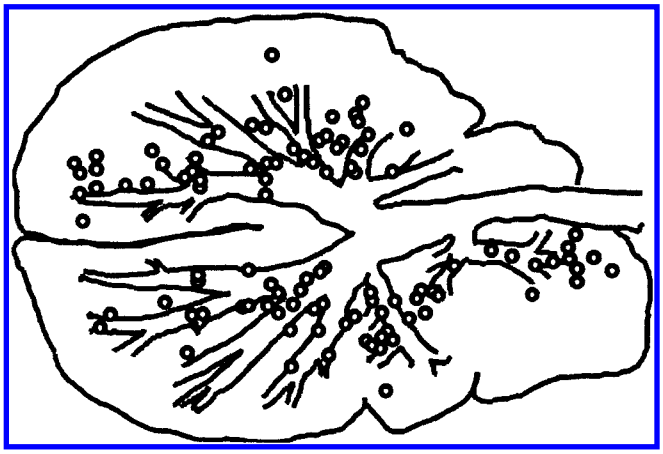


Fig. 6. Tracing from a postmortem CT scout film of the lungs of muskox 1, showing the distribution of *U. pallikuukensis* cysts. Not all cysts are visible in this film.



were mildly infiltrated by neutrophils, lymphocytes, and a few eosinophils. Similar inflammatory cells, together with fibroblasts, surrounded an adjacent bronchiole. The lumen of the bronchiole appeared normal. In the second section, parasite-associated changes extended from adjacent to a bronchiole to the pleura and contained at least one male and one female nematode surrounded by numerous eosinophils, lymphocytes, a few plasma cells, and multifocal hemorrhages (Figs. 9 and 10). Adjacent alveoli were filled or obliterated by lymphocytes and macrophages; many of the latter contained hemosiderin. Several foci of dense lymphocytic infiltration were also present. The adult parasites appeared larger than in the first section and eggs were present in at least one female. Free eggs and larvae were not present in either section.

Cysts from muskox 1 at day 791 p.i. were better defined and less variable than those from muskox 3 at day 97 p.i. Four cysts were examined histologically, three immediately beneath the pleura and one deeper in the parenchyma. Histological lesions were similar in all four. Most of the lung pathology was localized to the cysts, which contained a densely packed core of adult nematodes, eggs, and L1 (Fig. 11). A matrix of macrophages, fibroblasts, and fibrous tissue, as well as remnants of alveolar septa, extended among the nematodes, with occasional small foci of mineralization. In some cysts the L1 appeared deformed and degenerate but the adult parasites showed no evidence of degeneration. Surrounding the central core of each cyst, and separating it from the adjacent lung tissue, was a wall of macrophages, multinucleated giant cells, fibroblasts, and fibrous connective tissue, lymphocytes, plasma cells, and a few eosinophils. Many of the macrophages contained hemosiderin. Alveoli and compressed bronchioles, several of which communicated with the core of the cyst, were present through-

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Table 3. Distribution of *U. pallikuukensis* cysts among lung lobes of two experimentally infected muskoxen.

Muskox ID No.	Right ap/mid	Right diaph.	Accessory	Right total	Left apical	Left diaph.	Left total
1	30	24.6	5.5	60.1	9.8	30.1	39.9
	(55)	(45)	(10)	(110)	(18)	(55)	(73)
2	0	62.5	0	62.5	0	37.5	37.5
	(0)	(10)	(0)	(10)	(0)	(6)	(6)
Percentage of lung[a]	21.3	32.0	3.3	56.6	12.4	31.0	43.4
	(2.4)	(2.6)	(0.5)	(3.1)	(2.3)	(2.8)	(3.1)

Note: Values are given as the percentage, with the actual number of cysts in parentheses. "ap/mid," apical and middle lobes combined; "diaph," diaphragmatic.

[a]Each lobe is reported as percentage of total lung mass (n = 5). Numbers in parentheses show the standard deviation.

out the wall and often contained L1 (Fig. 12). In one cyst, a section of an adult nematode appeared to be incorporated into the inner part of the wall. Multifocal, moderate hemorrhage was evident throughout the cysts.

Several of the parasite-associated cysts completely filled the lung lobules in which they were located. Surrounding interlobular septa and adjacent lobules were often compressed or distorted but the cysts did not invade these adjacent lobules. Areas of a lobule not occupied by a cyst often contained a moderate inflammatory cell infiltration. The lung parenchyma adjacent to the lobules containing cysts was generally unaffected, although occasional L1 were 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 the amount of bronchiole-associated lymphoid tissue were observed, but the lumina of the airways appeared normal. Multifocal, small accumulations of inflammatory cells were not always associated with identifiable larvae.

Bacteriology

No bacteria of clinical significance were isolated from the cysts from muskox 1 with either aerobic or anaerobic cultures in enrichment broths.

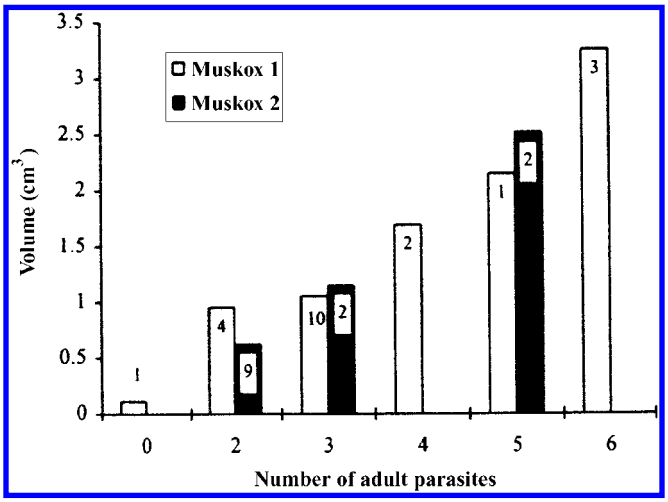
Monitoring parasite development and postmortem examinations: sheep

First-stage larvae were not recovered after any of the Baermann examinations of feces from either of the lambs. No radiographic, hematological, or clinical abnormalities consistent with parasitic infection were observed. No parasites were recovered from the experimentally infected lambs at postmortem on days 78 and 187 p.i. On the liver and lung surfaces of both animals were a small number of focal lesions, 1–7 mm in diameter, that contained a variety of inflammatory cells including eosinophils and, in a liver lesion in lamb A, multinucleated giant cells.

Discussion

In this study, development of patent *U. pallikuukensis* infection is documented for the first time in two experimentally infected muskoxen. Infection of a third muskox with larvae that had emerged from living gastropods resulted in lung cysts containing gravid adult female parasites and free eggs and larvae. On this basis, the infection in this animal was considered to be close to patency.

Fig. 7. Relationship between cyst size and adult *U. pallikuukensis* groupings in muskoxen 1 and 2. Numbers within the bars indicate the number of cysts examined (error bars show 1 standard deviation).



Prepatent period

The prepatent periods of 91 and 95 days for *U. pallikuukensis* are longer than those reported for any other protostrongylid lungworms in their typical definitive hosts (Boev 1975). Among other Muelleriinae these range from 25 to 59 days for *Cystocaulus ocreatus* and *Muellerius capillaris* (Davtyan 1949, cited in Boev 1975; Gerichter 1951; Rose 1959; Svarc and Zmoray 1960, cited in Boev 1975; Azimov et al. 1973, cited in Boev 1975). Among the distantly related Protostrongylinae of bighorn sheep (*Ovis canadensis*), prepatent periods of 45–54 days are typical for *Protostrongylus stilesi* and *P. rushi* (Fougere-Tower and Onderka 1988). Among the Protostrongylidae, the only prepatent periods comparable to those of *U. pallikuukensis* are observed among the Elaphostrongylinae, which is a sister-group for the Muelleriinae (Carreno and Hoberg 1999). These extended development periods may be due to the migration routes of larvae of these neural- and muscle-tissue-dwelling nematodes (Olsson et al. 1998). The route of migration of *U. pallikuukensis* larvae from the gastrointestinal tract to the lungs has yet to be determined.

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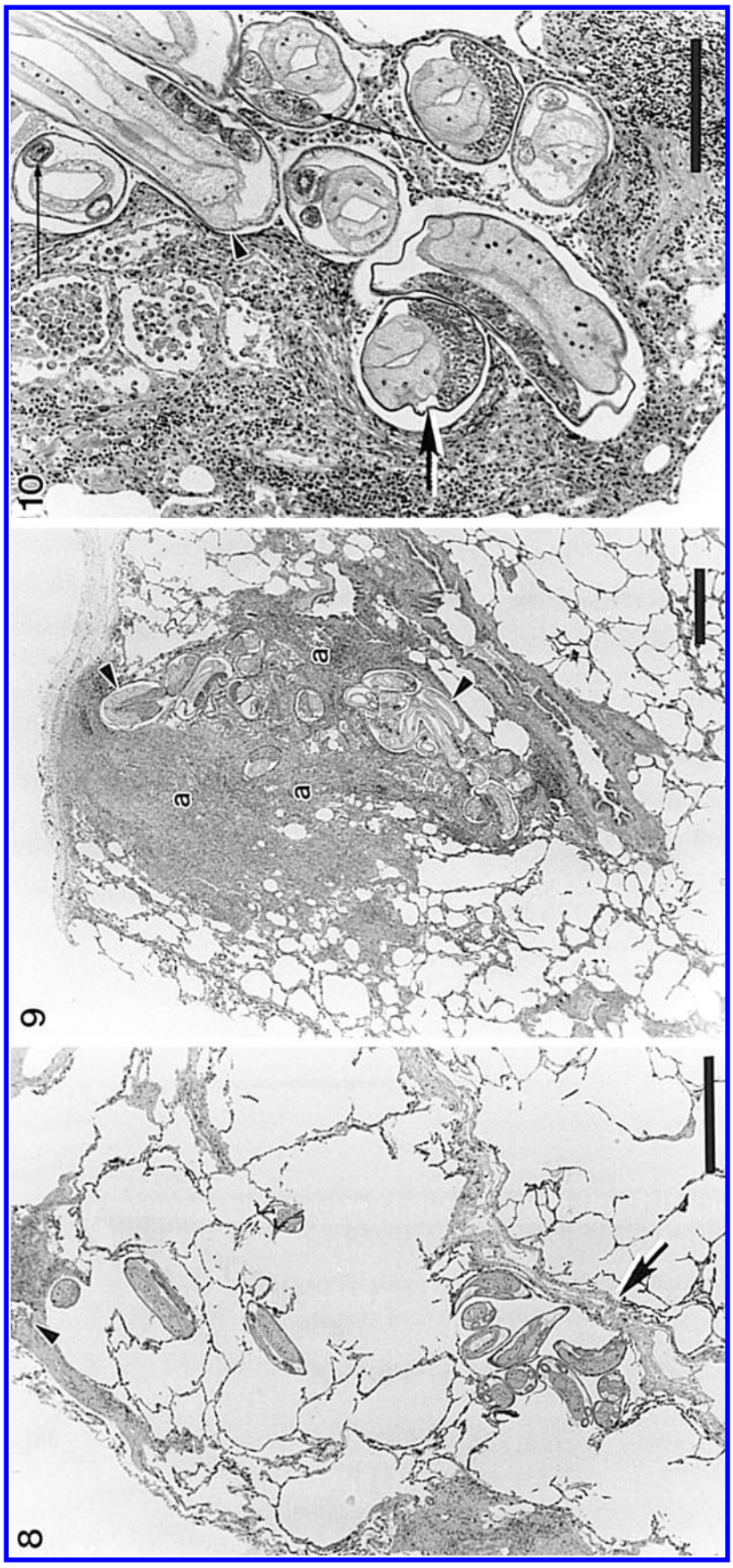

Figs. 8–10. Histological sections of the lungs of muskox 3 at day 97 p.i. Fig. 8. Two groups of nematodes in cross section; one is adjacent to an interlobular septum (arrow), the other to a bronchiole (arrowhead). Scale bar = 500 µm. Fig. 9. Nematodes (arrowheads) surrounded by numerous inflammatory cells (*a*). Scale bar = 500 µm. Fig. 10. Male (large arrow) and female (arrowhead) nematodes. Eggs are visible within the female (thin arrow). Scale bar = 200 µm.

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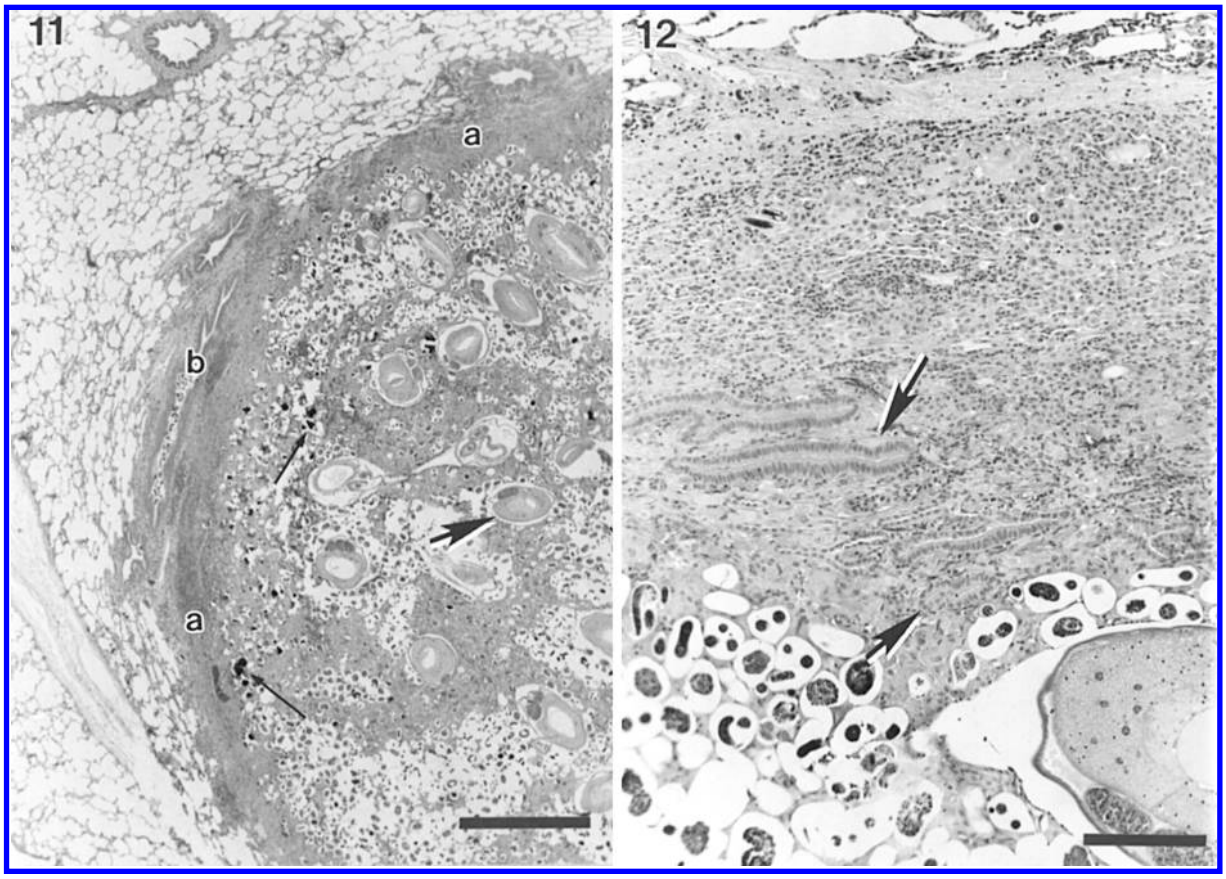
Figs. 11 and 12. Histological sections of the lung of muskox 1 at day 791 p.i. Fig. 11. Part of a cyst containing adult *U. pallikuukensis* (large arrow), eggs, and L1 surrounded by a thick cyst wall (*a*). L1 are visible in an adjacent bronchiole (*b*). Small foci of mineralization are visible within the cyst (thin arrows). Scale bar = 1000 µm. Fig. 12. Bronchioles (arrows) are visible extending through the inner part of a cyst wall. Scale bar = 200 µm.



Patency and larval production

Temporal patterns of L1 output were similar in muskoxen 1 and 2, although the hosts were of different ages and had experienced different histories of infection. If these patterns are characteristic of those that occur under natural conditions in the Arctic, infective larvae of *U. pallikuukensis* ingested during one summer may give rise to peak L1 production during the following summer. It is also possible, however, that the patterns observed in these experimental animals were influenced by the timing of the infections (both in the spring) or an altered host immune reaction resulting from the ingestion of large numbers of L3 on one or two occasions rather than periodically in small numbers, as prevalence and intensity data for the infective larvae of protostrongylids in their intermediate hosts (Robb and Samuel 1989) suggest may occur under natural conditions.

In the present study, larval output was quantified using a funnel Baermann technique. Recently, Forrester and Lankester (1997) reported that when such a technique was used for *Parelaphostrongylus tenuis*, only 13–14% of L1 in a sample were recovered, and it provided an unreliable estimate of larval numbers. In our study the daily LPG counts were estimated from three samples from each animal's total fecal production on that day and the methods used were consistent between animals and over time. Also, the patterns of larval production were similar for both muskoxen. The funnel Baermann technique may have underestimated larval numbers but it seems improbable that it affected the observed pattern of L1 production.

The patent periods of *U. pallikuukensis* in experimentally infected muskoxen were lengthy: at least 23 months in muskox 1 and 11 months in muskox 2. There was little indication from the condition of the cysts and adult parasites recovered and the lung pathology, that the nematodes in these two animals were becoming senescent. Adult *Elaphostrongylus rangiferi*, another northern protostrongylid, are believed to live for several years (Halvorsen at al. 1985). These prolonged life-spans may be an adaptation that enhances survival of the parasites from year to year when climatic conditions do not adequately support larval survival in the environment or development in the intermediate hosts.

Cyst distribution and characteristics

The distribution of cysts of *U. pallikuukensis* within the lung lobes contrasts with that for species in the related genera *Cystocaulus* and *Muellerius* in sheep (Gerichter 1951; Kassai 1957; Rose 1959; Boch and Nurnberg 1962; Sedlmeier et al. 1969). Whereas these parasites form primarily subpleural nodules most often along the obtuse margins of the diaphragmatic lobes, the majority of the cysts of *U. pallikuukensis* were found deeper in the parenchyma. Preliminary examinations, including CT scans, of lungs from naturally infected muskoxen

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suggest that this distribution is typical (S. Kutz, unpublished data).

Despite the approximately 1-year difference in age of infection at the time of euthanasia, and differences in numbers of L3 recorded, there was no significant difference in the size of cysts containing two parasites between muskoxen 1 and 2. Additional radiographic evaluations of muskox 1 showed an increase in cyst size between days 252 and 415 p.i., with little subsequent change at day 789 p.i. (Kutz et al. 1999). These findings suggest that cyst growth in this animal was determinate. The numbers of cysts detected in the experimentally infected animals are within the range reported by Gunn and Wobeser (1993), but cyst size did not approach the maximum (40 mm diameter) reported by Hoberg et al. (1995). Because of the strong positive correlation between cyst size and the number of parasites contained, individual cysts in naturally infected muskoxen may contain more parasites than were observed in the experimentally infected animals.

Lung pathology

This study provided an opportunity for comparing experimentally infected animals with animals from the wild, where infections are of unknown age and may represent an accumulation of many infection events over an extended period (Hoberg et al. 1995). Considerable variation in cyst size, mineralization, and reproductive condition of adult parasites have been described in naturally infected muskoxen (Gunn and Wobeser 1993; Hoberg et al. 1995), but was not observed in the experimentally infected animals.

Examination of lung lesions at days 97 (muskox 3, one infection) and 791 p.i. (muskox 1, two infections) provided some insight into lesion development. The inflammatory responses surrounding the non-encysted male and female adult parasites in muskox 3 at day 97 p.i. have not been reported in naturally infected animals and may represent the initial stages of cyst-wall formation. The cyst wall itself may act as a selective barrier by limiting the inflammatory reaction within the lung tissue and providing 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 tissues (Svarc 1984). Other than in an early infection (muskox 3), the principal lung pathology associated with reproductively active *U. pallikuukensis* is restricted to the cysts. This is in marked contrast to the more diffuse changes reported for other lung nematodes of ruminants such as those in the genera *Protostrongylus*, *Muellerius*, *Cystocaulus*, and *Dictyocaulus* (Rose 1961; Beresford-Jones 1967; Stockdale 1976; Seesee and Worley 1993).

Infection of sheep

Umingmakstrongylus pallikuukensis did not establish as adults in two domestic sheep, although histological lesions that may have been associated with parasite 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 discourage their use as an experimental model for this parasite.

Host–parasite biology

We have confirmed in two muskoxen that L3 of *U. pallikuukensis*, artificially digested from the gastropod intermediate hosts *D. reticulatum* and *D. laeve*, can develop to reproductively mature adults in the natural definitive host. In a third muskox we have demonstrated that L3 which had emerged from live *D. reticulatum* are also infective. Larval emergence from gastropods has been reported for the protostrongylids *P. stilesi*, *M. capillaris*, and *C. ocreatus* and for *Angiostrongylus costaricensis* (Davtyan 1950, cited in Boev 1975; Rose 1957; Monson and Post 1972; Ubelaker et al. 1980). Larval emergence has also been reported for *Protostrongylus boughtoni* in snowshoe hares, and although the emerged L3 are infective for domestic rabbits, they are considered an unlikely source of infection for the natural definitive hosts (Kralka and Samuel 1984). For the other protostrongylids, and for *U. pallikuukensis*, the epidemiological significance of emerged L3 under natural conditions has not been determined.

The effect of *U. pallikuukensis* on muskox populations in the Arctic is unknown. Among other lung nematodes, *Dictyocaulus viviparus* has significant metabolic costs for cattle (Verstegen et al. 1989), and mixed infections with protostrongylid lungworms (species of *Muellerius*, *Cystocaulus*, *Protostrongylus*, and *Neostrongylus*) can result in impaired respiratory exchange in domestic goats (Berrag and Cabaret 1996). Pulmonary compromise caused by *Protostrongylus* spp. in bighorn sheep has been implicated as a predisposing factor in bacterial and viral pneumonia outbreaks (Forrester 1971; Uhazy et al. 1972; Spraker et al. 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 to be localized to the cysts, leaving most of the lung tissue in adult muskoxen unaffected. No clinical signs of pulmonary disease were observed in the experimental animals, but the exercise intolerance observed in naturally infected muskoxen (C. Adjun and G. Atatahak, personal communication) suggests pulmonary compromise. Hoberg et al. (1995) suggested that the greatest effect of the parasites may be that of space-occupying lesions, with the cysts causing displacement and compression of lung tissue. Additionally, it is possible that there are significant metabolic costs associated with heavy *U. pallikuukensis* infections. Increased mortality rates in muskox populations where the parasite is present could result from these factors, in combination with other infectious agents, limiting environmental conditions in the Arctic, and predation. Grizzly bears prey on muskoxen by stampeding herds and killing the slower moving animals (A. Gunn, personal communication), which indicates that the exercise intolerance in naturally infected animals may be significant.

Umingmakstrongylus pallikuukensis is an intriguing parasite differing considerably from its putative sister-taxon *Cystocaulus* and 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

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L3 given developing to reproductively active adults in one animal. The long patent period, combined with high larval output, probably produces extensive environmental contamination over an extended period. Yet, to complete its life cycle, the parasite must survive under harsh arctic conditions, penetrate a susceptible gastropod IH, develop to infective L3 at low summer temperatures, and then be ingested by a muskox. The high prevalence and intensity of *U. pallikuukensis* in the Kugluktuk region, together with the localized lung pathology, is indicative of a parasite well adapted to its current host and the rigours of the arctic environment and suggest a long-standing host–parasite relationship. Current phylogenetic evidence suggests, however, that the origin of the genus *Umingmakstrongylus* can be linked to colonization of muskoxen by a protostrongylid nematode, probably from a caprine source in the Pleistocene or in the late Tertiary (Hoberg et al. 1995; Carreno and Hoberg 1999).

Wildlife resources are extremely important for the people of remote communities of Nunavut and the Northwest Territories and across high latitudes of the Holarctic region. Through subsistence hunting, sport hunting, commercial harvests, and tourism, wildlife produces considerable cultural, nutritional, and economic benefits (Gunn et al. 1990). Yet despite the integral role played by wildlife, relatively little is known about the biodiversity of the parasite faunas 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. 2000). The 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).

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