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BIONOMICS OF LARVAE OF *PARELAPHOSTRONGYLUS ODOCOILEI* (NEMATODA: PROTOSTRONGYLIDAE) IN EXPERIMENTALLY INFECTED GASTROPOD INTERMEDIATE HOSTS

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ABSTRACT: *Parelaphostrongylus odocoilei* is a protostrongylid parasite that has recently been recognized at several locations in sub-Arctic, but not Arctic, North America. We investigated factors that may determine the distribution of *P. odocoilei*, including suitable gastropod intermediate hosts, temperature requirements for larval development in gastropods, and larval emergence facilitating overwinter transmission. We collected and experimentally infected gastropods from a site in the sub-Arctic where *P. odocoilei* is at the northern limit of its distribution. *Deroceras laeve*, *Catinella* sp., and *Euconulus* cf *fulvus*, but not members of the Pupillidae, were suitable intermediate hosts. We describe bionomics of larvae of *P. odocoilei* in *D. laeve* and *Catinella* sp. Infective larvae emerged from all slugs (*D. laeve*) and 60% of *Catinella* sp. snails, and emergence from *D. laeve* was intensity dependent. Emerged infective larvae survived up to 6 mo under conditions approximating that of the subnivean environment. In *D. laeve*, there was a direct relationship between temperature and development rate of larvae of *P. odocoilei*. Larvae of *P. odocoilei* did not develop to infective stage below the theoretical threshold (8.5 C), and required a minimum of 163 degree days to complete development. These developmental parameters can be incorporated into a model to predict larval development in the field. Knowledge of the factors influencing larval bionomics provides the foundation for predicting temporal and spatial patterns of parasite distribution, abundance, and transmission.

Parelaphostrongylus odocoilei, a protostrongylid muscleworm, has traditionally been considered a parasite of temperate and coastal regions in western North America. Recently, however, it was discovered in populations of thinhorn sheep (*Ovis dalli*), mountain goats (*Oreamnos americanus*), and woodland caribou (*Rangifer tarandus caribou*) in sub-Arctic, but not Arctic, North America (Kutz, Veitch et al., 2001; Jenkins, Appleyard et al., 2005). Similar to other protostrongylids, *P. odocoilei* has an indirect life cycle, where first-stage larvae (L1) shed in the feces of a mammalian definitive host penetrate the foot of a gastropod intermediate host, develop to second-stage larvae (L2), and then to infective third-stage larvae (L3). Native gastropod species naturally infected with larvae morphologically indistinguishable from those of *P. odocoilei* have been described in a temperate region, in Jasper, Alberta, Canada (52°N, 118°W) (Samuel et al., 1985). Prevalence of infection was less than 5%, necessitating the capture of more than 10,000 gastropods to determine suitable intermediate host species. Most logistically feasible sampling techniques underestimate gastropod abundance and the prevalence of protostrongylid infection (McCoy and Nudds, 1997; Hawkins et al., 1998). In addition, species of protostrongylid larvae cannot reliably be identified on the basis of morphology alone. Therefore, to determine gastropod species potentially important for natural transmission, experimental infection of native gastropods is an effective and attractive alternative (Platt and Samuel, 1984).

Definitive hosts may become infected by ingesting gastropods containing L3, or L3 that have emerged from gastropods. Reports of larval emergence vary among different species of protostrongylids and gastropod hosts, and the significance of this phenomenon is controversial. Other than a single report of larval emergence of *P. odocoilei* of thinhorn sheep origin (Kutz, Veitch et al., 2001), emergence has not been reported for any elaphostrongyline or considered an important transmission route for most protostrongylids (Anderson, 2000). In contrast, recent work on a protostrongylid lungworm of muskoxen, *Umingmakstrongylus pallikuukensis* (Kutz et al. 2000), and older, less-accessible studies in the Russian literature (summarized by Boev, 1975), indicate that emergence is important for transmission of some protostrongylids, especially in northern regions where gastropods are seasonally unavailable for up to 8 mo of the year. Further work is needed to determine the significance of larval emergence for *P. odocoilei*, including survival of emerged larvae under simulated natural conditions.

Temperature is probably the most important abiotic factor affecting rates of development of parasites with free-living developmental stages, or larval stages in poikilothermic intermediate hosts, such as gastropods (Halvorsen and Skorping, 1982; Shaw et al. 1989). Degree-day (DD) models, which describe the amount of heating above a critical threshold temperature, are widely used to determine conditions allowing parasite development and can be incorporated into deterministic, predictive models for rates of parasite development under different climatic conditions (Campbell et al., 1974; Harvell et al., 2002; Saunders et al., 2002). Early workers began to examine the effects of weather and climate on parasite transmission (Levine, 1963), but "this became a neglected and low prestige area over the last 2 decades as our ability to study the . . . more molecularly glamorous aspects of the parasite life-cycle increased" (Smith et al., 1995). As a result, there are few empirical studies available for protostrongylids (Halvorsen and Skorping, 1982; Samson and Holmes, 1985; Kutz, Hoberg et al., 2001), and the effects of temperature on larval development have not been investigated for *P. odocoilei*.

Many biotic and abiotic factors, including species of gastropod, infection intensity, and temperature, influence larval bionomics, including development, emergence, and survival of larval stages (Gerichter, 1948; Halvorsen and Skorping, 1982; Skorping, 1984; Kutz et al. 2000). In turn, factors affecting larval bionomics drive temporal and spatial patterns of trans-

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TABLE I. Emergence of infective third-stage larvae of *Parelaphostrongylus odocoilei* from *Deroceras laeve* and *Catinella* sp. experimentally exposed to first-stage larvae (L1).

Trial	Species (number)	L1 dose	Mean L3/gastropod (range)*	Mean %L3 emerging (range)†	% gast.‡	% L3/L positive controls§	% L3/L at end of trial§
E1	*D. laeve* (10)	1,000	85 (54–127)	29 (14–49)	100	99	99
E2	*Catinella* (10)	500	53 (34–72)	4 (1–9)	60	34	97
E3	*D. laeve* (12)	500	34 (14–64)	21 (6–33)	100	84	100

Note that E2 and E3 ran concurrently.
* Total number of L3, including emerged and recovered at digest.
† L3 emerging as a percentage of total number of L3.
‡ Percentage of gastropods experiencing emergence by the end of the trial.
§ Percentage of larvae (L) that were infective L3 at digest.

mission for protostrongylid parasites. To explore the determinants of the distribution of *P. odocoilei* in North America, therefore, our objectives were: (1) to identify gastropod intermediate hosts native to the sub-Arctic; (2) to determine the significance of larval emergence, including survival of emerged larvae; (3) to determine the effects of temperature on development of larvae; and (4) to develop a DD model that can be used to predict larval development under different climatic conditions.

MATERIALS AND METHODS

Infection of gastropods with *P. odocoilei*

L1 of *P. odocoilei* were obtained from feces of captive Stone's sheep (*O. d. stonei*) experimentally infected with *P. odocoilei* from naturally infected Dall's sheep (*O. d. dalli*) in the Mackenzie Mountains, Northwest Territories, Canada (65°01′N, 127°35′W). Adult parasites and L1 from the captive sheep were confirmed as *P. odocoilei* on the basis of morphological and molecular data (Jenkins, Hoberg et al., 2005). Feces were held at −20 C for 5–33 mo before L1 were recovered using a modified beaker Baermann technique (Forrester and Lankester, 1997). Only motile L1 were used to calculate infection doses. Gastropods were fasted 24 hr, housed 6 to a medium petri dish (9 cm in diameter), and exposed to L1 suspended in a small amount of water at room temperature (Hoberg et al., 1995). Gastropods that moved away from the L1 suspension were returned to the suspension every 15–20 min for 3 hr. To synchronize infection in development trials, gastropods were rinsed with water at the end of the infection period to remove L1 that had not penetrated the gastropod.

Native intermediate hosts

Native gastropods were collected in July and August 2002 in the Mackenzie Mountains (64°28′N, 129°37′W) and snails identified on the basis of shell morphology (Pilsbry, 1946, 1948; Burch, 1962). Twelve specimens of *Catinella* sp., 6 specimens of *Euconulus* cf *fulvus* (possibly *E. fulvus alaskensis*), and 5 specimens of the Pupillidae (*Vertigo modesta* or *V. alpestris oughtoni*, or both, and *Columella edentula* or *C. alticola*, or both) were each exposed to 500 L1 of *P. odocoilei* and group-housed at 20 C (9–28 C) in large plastic petri dishes (15 cm in diameter) containing native vegetation and dried leaf litter. Equal numbers of uninfected control snails of each species were treated in a similar fashion, except they were not exposed to L1. As part of a separate experiment, 10 slugs (*Deroceras laeve*) were also collected from the same site, each exposed to 250 L1 of *P. odocoilei*, and housed under the same conditions. At 20 days postinfection (dpi), groups of 5–6 snails (including mortalities) were digested in a pepsin and hydrochloric acid solution for 1.5–3 hr to recover larvae (Hoberg et al., 1995). All larvae were examined and quantified at ×100–×400, and larval stages determined using morphological criteria established for *U. pallikuukensis* (Kutz, Hoberg et al., 2001). The L1, L2, and early L3 (dead and live early L3, which had poor motility and survival in digest fluid) were considered preinfective larvae, whereas intermediate L3 (partially ensheathed in the L2 cuticle) and late L3 (exsheathed) were considered infective larvae (as defined by Gerichter, 1948; Rose, 1957; Platt, 1978).

Larval emergence and survival

Three larval emergence trials (E1–E3) were established from September 2001 to September 2003 (Table I). *Deroceras laeve* were obtained from protostrongylid-free laboratory colonies and *Catinella* sp. were collected from the Mackenzie Mountains in August 2003. Gastropods were each exposed to 1,000 (E1) or 500 (E2 and E3) L1 and housed at room temperature (20–21 C). In E1 from 2 to 14 dpi, 6 gastropods were group-housed in each of 3 large plastic petri dishes (15 cm in diameter), which were examined for emerged larvae at 14 dpi. In E1 after 14 dpi, and in E2 and E3 after 4 dpi, gastropods were housed individually in small glass petri dishes (6 cm in diameter) containing 1–2 ml of tap water and maintained as per Kutz et al. (2000). Dishes were examined using a dissecting microscope for emerged larvae every 2 days from 14 to 60 dpi in E1, every 4 days from 6 to 18 dpi in E2 and E3, and every 2 days from 18 to 84 dpi in E2 and E3. For examination, gastropods were moved to new dishes, and tops and bottoms of vacated dishes were half-filled with tap water and examined immediately, or dishes were refrigerated for up to 4 days before examination. In E1 only, at each transfer the feet of slugs were examined using a dissecting microscope for lesions associated with larvae. As positive controls, 3 gastropods were digested at 26 dpi (E1) or 21 dpi (E2 and E3). At the end of each emergence trial, all gastropods were individually digested, and larvae counted and classified as above.

The cumulative percentage of L3 that emerged was calculated by dividing the number of L3 that emerged from an individual gastropod up to and including a given dpi by the sum of the number of L3 that emerged and the number of L3 recovered from digest of that gastropod at the end of the trial. The group mean cumulative percentage included only those gastropods experiencing emergence by the end of the trial. Data from 22 slugs in E1 and E3 were used for regression analysis of intensity of infection (L3/slug) on the percentage of infective L3 emerging from each slug by 60 dpi.

Preinfective larvae that emerged from slugs in E1 were held in 10 ml of tap water in a small glass petri dish at room temperature and monitored for survival for up to 7 days. Infective L3 that emerged from slugs in E3 were refrigerated in 10 ml of tap water in a small glass petri dish until the end of the experiment. From these, 60 motile or tightly coiled L3 was hand-picked and held in darkness at 1.6 C (range −3.4 to 9.8 C) in 10 ml of tap water in 25-ml Erlenmeyer flasks with a foam cork, 25 larvae/flask (one with 10 emerged L3/flask). The numbers of dead (visibly dead or missing and presumed dead) and live (tightly coiled or motile) L3 were recorded at 3, 6, and 12 mo.

Larval development and temperature

Six larval development trials (D1–D6) were established from November 2002 to March 2003 (Table II). *Deroceras laeve* were obtained from laboratory colonies, each exposed to 250 L1 of *P. odocoilei*, and group-housed in containers filled with moist soil and vermiculite at the temperatures indicated in Table II (Kutz, Hoberg et al., 2001). Temperatures were recorded every 30–60 min using HOBO XT external or H08 internal temperature sensors (Onset Computer Corporation, Pocasset,

TABLE II. Effects of temperature (range 7.8–24.3 C) on development of larvae of *Parelaphostrongylus odocoilei* in *Deroceras laevae* each exposed to 250 first-stage larvae.

Trial	Mean temperature ± SD (C)	Monitoring period (dpi*)	Number of slugs	Mean slug weight ± SD (mg)	Mean larvae/slug at digest (range)	Dpi first L3†
D1	7.8 ± 0.2	49–91	25	91 ± 41	38 (14–79)	Not observed
	20.6 ± 0.4	91–105‡				105 (1/1)
D2	9.8 ± 0.3	49–91	21	150 ± 53	15 (7–29)	70 (1/3)
						91 (3/3)
D3	12.4 ± 0.2	28–56	15	123 ± 34	12 (4–28)	49 (3/3)
D4	16.4 ± 0.4	6–26§	26	95 ± 31	20 (8–40)	24 (1/1)
D5	20.0 ± 0.4	4–18	24	90 ± 35	25 (5–44)	14 (2/3)
						16 (3/3)
D6	24.3 ± 0.8	3–14‖	18	89 ± 41	21 (10–39)	10 (1/3)
						12 (3/3)

* Days post infection.
† Infection third-stage larvae as defined in text.
‡ At each of 98, 102, and 105 dpi, 1–2 slugs collected, including mortalities.
§ At each of 20, 22, 23, 24, and 26 dpi, 1 slug collected, including mortalities.
‖ Slugs were collected at 3 dpi, then every 2 days starting at 6 dpi.

Massachusetts). Three slugs were collected every 7 days (D1, D2, and D3) or 2 days (D4, D5, and D6) for the monitoring periods indicated in Table II. Collected slugs were weighed and individually digested immediately or after refrigeration for up to 4 days. Larvae were counted and classified to developmental stage as described above.

Development rate was defined as the inverse of the first dpi that infective L3 were observed. The theoretical threshold temperature (T_0) and the thermal constant (DD, degree days, or the minimum amount of heating above threshold necessary for development of infective L3) were calculated from the intercept and slope of the line generated by a regression analysis of temperature on larval development rates for D2 to D6 [development rate = ($b \times$ temperature) $- a$, $T_0 = -a/b$, DD = $1/b$] (Campbell et al., 1974; Samson and Holmes, 1985; Kutz, Hoberg et al., 2001). The number of days at a given temperature necessary to obtain infective larvae was predicted using [dpi = DD/($T - T_0$)].

RESULTS

Native intermediate hosts

All specimens of *E.* cf *fulvus*, 75% of *Catinella* sp., and 40% of the Pupillidae were alive at 20 dpi. There were no differences in survival between infected and uninfected snails. At digest at 20 dpi, 93% (26/28) of larvae recovered from 6 *E.* cf *fulvus* and 11% (18/171) of larvae recovered from 12 *Catinella* sp. were infective L3. For comparison, at 19 dpi, 76% (295/386) of larvae were infective L3 from 9 experimentally infected native *D. laeve*. Only 3 dead preinfective larvae were recovered from 5 pupillid snails. Nine infective L3 were recovered from 5 uninfected control *Catinella* sp. snails.

Larval emergence and survival

Small numbers of preinfective larvae (36 in E1, 5 in E2, and 2 in E3) emerged from ~20% of gastropods, and the first infective L3 emerged between 22 and 26 dpi in all trials. Emergence of infective larvae occurred from 100% of *D. laeve*, but only 60% of *Catinella* sp. snails, and the mean percentage of infective L3 emerging from *D. laeve* was greater (Table I). The percentage of infective larvae emerging from *D. laeve* correlated with infection intensity ($F = 6.136$, $P = 0.022$) (Fig. 1). The mean number of larval-associated foot lesions visible in *D. laeve* in E1 increased from 14 to 44 dpi, then decreased steadily until the end of the trial at 60 dpi (Fig. 2).

Both preinfective and infective larvae emerged in high numbers from sick *D. laeve* and *Catinella* sp., often in the days

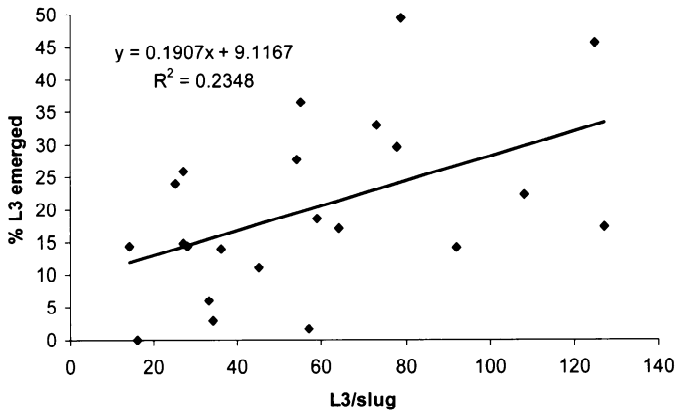

Figure 1. Intensity-dependence of emergence of infective larvae (L3) of *Parelaphostrongylus odocoilei* from *Deroceras laeve* in E1 and E3.

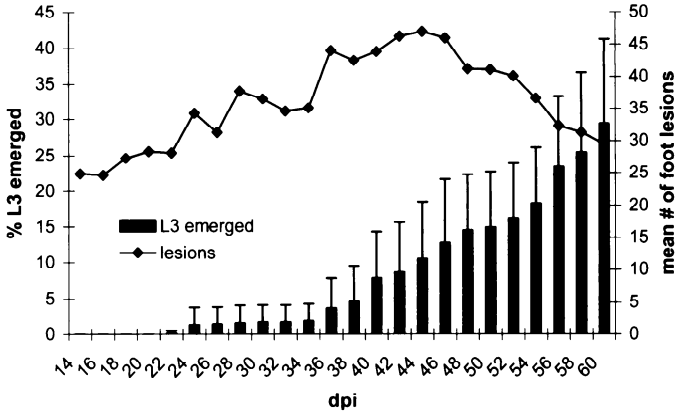

Figure 2. Mean cumulative percentage of infective larvae (L3) emerging from *Deroceras laeve* in E1, and mean number of foot lesions per slug. Error bars = 1 SD.

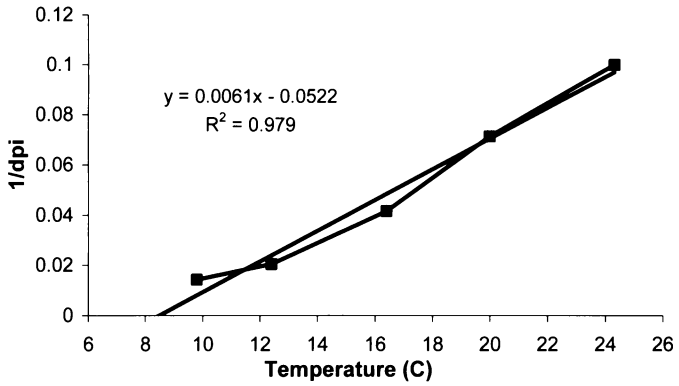

Figure 3. Relationship between temperature (C) and rate of development of larvae of *Parelaphostrongylus odocoilei* in *Deroceras laeve* (1/dpi = the inverse of the day post infection that the first infective larvae was observed). Note that although the linear model fits the data well, the relationship may actually be exponential.

immediately before death. In E1, 11 preinfective larvae emerged from a slug 2 days before the death of the slug at 16 dpi, and 11 preinfective larvae and 46% of infective L3 emerged from a slug that was sick for the duration of the monitoring period. In E2, 2 L3 emerged the day before death of a *Catinella* sp. snail at 72 dpi. *Catinella* sp. snails aestivated and fasted occasionally.

Preinfective larvae that emerged did not survive for longer than a few days, nor did they undergo further development. At 3, 6, and 12 mo, 83, 65, and 0% of emerged infective L3 were alive, respectively.

Larval development and temperature

Development rates of larvae of *P. odocoilei* in *D. laeve* had a direct relationship with temperature, which could be described by a linear model ($y = 0.0061x - 0.0522$, $R^2 = 0.979$, $F = 139.75$, $P = 0.001$) (Fig. 3). An exponential model also fit the observed data ($y = 0.0038e^{0.1399x}$, $R^2 = 0.981$, $F = 154.8$, $P = 0.001$), whereas a power model had the best fit ($y = 0.00008x^{2.244}$, $R^2 = 0.992$, $F = 356.7$, $P = 0.000$). In D2 (9.8 C), development occurred more rapidly than predicted by the linear model and was less synchronous, with infective L3 not present in all 3 slugs from each collection until 91 dpi (Table II). In D1 at 7.8 C (range 7.4 to 10.2 C), development to L2 but not to L3 occurred by 91 dpi (Fig. 4). After 91 dpi, when slugs were housed at 20.6 C, the first infective larvae were observed at 14 days (105 dpi on the D1 timeline), similar to D5 at 20 C (Table II, Fig. 4). The T_0 was 8.5 C, and the thermal constant was 163 DD.

DISCUSSION

Design

Bionomics of larvae of *P. odocoilei* in intermediate hosts potentially important in transmission at sub-Arctic latitudes have not been previously investigated. We explored factors that may determine the distribution of *P. odocoilei*, including suitable gastropod intermediate hosts, larval emergence (which creates the potential for overwinter transmission), and temperature requirements for larval development in gastropods. By comparing our findings to those for *P. odocoilei* in temperate and coastal regions, and to protostrongylid parasites limited to Arctic latitudes such as *U. pallikuukensis*, we gain insight into parasite life histories, as well as abiotic and biotic constraints on parasite development and transmission.

Figure 4. Distribution (as a percentage of total number of larvae) of larval stages of *Parelaphostrongylus odocoilei* recovered from *Deroceras laeve* digested on progressive days post infection (dpi) in D1. After 91 dpi, temperature increased from 7.8 to 20.6 C (arrow). L1 = first-stage larvae, L2 = second-stage larvae, eL3 = early third-stage larvae, infL3 = infective larvae, and ? = unidentifiable larvae.

Native intermediate hosts

On the basis of experimental infections, several species of gastropods native to the Mackenzie Mountains in sub-Arctic North America are suitable intermediate hosts for *P. odocoilei*, including *D. laeve*, *Catinella* sp., and *E.* cf *fulvus*. This is the first published report that *Catinella* sp., or indeed any member of the Succineidae (Heterurethra), can serve as a suitable intermediate host for *P. odocoilei*, although succineid snails are suitable hosts for other protostrongylids (Lankester and Anderson, 1968; Kutz et al., 2000). After exposure to similar numbers of L1, more larvae established in *Catinella* sp. than *E.* cf *fulvus* and *D. laeve*, but larvae required more time to develop to infective stage. This may reflect delayed development due to aestivation of *Catinella* sp. snails, as almost all larvae eventually reached infective stage in E2 (Table I) (Lankester and Anderson, 1968; Solomon et al., 1996; Kutz et al., 2000; Kutz, Hoberg et al., 2001), but see Gerichter (1948). Interestingly, infective L3 morphologically indistinguishable from *P. odocoilei* were recovered from uninfected control specimens of *Catinella* sp. collected in the Mackenzie Mountains, which may represent contamination or, possibly, natural infection of one or more snails with a protostrongylid native to this region. If the latter, this likely had negligible effects on our results from experimentally infected snails, as prevalence of naturally infected gastropods is low, less than 5% in Samuel et al. (1985), and mean intensity of protostrongylid larvae is much lower in natural versus experimental infections: 3 larvae/gastropod in Lankester and Anderson (1968) as compared to 53 larvae/*Catinella* sp. snail in E2 in the current study.

Both *D. laeve* and *E. fulvus* are considered important intermediate hosts for *P. odocoilei* in temperate regions on the basis of abundance and prevalence of larvae in naturally infected gastropods (Samuel et al., 1985). In addition, the snails *Vitrina limpida*, *Zonitoides* spp., and *Discus* spp. in temperate regions are potentially important in transmission (Platt and Samuel, 1984; Samuel et al., 1985). Members of the Pupillidae appear to be refractory to infection with *P. odocoilei*, and had poor survival in captivity, as observed in subsequent trials with larger numbers of snails (n = 48; data not shown) and elsewhere (Gray et al., 1985; Samuel et al., 1985). These findings suggest that *D. laeve* and *Catinella* sp. are the most important intermediate hosts for *P. odocoilei* in the sub-Arctic, with *E.* cf *fulvus* possibly playing a lesser role on the basis of lower larval establishment. Both *D. laeve* and *Catinella* sp. are hydrophilic gastropods, abundant in sedges, grasses, and willow stands, whereas *E. fulvus* is more xerotolerant and can be found in a wider variety of habitats in temperate regions (Boag and Wishart, 1982) and in the Mackenzie Mountains (data not shown). Thinhorn sheep primarily encounter habitat suitable for gastropods on their winter range, and possibly at heavily used, naturally occurring mineral licks and water sources on summer range (Hoefs and Cowan, 1979; Simmons, 1982). Such habitat patches may serve as foci of infection (Lankester and Anderson, 1968; Anderson, 1972).

North of the Arctic Circle, larvae of *P. odocoilei* were not present in fecal samples from thinhorn sheep, barren-ground caribou, or musk oxen, although other protostrongylid species are present in, and frequently shared among, these hosts (Hoberg et al., 2002; Jenkins, Appleyard et al., 2005; data not shown). The slug *D. laeve* has been reported from Alaska and the Arctic Islands, and *D. laeve*, *Catinella* sp., and *E. fulvus* have been recovered from the coast of the Arctic mainland at 68°N (Pilsbry, 1948; Kutz, 2000). Both *D. laeve* and *E. fulvus* have Holarctic distributions (Burch, 1962). Therefore, at least some of the gastropod species important for transmission of *P. odocoilei* in sub-Arctic and temperate regions are present in the Arctic. The apparent absence of *P. odocoilei* from these regions suggests that either this parasite has not been introduced into the historically isolated Dall's sheep populations in the Arctic, or other factors may currently be limiting its distribution, i.e., abiotic conditions unfavorable for survival of L1 or development of larvae in gastropods.

Larval emergence and survival

Emergence of infective larvae from intermediate hosts can be viewed as 'bet-hedging,' where a parasite produces offspring that differ in infection strategy (Fenton and Hudson, 2002). Infective larvae of *P. odocoilei* emerged from healthy specimens of *D. laeve* and *Catinella* sp., both important intermediate hosts for *P. odocoilei* in the sub-Arctic. Emergence occurred less commonly from *Catinella* sp. despite higher larval establishment rates, perhaps because of delayed larval development and aestivation (Kutz et al., 2000). Larvae emerged in disproportionately large numbers from sick or dying gastropods (similar to observations by Rose, 1957 and Kutz et al., 2000), suggesting that protostrongylid larvae are sensitive to changes in the physical milieu or immunity of the host. Marked host reactions were associated with larvae in the feet of *D. laeve*, with lesions similar in appearance to those reported for *P. odocoilei* in *Triodopsis multilineata* (Platt, 1978), and for *Elaphostrongylus rangiferi* in *Arianta arbustorum* (Skorping, 1984). Visibility of lesions initially increased, possibly due to increasing host reaction surrounding larvae, then decreased, correlating with emergence of a greater percentage of larvae and resolution of lesions (Fig. 2).

Emerged infective larvae of *P. odocoilei* survived 6 mo in darkness in water at near-freezing temperatures (at times below freezing) under conditions simulating the subnivean environment, where there is little direct sunlight, 100% relative humidity, and temperatures are stable at or just below 0 C, despite much colder air temperatures (Marchand, 1996; Forrester and Lankester, 1998). Survival of emerged infective larvae has been observed under various environmental conditions for other protostrongylids (Rose, 1957; Boev, 1975; Cabaret and Pandey, 1986; Kutz et al., 2000). Emergence of infective larvae may, therefore, be an important aspect of transmission of some protostrongylids, especially in northern regions where gastropods are seasonally unavailable for much of the year (Kutz et al., 2000). Gastropods become inactive when temperatures fall below 0 C and when snow cover becomes continuous, although some activity may occur in the subnivean space in early winter and late spring (Aitchison, 1979). Larval emergence may facilitate overwinter transmission of free-living L3 of *P. odocoilei* to ungulates foraging beneath the snow pack, as suggested for *U. pallikuukensis* in the Canadian Arctic (Kutz et al., 2000), and for free-living L3 of gastrointestinal nematodes in the Eurasian Arctic (Halvorsen et al., 1999).

Preinfective larvae of *P. odocoilei* that emerged did not survive or continue development, and likely have no significance for transmission. Emergence of preinfective larvae has not been previously reported for any protostrongylid species, nor has it been considered a mechanism of host resistance (Cabaret, 1979; Kralka and Samuel, 1984; Kutz et al., 2000). Emergence of preinfective larvae of *P. odocoilei*, which occurred from both healthy and sick gastropods, likely reflects "spillover" at high infection intensities, or expulsion by the intermediate host. This may also occur with some infective larvae at high intensities, as the percentage of infective larvae of *P. odocoilei* that emerged from *D. laeve* was correlated with intensity of infection. Although mean infection intensities were artificially high in experimentally infected gastropods, 295 larvae of *P. odocoilei* have been reported in a naturally infected slug (Platt, 1978). Infective L3 were significantly smaller in *D. laeve* in E1 at high infection intensity (Jenkins, 2005), suggesting that larval growth may be intensity dependent, as observed for larvae of other protostrongylid species (Boev, 1975; Skorping, 1984).

Even at high infection intensities, the percentage of infective larvae that emerged from *D. laeve* never exceeded 50%, similar to *Protostrongylus boughtoni* and *P. stilesi* (Monson and Post, 1972; Boev, 1975; Kralka and Samuel, 1984). In contrast, 100% of L3 of *U. pallikuukensis* emerged from some slugs regardless of infection intensity (Kutz et al., 2000). This may reflect specific adaptations to different environmental constraints; *P. odocoilei* is present at latitudes ranging from 36° to 65°N (Jenkins, Appleyard et al., 2005), whereas *U. pallikuukensis* is present at latitudes ranging from 65° to 69°N (Kutz, 2000). The possibility of latitudinal differences in the proportion of larvae that emerge within and among species of protostrongylids warrants further

investigation. Where overwinter transmission is not limiting, i.e., in warm temperate or tropical regions, few or no larvae may emerge, which may in part account for the controversy over the significance of this phenomenon.

Larval development and temperature

Heating above the threshold temperature (T_0) may be most important for development of *P. odocoilei* from L2 to L3. Development to infective L3 did not occur below T_0 (8.5 C), but larvae did develop from L1 to L2. When warmed above T_0, L2 developed to L3 at a rate similar to that predicted if the larvae had started as L1, as observed for other protostrongylids (Gerichter, 1948). At or below T_0, development from L1 to L2 occurred for *U. pallikuukensis* (Kutz, 2000), but not *E. rangiferi* (Halvorsen and Skorping, 1982). It is possible that stochastic variation in temperature may trigger development from L1 to L2 when temperatures briefly reach or exceed a critical threshold (Saunders et al., 2002). Alternatively, the threshold temperature for development from L1 to L2 may be lower than that for L2 to L3.

The threshold temperature of protostrongylids from temperate and northern regions, including *P. odocoilei*, *E. rangiferi*, *U. pallikuukensis*, and *Protostrongylus* spp., appears to be strongly conserved, ranging from 8 to 10 C (Samson and Holmes, 1985; Kutz, Hoberg et al., 2001). In contrast, T_0 of *Muellerius capillaris*, a protostrongylid parasite of more temperate climates, is 4.2 C (Rose, 1957; Kutz, Hoberg et al., 2001). The paradoxically higher T_0 in protostrongylids in northern climates may be an adaptation to strong seasonal patterns of transmission, delaying onset of development until temperatures are consistently warmer and development to infective stage occurs rapidly (Schjetlein and Skorping, 1995). Slowly developing larval stages may place increased demands on their gastropod hosts for a longer period of time, or may elicit stronger host reactions, decreasing survival of both gastropods and larvae.

Using the DD concept, or the amount of heating above threshold, larvae of *P. odocoilei* needed 163 DD to develop to infective stage, similar to *U. pallikuukensis* and *M. capillaris*. Higher values have been reported for *Protostrongylus* spp. (305) and *E. rangiferi* (250) (Samson and Holmes, 1985; Kutz, Hoberg et al., 2001). For *Protostrongylus* spp., Samson and Holmes (1985) used a more conservative end point (>50% of gastropods with infective larvae) that minimized the effects on the model of asynchronous development at low temperatures. Further investigation is needed to determine the influence of intermediate host species, phylogenetic relationships, and environment, as well as methods used by investigators, on developmental parameters of protostrongylid larvae.

Developmental parameters of *P. odocoilei* may vary across its broad host range and geographic distribution extending from California to Alaska. Using T_0 and DD of *P. odocoilei* of sub-Arctic origin, larvae would need 17 days at 18 C to reach infective stage in *D. laeve*, an intermediate host native to both temperate and sub-Arctic regions. At this temperature, larvae of *P. odocoilei* from mule deer in temperate regions (Jasper, Alberta, Canada) needed a minimum of 22 days to develop to infective L3 in *T. multilineata* (Platt, 1978), whereas larvae of *P. odocoilei* from black-tailed deer in milder coastal regions (Vancouver Island, British Columbia, Canada) needed 35–38 days in various gastropods, including *T. multilineata*, *Deroceras reticulatum* (previously *Agriolimax agrestis*), and *Helix aspersa* (Hobmaier and Hobmaier, 1934; Shostak and Samuel, 1984). Establishment and development rates of larvae of *P. odocoilei* may be suboptimal in these gastropod species, which are not native intermediate hosts for *P. odocoilei* (Platt and Samuel, 1984; Shostak and Samuel, 1984; Kutz, Veitch et al., 2001). Nonetheless, the possibility of a continuum of developmental capacity of *P. odocoilei* across its geographic and host range warrants further exploration. For example, the prepatent period of *P. odocoilei* in thinhorn sheep was approximately 20 days longer than that of *P. odocoilei* in mule deer given similar doses of infective L3 (Jenkins, Hoberg et al., 2005). Genetic differences at the COX II locus have been observed in mitochondrial DNA of *P. odocoilei* from different locations, but were not restricted latitudinally (unpubl. obs.).

Our work suggests that a DD model (which assumes a linear relationship) may adequately predict development rates of larvae of *P. odocoilei*. However, an exponential or power relationship cannot be excluded, and larval development at cooler temperatures may be faster than predicted by the linear model (Saunders et al. 2002); it is not known whether the amount of development that occurs at or below threshold temperature is biologically significant. For this reason, and in light of the possibility of biological differences in *P. odocoilei* across its broad geographic range, the DD model needs to be validated using observations of larval development in the field before it can be broadly applied (Kutz et al., 2002). Nonetheless, it is instructive to compare temperatures across the range of *P. odocoilei* to determine if climate currently determines the northern limits of its distribution in North America. At most sites in temperate Canada where *P. odocoilei* is established, daily average temperatures exceed T_0 (8.5 C) for 5–7 mo (May–September in temperate regions, or April–October in the coastal regions). In the western Canadian sub-Arctic and Arctic, daily average temperatures exceed T_0 for only 3 mo (June–August), and in the Arctic, never exceed 14 C (vs. 17 C in the sub-Arctic) (http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html, accessed April 2005). Therefore, *P. odocoilei* may be restricted from northward range expansion by temperature-dependent limitations on development of larvae in intermediate hosts.

It is essential to develop models on the basis of empirical observations in the laboratory and validate them in the field before applying such models to determine the effects of climate, and by extension climate change, on parasite distribution, abundance, and transmission (Hoffmann and Blows, 1994; Harvell et al., 2002; Kutz et al., 2005). Laboratory-based investigations, such as the current study, are key to understanding the biology and ecology of host–parasite systems, interpreting epidemiological patterns, and predicting shifts in the host–parasite equilibrium.

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