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Disease transmission in an extreme environment: Nematode parasites infect reindeer during the Arctic winter

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ABSTRACT

Parasitic nematodes are found in almost all wild vertebrate populations but few studies have investigated these host–parasite relationships in the wild. For parasites with free-living stages, the external environment has a major influence on life-history traits, and development and survival is generally low at sub-zero temperatures. For reindeer that inhabit the high Arctic archipelago of Svalbard, parasite transmission is expected to occur in the summer, due to the extreme environmental conditions and the reduced food intake by the host in winter. Here we show experimentally that, contrary to most parasitic nematodes, *Marshallagia marshalli* of Svalbard reindeer is transmitted during the Arctic winter. Winter transmission was demonstrated by removing parasites in the autumn, using a novel delayed-release anthelmintic bolus, and estimating re-infection rates in reindeer sampled in October, February and April. Larval stages of nematodes were identified using molecular tools, whereas adult stages were identified using microscopy. The abundance of *M. marshalli* adult worms and L4s increased significantly from October to April, indicating that reindeer were being infected with L3s from the pasture throughout the winter. To our knowledge, this study is the first to experimentally demonstrate over-winter transmission of a gastro-intestinal nematode parasite in a wild animal. Potential mechanisms associated with this unusual transmission strategy are discussed in light of our knowledge of the life-history traits of this parasite.

1. Introduction

Parasitic nematodes of vertebrates have successfully exploited a wide variety of habitats and hosts, from polar regions to the tropics, and are remarkable in that they must adapt to two distinct environments, the external conditions during their free-living stages and the internal conditions of the host they parasitize. For the free-living stages, the external conditions, including soil moisture, humidity and temperature, have a major influence on the life history parameters of the parasite (Anderson, 1992). Most of what is known about the population ecology of these nematodes is based on parasites infecting domesticated animals (Stromberg, 1997; ÓConnor et al., 2006). However, the role of macroparasites in wildlife ecology is equally important and, in recent years, has received increasing attention for two main reasons (Thompson et al., 2010; Tompkins et al., 2011). First, accumulating evidence suggests that they have the potential to regulate host populations through effects on reproduction and/or survival (Anderson and May, 1978; May and Anderson, 1978; Dobson and Hudson, 1992; Hudson et al., 1992; Albon et al., 2002; Gunn and Irvine, 2003; Irvine, 2006). Second, climate change could significantly alter the parasite–host relationship leading to the spread of disease and increased pathogenicity (Kutz et al., 2005; Hudson et al., 2006; Jenkins et al., 2006; Morgan and Wall, 2009; Laaksonen et al., 2010). Given that current and predicted climate warming is particularly pronounced in the Arctic (Kattsov and Källén, 2005), improving our understanding of host–parasite ecology in that area, may be of wider relevance (Kutz et al., 2005, 2009a; Davidson et al., 2011).

Polar, alpine and temperate environments all exhibit strong seasonality in climate, where temperatures drop below zero on a regular basis. Parasites are expected to have adapted to these conditions by, for example, cold hardiness of the free-living stages and winter survival in the host by the parasitic stages (Wharton, 1999).

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However, for domesticated animals in these regions high infection intensities are experienced during spring and summer whilst transmission rates during the winter are typically low. This is in part due to the practice of housing animals during winter but mainly due to the reduced survival and development of the free-living nematode stages at sub-zero temperatures (Stromberg, 1997; ÓConnor et al., 2006). The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) which are found in the Svalbard archipelago in the high Arctic (76–81° N, 9–33° E) and their nematodes provide an ideal system for the study of host–parasite interactions in the Arctic because it is a relatively simple system with few confounding factors complicating the interpretations of the host–parasite relationships; in addition experimental manipulations have proved possible (Albon et al., 2002; Stien et al., 2002b). The environment on Svalbard is harsh for most of the year, with low temperatures, humidity and precipitation combined with high winds (Halvorsen and Bye, 1999). During winter (October–May), Svalbard reindeer spend less time grazing and have reduced food intake, partly due to a need to conserve energy (Loe et al., 2007), but also due to difficulties in accessing forage plants buried under snow and ice (Hansen et al., 2009). Thus, the main grazing season for the reindeer occurs during the short summer.

Given the extreme environmental conditions that prevail on Svalbard and the reduced food intake by the host during winter, peak egg output and nematode transmission is expected to occur between June and August (Halvorsen and Bye, 1999; Halvorsen et al., 1999; Irvine et al., 2000), when the mean monthly air temperature is above 0 °C (Statistisk-sentralbyra, 2005). Surprisingly, studies on the population dynamics of the two most abundant nematodes in the system have revealed contrasting life-histories (Irvine et al., 2000; Irvine, 2001; Stien et al., 2002b). Whilst summer transmission has been demonstrated experimentally for *Ostertagia gruehneri* infection in Svalbard reindeer (Stien et al., 2002a,b), cross-sectional data suggest that a second abundant gastrointestinal nematode in this host, *Marshallagia marshalli*, is most likely to be transmitted over the winter (Halvorsen et al., 1999; Irvine et al., 2000).

Here we report on an experiment to determine the extent to which *M. marshalli* accumulates in the reindeer host during the winter months, despite the severe conditions. To do this, the logistical problems of working during the Arctic winter were overcome by developing a delayed-release anthelmintic bolus administered in April, but activated in late autumn (Carlsson et al., 2012). Specifically, the prediction that the abundance of all stages of the nematode in the host are low at the start of winter in treated animals but that the number of adult *M. marshalli* worms increases as the winter progresses was tested. In addition, we predicted that the other nematode species, *O. gruehneri*, would show no over-winter transmission and that the abundance of this parasite would remain stable over winter. Finally the potential mechanisms and evolutionary adaptations associated with over-winter transmission of this species are discussed.

2. Materials and methods

2.1. Study area and reindeer hosts

The climate on Svalbard is severe, with a mean annual air temperature at Svalbard airport (1981–2010) of −5.4 °C, ranging between a mean of −12.9 °C (range −22.0 °C to 2.7 °C) in January to 6.4 °C (range 6.1 °C −8.2 °C) in July. Svalbard reindeer are widespread and the only large free-living herbivores on the island. Previous studies have shown large inter-annual variations in reindeer population size: in years with "bad winters" (high precipitation and icing) the population experiences poor survival with low birth rates the following summer (Solberg et al., 2001; Tyler et al., 2008). Svalbard reindeer are especially vulnerable during winter due to starvation. They lose up to 50% of their body weight between October and April, and although they store large reserves of subcutaneous fat during summer and autumn, these are not sufficient to meet their energy requirements during winter (Oritsland, 1986; Tyler, 1987). The reindeer do not undertake seasonal migration, but may move locally in search of more accessible forage (Stien et al., 2010). The present study was undertaken in the Colesdalen–Reindalen valley system, Nordenskiöldland, Spitsbergen, Norway (77°50–78°20 N, 15°00–17°30 E) during the winters of 2006–07, 2007–08 and 2008–09. Female reindeer have been marked and caught annually in this valley system since 1994, allowing treatment and recapture of known individuals (Albon et al., 2002). Experimental manipulation of parasite burdens was undertaken by administering an anthelmintic to chemically remove the resident worm burden (see Section 2.2).

2.2. Anthelmintic treatment

To experimentally manipulate worm abundance during the winter, a delayed-release intra-ruminal bolus was administered orally to female adult reindeer caught in April. The bolus released a placebo for c. 150 days followed by an anthelmintic drug for c. 30 days (abamectin, MSD Agvet, NZ). Thus, nematodes were eliminated in October (Carlsson et al., 2012). The protection against re-infection was expected to be short-term (4–5 weeks) rather than the whole winter, and treated animals culled in February and April the following year were predicted to have become re-infected.

2.3. Nematode samples and reindeer population size

To estimate the abundance of gastrointestinal nematodes, abomasa were collected from a total of 43 adult females. Non-treated and treated animals were culled in five sampling periods; in October 2006 (sample sizes: control = 8, treated = 2), October 2007 (control = 8, treated = 2), February 2007 (control = 4, treated = 4), February 2009 (control = 4, treated = 4), and April 2009 (control = 4, treated = 3). All animal procedures on Svalbard were performed under license from the Norwegian National Animal Research Authority and the Governor of Svalbard with a veterinary surgeon in attendance. The estimated reindeer population size in the study area was obtained from an annual census conducted in July by the Norwegian Institute for Nature Research (NINA). Based on earlier analyses of the relationship between reindeer densities and *O. gruehneri* abundance (Albon et al., 2002), as well as the proposed transmission strategy of *M. marshalli* (Irvine et al., 2000), a 1 year time-lag was assumed in the response of *M. marshalli* infection to host population size. The estimated population size used in the analysis, in relation to worm burdens during the winters of 2006–07, 2007–08 and 2008–09, were therefore from the summers of 2005 (415), 2006 (389) and 2007 (506), respectively.

2.4. Parasite populations

Both *O. gruehneri* and *M. marshalli* are gastro-intestinal nematodes and have direct life-cycles in which transmission of the infective stage to its host occurs during grazing. Eggs are passed in faeces and develop on the ground through three larval stages. The infective L3s are protected by a sheath based on the L2 cuticle, which serves to protect the L3 from adverse conditions. Following transmission to the host through ingestion, the L3s invade the abomasal mucosa, develop into L4s and then return to the abomasal lumen where they undergo a final moult to the adult stage. In some species, L4s in the mucosa enter a state of arrested development and may accumulate in high numbers. This usually occurs during late summer and autumn and is thought to be an adaptation

by the parasite to survive periods when environmental conditions are unfavourable for the development and survival of the free-living stages. These arrested larvae may then resume development to become reproducing adults when environmental conditions are more favourable for transmission (Anderson, 1992; ÓConnor et al., 2006). To ascertain whether any observed increase in worm abundance was in fact due to a new infection acquired from L3s on the tundra, all three parasite stages in the host were extracted and identified, namely L4s, L5s and adult worms. Abomasa from culled reindeer and nematodes from the abomasal lumen were sampled and counted as described in Halvorsen et al. (1999) and Irvine et al. (2000). Larvae recovered from the abomasal lumen of Svalbard reindeer are generally much longer than those recovered from the mucosa (mean length ± S.D: lumen-dwelling larvae = 2,571 μm ± 828, $n = 79$; mucosal-dwelling larvae = 1,337 μm ± 438, $n = 438$; t-test: $t = 13.15$, df = 80.05, $P < 0.001$) and were therefore assumed to be L5s (Irvine et al., unpublished data). Thus, mucosal-dwelling larvae are henceforth referred to as L4s and lumen-dwelling larvae as L5s.

Abomasa were removed and frozen within 3 h of culling and after thawing the abomasa were opened along the greater curvature. Adults and L5s were recovered from the abomasa and counted as described in Halvorsen et al. (1999). Adult worms were identified based on morphological features of a subset of adult male nematodes (Dróżdź, 1965), with minor and major morphs grouped according to recent molecular findings (Dallas et al., 2000a, 2001). For recovery of L4s, the empty, washed abomasum was cut in half from the duodenum down to the reticulo-omasal orifice. To estimate L4 abundance, one half the mucosa was digested in an acid-pepsin solution for 12 h as described previously (Irvine and Dallas, 2002). Larval extraction for species identification was performed using the remaining mucosa in a 5–7 h neutral acetyl-cystine digestion (60 mM N-Acetyl Cystine in a 10× PBS solution) as described previously (Irvine and Dallas, 2002). Larvae were then isolated from the digest and counted as described in Halvorsen et al. (1999). For DNA isolation, the first 150 L4s (where available) were collected and stored in 70% ethanol and of those, 48 larvae were randomly selected for species identification. The conditions for DNA isolation and identification of L4s and L5s using species-specific primers for *M. marshalli* and *O. gruehneri* in a PCR were as previously described (Dallas et al., 2000b; Irvine and Dallas, 2002), with minor adjustments. Briefly, DNA from a minimum of 48 individual larvae from each animal, where available, was isolated by overnight incubation at 55 °C with 2 μL proteinase K in a 20 ml 10 mM Tris–HCl, 0.1 mM EDTA solution. One PCR primer pair, diagnostic for *M. marshalli* (Mm-ITS2-F and MM-ITS2-R) or *O. gruehneri* (Og-ITS2-F and Og-ITS2-R) (Dallas et al., 2000b; Irvine and Dallas, 2002), was used in each PCR assay. The PCR volume was a total of 10 μL containing: 1× NH4 extraction buffer (Bioline Ltd., United Kingdom), 2.5 Mm $MgCl_2$, 200 μM dNTP, 0.5 μ Taq polymerase (Bioline Ltd.), 1 μM F/R primers and 5 μL undiluted larval DNA. The PCR programme was 92 °C for 2 min, (90 °C for 15 s, 50 °C for 15 s) × 40 cycles, 72 °C for 3 min and 20 °C for 1 min. The annealing temperature was 48.5 °C for the PCR assay diagnostic for *M. marshalli* as opposed to 50 °C. Earlier work has established that the mucosal-dwelling larvae are dominated by *O. gruehneri* and the lumen-dwelling larvae by *M. marshalli* (Irvine, 2001). Therefore, for each larval sub-population, PCR-based identification was performed for the dominant species first; unsuccessful samples were then repeated using a PCR assay specific for the second species. Not all larvae gave a PCR product that could be used to identify species. In a few cases where the PCR success rate was unusually low, a second batch of 48 larvae, where available, was tested. In all analyses, it was assumed that the probability of success with PCR-based species identification was equal for *O. gruehneri* and *M. marshalli*.

Adult and larval nematodes were successfully extracted and counted from all 43 animals. However, for five animals (all culled in October 2006, two treated and three control animals) the L4s were unsuitable for species identification because they had been extracted using a pepsin acid digest; these animals were not included in the analysis. Species identification of L5s was unsuccessful for two animals (controls, culled October 2006 and February 2007) and these were not included in the analysis. The abundance of each species and each parasite fraction was estimated using the proportion of identified worms and scaling it up to the infra-population level on the basis of worm counts, assuming an equal proportion of male and female worms in the adult section (Stien et al., 2005).

2.5. Statistical analyses

All analyses were performed using *R* version 2.13.0 (R Development Core Team (2012). The abundance of nematodes was analysed using generalised linear mixed effects models (GLMM) with a negative binomial error distribution and a log link function (Wilson and Grenfell, 1997; Paterson and Lello, 2003; Pollitt et al., 2012). The function glmmADMB from the glmmADMB library in *R* was used for this analysis. The effects of treatment, time (during winter) and host population size on the estimated nematode abundance were analysed separately for the three worm fractions (adult worms, L5s and L4s) for both *M. marshalli* and *O. gruehneri*. Time (month: October = 0, February = 4 and April = 6) and host population size (population size counted year $t - 1$) were fitted as continuous variables and treatment was included as a two-level factor (treated with anthelmintic or not). The effects of host population size in years t and $t - 2$ were also tested, but these explained less variation in worm counts than host population size in year $t - 1$. Year was fitted as a random effect to account for any temporal auto-correlation in worm abundances, where each winter sampled (i.e., 2006–07, 2007–08 and 2008–09) corresponded to 1 year. Subsequent model selection was based on stepwise deletion, retaining variables at $P < 0.05$, and ANOVA using Chi-squared test statistics. For analysis of L4 *M. marshalli* burdens, a large proportion of treated animals had an estimated L4 abundance of zero and these points had a strong effect on the slope estimates for change over time in the treated group, generating spurious estimates. To account for this, a set of random numbers were generated from the negative binomial distribution with a mean that was half of the lowest observed *M. marshalli* L4 abundance above zero and a theta (or k) of 0.4, and inserted these values in the model instead of the zero counts.

3. Results

3.1. Ostertagia gruehneri

The anthelmintic treatment significantly reduced the number of *O. gruehneri* L4s ($P < 0.001$) and the number of adult worms ($P = 0.004$; Table 1; Fig. 1A and C). The abundance of both of these worm fractions remained stable throughout the winter in both treated and control animals (adults: $P = 0.343$; L4: $P = 0.243$), and there was no interaction between treatment and time (adults: $P = 0.543$; L4: $P = 0.354$), with the treated animals having low worm abundance throughout the winter and the control group having high burdens (Table 1; Fig. 1A and C). There was no relationship between burdens of *O. gruehneri* and reindeer population size in year $t - 1$ for any of the life-stages: adult worms ($P = 0.307$), L4s ($P = 0.420$) or L5s ($P = 0.421$; Table 1). The abundance of *O. gruehneri* L5s was equally low in the control and treated groups in October ($P = 0.712$), and did not change over winter ($P = 0.243$,

Table 1
Likelihood ratio statistics, parameter estimates with standard errors and sample sizes for the generalised linear mixed effects models analysing the influence of time, treatment and density on the abundance of adult worms, L5s and L4s for *Ostertagia gruehneri* and *Marshallagia marshalli*. The *P*-values and deviance estimates are from a likelihood ratio test, when comparing the minimal model to a model with (non-significant predictors) or without (significant predictors) the specified fixed effect. Reported estimates of fixed effects for significant predictors (bold values), standard deviation of the random effect and theta (the negative binomial dispersion parameter) are all from the minimal model.

Predictor	*Ostertagia gruehneri*					*Marshallagia marshalli*				
	Estimate	S.E.	df	Deviance	$P(\chi^2)$	Estimate	S.E.	df	Deviance	$P(\chi^2)$
Adult worms										
Intercept	9.129	0.241				8.23	0.146			
Time	0.077	0.081	1	0.898	0.343	0.009	0.049	1	1.566	0.210
Treatment	−4.107	0.408	1	36.202	**<0.001**	−1.64	0.251	1	16.222	**<0.001**
Reindeer population size	0.004	0.004	1	1.04	0.307	0.008	0.002	1	10.06	**0.001**
Time:Treatment	0.091	0.159	1	1.22	0.543	0.255	0.068	1	11.503	**0.001**
Theta Random effect	0.617 ± 0.13					5.058 ± 1.059				
	S.D. < 0.001					S.D. < 0.001				
L5s										
Intercept	5.995	0.438				7.489	0.263			
Time	−0.176	0.139	1	1.362	0.243	−0.072	0.068	1	10.530	**0.042**
Treatment	−0.343	0.921	1	0.136	0.712	0.3346	0.427	1	2.104	0.144
Reindeer population size	−0.006	0.006	1	0.65	0.420	<0.001	0.005	1	0.039	0.842
Time:Treatment	−0.400	0.304	1	3.258	0.354	−0.283	0.122	1	6.054	**0.013**
Theta Random effect	0.175 ± 0.039					1.418 ± 0.296				
	S.D. = 0.438					S.D. ⩽ 0.001				
L4s										
Intercept	9.384	0.115				7.834	0.398			
Time	0.041	0.041	1	1.002	0.316	0.125	0.095	1	2.766	0.096
Treatment	−0.781	0.196	1	13.368	**0.004**	−4.934	1.28	1	9.444	**0.002**
Reindeer population size	0.001	0.001	1	0.648	0.421	0.009	0.005	1	2.912	0.087
Time:Treatment	−0.013	0.095	1	1.02	0.600	0.624	0.260	1	4.234	**0.039**
Theta Random effect	3.044 ± 0.663					0.529 ± 0.127				
	S.D. ⩽ 0.001					S.D. < 0.001				

Table 1; Fig. 1B). These data are consistent with a lack of over-winter transmission in this species.

3.2. Marshallagia marshalli

The anthelmintic treatment significantly reduced *M. marshalli* adult worm abundance ($P = 0.001$; Table 1; Fig. 1D and F). In line with our predictions, and in contrast to *O. gruehneri*, there was a clear increase in adult *M. marshalli* burdens in the treated group over the winter: the abundance of adult worms was low in October, increased over winter and converged with the controls in April ($P < 0.001$; Table 1; Fig. 1D). In the control group, there was no significant trend over winter ($P = 0.210$; Table 1; Fig. 1D). Anthelmintic treatment did not result in a significant decline in the overall abundance of *M. marshalli* L5s, but there was a significant interaction between time and treatment ($P = 0.013$; Table 1). This reflects the fact that whilst there was no significant change in the abundance of L5s in the control group, there was a significant decline in the treated group, such that in both February and April the treated animals had a lower abundance of L5s in the lumen than the controls (Fig. 1E). The abundance of L4s followed a similar pattern to that of the adult worms, with the anthelmintics significantly reducing larval burdens ($P = 0.002$) and treated animals having fewer L4s than controls in October, but with larval burdens increasing over winter to converge on those of the control group in April (time:treatment interaction: $P = 0.039$; Table 1; Fig. 1F). Neither the abundance of L5s nor L4s was related to reindeer density during the previous summer (L5: $P = 0.842$; Table 1; L4: $P = 0.184$; Table 1), but adult *M. marshalli* infection was related to host density ($t - 1$), with worm abundance increasing with host density ($P < 0.001$; Table 1).

4. Discussion

To our knowledge, this is the first study to experimentally demonstrate over-winter transmission of a gastrointestinal nematode in a wild mammal. Studying the population dynamics of parasites in wild hosts is difficult and sample sizes are often limited due to logistical and management constraints associated with working with free-ranging wild animals. However, despite the relatively small sample sizes, by conducting a carefully controlled long-term experimental manipulation, analysed using robust statistical methods, this study clearly shows that *M. marshalli* is transmitted during the harsh Arctic winter, whereas *O. gruehneri* is not.

Many directly-transmitted parasitic nematodes have the ability to arrest their development as L4s in the abomasal mucosa and resume development at a later date (Anderson, 1992). Thus, the observed increase in adult worm abundance could be due to emerging L4s. However, by using molecular tools that allow identification of the larval stages of gastro-intestinal nematodes (Dallas et al., 2000b; Irvine and Dallas, 2002), it was established that the abomasal mucosa of treated animals contained no *M. marshalli* L4s in October, but as winter progressed these larvae accumulated. Therefore, these results do not support the hypothesis that an increase in adult worm burden was due to emerging immature larvae but indicate that reindeer were re-infected with viable infective larvae from the tundra, from October to April, after effective removal of worms at the start of the winter.

Identification of the larval stages also gave further insight to the life-history of this parasite. Previous work has suggested that *M. marshalli* adults have a short lifespan, based on the strong seasonal pattern in abundance of adult worms, which peaks in April and is close to zero in July (Irvine et al., 2001 as cited earlier; Stien et al., 2002b). In a short-lived nematode, one would expect a short prepatent period in which the nematode matures quickly to the adult stage in order to maximise its reproductive success (Morand and Sorci, 1998). In line with previous observations (Irvine, 2001), the proportion of *M. marshalli* larvae was much higher in the lumen than the mucosa, suggesting that *M. marshalli* moves quickly from L4s in the mucosa to L5s in the lumen and then rapidly develops into mature adults. The abundance of *M. marshalli* L4s increased over winter, with the anthelmintic-treated animals having a lower abundance immediately post-treatment, whilst the abundance of *M. marshalli* L5s in treated animals was lower than that in the

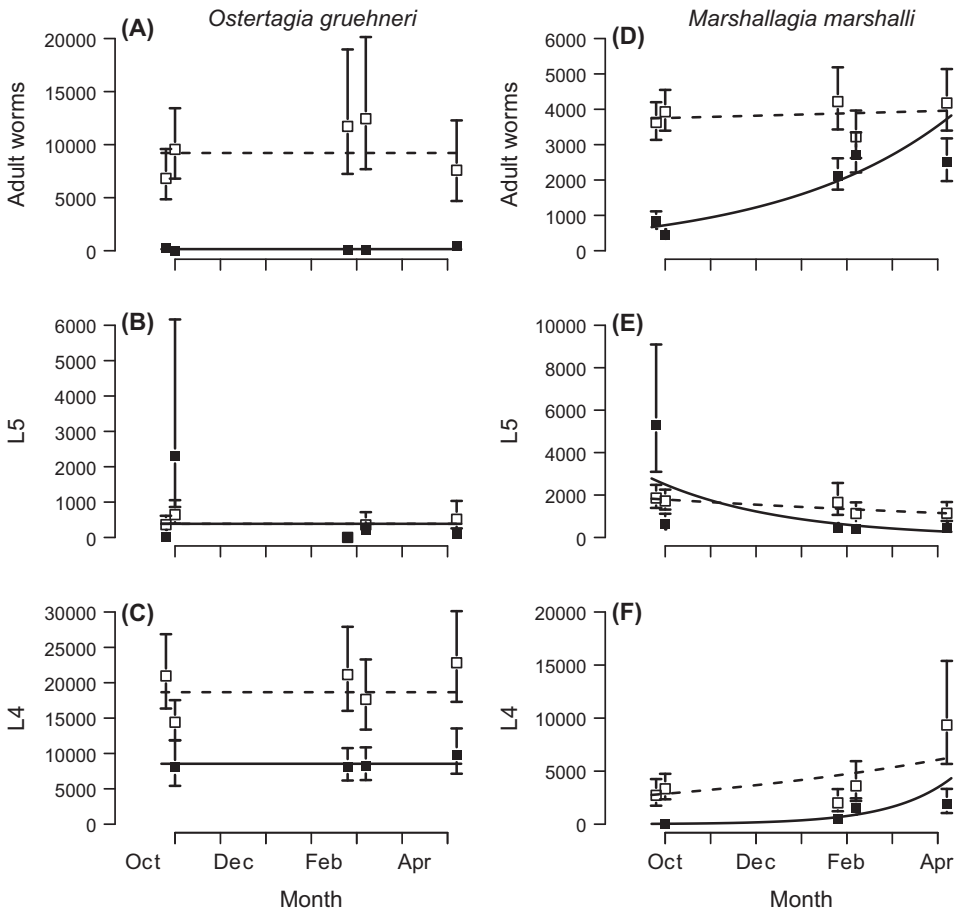

Fig. 1. Estimated and predicted abundance of adult worms (A, D), L5s (B, E) and L4s (C, F) for *Ostertagia gruehneri* (A–C) and *Marshallagia marshalli* (D–F) in October, February and April (2006–2009) for adult female reindeer not treated (open symbols, broken lines) and treated with anthelmintics (filled symbols, solid lines). Treated animals sampled in October were culled shortly after effective release of the drug, and February and April animals 5 and 7 months later, respectively. From left to right, symbols show the estimates and standard errors for the abundances of each worm fraction for each treatment for animals culled in October 2006 and 2007, February 2007 and 2009 and April 2009 (when samples were available). Lines represent the predicted abundance of nematodes over time from the minimal model. For *M. marshalli* adult worms, the predictions are given for the average reindeer population size. Overall sample sizes and samples from successfully identified nematodes in each worm fraction and month are as follows: October $n = 20$ hosts, (controls: adult = 16, L5 = 15, L4 = 13; treated: adult = 4, L5 = 4, L4 = 2 (2007 samples only)), February $n = 16$ hosts (controls: adult = 8, L5 = 7, L4 = 8; treated: adult = 4, L5 = 8, L4 = 8) and April $n = 7$ hosts (controls: adult = 4, L5 = 4, L4 = 4; treated: adult = 3, L5 = 3, L4 = 3).

control animals only in the latter 2 months. This could reflect intrinsic density-dependent regulation of development in these parasites, in which the transition from L4 to L5 occurs faster in the treated animals at the start of winter due to lower burdens of *M. marshalli* in this group. By April, the number of adult *M. marshalli* in the abomasum was similar between the treated and the control animals. If development to the adult stage is density dependent then the rate of development in the treated animals may decrease, leading to the observed build-up of larvae in the mucosa and a decrease in the lumen. The accumulation of larvae in the mucosa would be consistent with more L4s entering a hypobiotic stage as the density of adults increases (Armour and Bruce, 1974; Eysker, 1997). However, previous studies found no evidence of arrested development in *M. marshalli* (Irvine et al., unpublished data).

Density-dependent regulation of the establishment of infection has been shown in other nematode species (Michael and Bundy, 1989; Goater, 1992; Norozianamiri and Behnke, 1993), and has been postulated as the result of competition for space or food resources in a host gut with a finite carrying capacity (Keymer, 1982). Such a regulatory mechanism could explain the contrasting dynamics of *M. marshalli* adult worm abundances in the two treatment groups; where the carrying capacity in the non-treated reindeer, which had significantly higher abundances of adult *M. marshalli* in October than the treated reindeer, may have been reached earlier in the season and consequently no significant increase in abundance was observed over winter. In contrast, in a neighbouring valley system where the abundance of *O. gruehneri* is lower, the carrying capacity for *M. marshalli* could be higher, allowing more time to pass before reaching the saturation point, enabling detection of an increase over winter, as shown in earlier work (Halvorsen et al., 1999; Irvine et al., 2000). An alternative hypothesis for nematode population control is through the host immune response, but this is unlikely since previous work has found no evidence for acquired immunity in response to *M. marshalli* in Svalbard reindeer (Irvine et al., 2000, 2001 as cited earlier).

Although Hoar et al. (2012) recently demonstrated that *O. gruehenri* L2s and L3s have high over-winter survival on the Canadian tundra, there was no significant change in the burdens of any of the worm fractions of *O. gruehneri* from October to April in either the treated group or the untreated, control group, demonstrating that this parasite is not transmitted during the winter on Svalbard. The abundance of *O. gruehneri* adults and L4s was, however, high throughout the winter in the untreated control group, supporting the hypothesis that *O. gruehneri* is long-lived (Irvine et al., 2000; Stien et al., 2002b).

Previously, the abundance of *O. gruehneri* was shown to be significantly related to reindeer population density (Albon et al.,

2002). Our results indicate a significant positive relationship between the abundance of adult *M. marshalli* in winter and reindeer population size during the summer of the previous year (i.e., 16–22 months earlier). This is consistent with the evidence that *M. marshalli* develops rapidly from L3 to adult stages, which limits the ability to be able to detect a strong influence of host density on larval burdens. This result supports previous research suggesting a 1 year time-lag in the transmission of *M. marshalli* based on the hypothesis that the parasite remains as an egg through the winter and develops into the infective stage during the following summer when conditions are more favourable (Halvorsen et al., 1999; Irvine et al., 2000). During winter, reindeer graze on the wind-blown ridges where snow cover is thin and food more accessible, whereas during the summer they forage over wide areas in the valley floors (Van der Wal et al., 2000). Thus, reindeer are unlikely to ingest infective larvae until they return to the winter grazing grounds the following winter, which could explain the time-delay in the response of the parasite to changing host densities.

Ostertagia gruehneri is a parasite of reindeer and as such is expected to have adapted to the harsh environments in which these hosts persist. The prevalence of adult and mucosal-dwelling *O. gruehneri* is high throughout the year but during the winter there is no or very little egg output. This is thought to be an adaptation by the parasite to focus egg production during the summer, thus avoiding the winter when egg development is retarded and survival is unlikely (Halvorsen and Bye, 1999; Irvine et al., 2000; Stien et al., 2002b). The ability of L2s and L3s to persist in the environment over winter may also be an adaptation that has evolved in this parasite in order to survive in the environment in which its host resides (Hoar et al., 2012). *Marshallagia marshalli*, on the other hand, has a wide geographical and host distribution, infecting both cervid and bovid species and is common in wild ruminants in North America, sheep in Saudi Arabia and Syria (Elazazy, 1995), goats in Turkey (Umur and Yukari, 2005), Saiga and sheep in Kazakhstan (Morgan et al., 2007), as well as reindeer in the Arctic. It is therefore surprising to find that it is this generalist parasite that has evolved a life-cycle that exposes it to the most extreme conditions of the Arctic. This life-history strategy may have evolved through inter-specific competition with *O. gruehneri*, as has been found for parasitic nematode species assemblages elsewhere (Diez-Banos et al., 1992; Lagrue and Poulin, 2008), a hypothesis that is in part supported by the inverse relationship in abundance between the two species, as mentioned above. Furthermore, by evolving a winter-transmission strategy, *M. marshalli* infects its host in their first year of life and infection increases rapidly, which could confer a further competitive advantage over *O. gruehneri* which, on Svalbard, mainly infects its host during their second summer (Irvine et al., 2000). However, the life-history strategy of this parasite may not be a product of specific adaptations to the high Arctic and interactions with *O. gruehneri* and Svalbard reindeer, but may instead be an ancestral trait. *Marshallagia marshalli* is mainly a parasite of dry deserts and it has been argued that it exhibits a similar transmission strategy in Kazakhstan, where cross-sectional data and mathematical modelling suggest that the parasite infects Saiga antelope only in the winter when they are in the south of their range (Morgan et al., 2007). It is worth noting that the genus *Marshallagia* needs revision and Dróźdź (1965) lists three more species in the genus, raising the possibility that it is a species complex. Thus, whether the parasite's unique life-cycle is due to derived or inherited traits cannot be elucidated until the taxonomy has been resolved.

Finally, it is generally recognised that the Arctic is particularly sensitive to the effects of climate warming, with models projecting an increase of up to 5 °C in mean annual surface air temperatures by the end of the 21st century (Kattsov and Källén, 2005). The impact of this warming is unclear and is likely to vary with the nature of the host–parasite interaction (Kutz et al., 2009b; Hoar et al., 2012). An increase in winter temperatures may lead to higher infection levels of *M. marshalli* due to increased development rates and survival of free-living stages (Kutz et al., 2005; Hudson et al., 2006). These changes could, in turn, increase competition with *O. gruehneri*, with unknown consequences for the fitness of the reindeer host. However, without data for development and survival rates of the free-living stages of *M. marshalli* it is difficult to interpret the impact of climate change on this parasite–host system.

In conclusion, the remarkable adaptation of *M. marshalli* has only been revealed by carefully controlled long-term experimental studies, using a novel delayed-release anthelmintic bolus. This study illustrates the importance of experimentally manipulating parasite loads, which when the treatment effect is strong, can reveal important insights into the transmission dynamics of parasites in the wild despite small sample sizes. In addition, it serves to highlight the importance of considering all aspects of the host–parasite system, from the life-history of the parasite to the density and distribution of hosts in space and time.

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