

# Identifying disease reservoirs in complex systems: mountain hares as reservoirs of ticks and louping-ill virus, pathogens of red grouse

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## Summary

1. We examined the role of mountain hares in the louping-ill virus/*Ixodes ricinus* tick system to determine whether hares were reservoirs of these pathogens for red grouse. A field experiment, which involved reducing mountain hare densities was undertaken and changes in tick abundance, louping-ill virus seroprevalence and red grouse densities recorded.

2. Hares were found to be important hosts for all stages of ticks at two study sites and, where sheep were frequently treated with acaricide, hares fed the greatest proportion of adult ticks. Hare densities were reduced at the experimental site between 1993 and 2001 but remained relatively constant at a control site. Both nymph and larvae tick burdens on red grouse chicks declined over this period to very low levels at the treatment site, but not at the control site. The estimated size of the tick population at the treatment site decreased by more than 99% by 1999.

3. Louping-ill prevalence, as measured by antibody prevalence in shot young red grouse, also declined at the treatment site, lagging behind the tick decline by approximately 2 years. The number of young grouse produced per hen grouse at this treatment site increased in comparison to a second control area. However, no change was found in summer grouse densities and thus we cannot demonstrate parasite-mediated competition.

4. A deterministic SIR-type model describing the dynamics of this pathogen/vector/host system was constructed and used to examine the role of mountain hares in louping-ill virus and tick dynamics. The model predicted a decline in tick numbers and seroprevalence as hare densities reduced. The inclusion of hares as both tick hosts and vector–host–vector transmitters of louping-ill virus gave the best fit to the observed data.

*Key-words:* disease reservoir, *Ixodes ricinus*, louping-ill virus, mountain hare, red grouse, tick.

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## Introduction

Infectious agents that infect more than one host species are both ubiquitous and important. About 60% of human pathogens are zoonoses and up to 90% of domestic animal pathogens can infect more than one host species (Cleaveland, Laurenson & Taylor 2001;

Taylor, Latham & Woolhouse 2001). Generalist pathogens maintained in one species can spill over and cause potentially devastating and repeated epidemics. Alternatively, they may provide a more sustained force of infection in another more susceptible species, leading to localized extinction (Holt & Pickering 1985). Moreover, multi-species pathogens are more likely to cause emerging diseases in both humans and domestic mammals than single-species pathogens (Cleaveland *et al.* 2001; Taylor *et al.* 2001).

Understanding these complex host-pathogen systems is fundamentally difficult but of critical importance. A key issue in these systems is to identify the reservoir of infection, that is the epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population (Haydon *et al.* 2002). In many cases, diseases are poorly controlled when our knowledge of the structure and dynamics of reservoir systems is poor (e.g. Bingham *et al.* 1999; Portaels *et al.* 2001).

The ultimate proof of the existence of a reservoir requires the isolation of the target population from transmission from the putative reservoir, with subsequent monitoring of disease incidence as it declines to zero in the target population (Haydon *et al.* in press). In reality, these experiments are difficult to carry out on a meaningful scale with sufficient replication. For example, in one of the very few cases where this has been attempted – experiments to examine the role of badgers in the epidemiology of bovine tuberculosis – the associated difficulties and controversy has raised concerns over the power and quality of the data (Krebs *et al.* 1998). As a consequence, quasi-experiments (May 1999) or the assembly of a range of other strands of evidence that point to the existence of a reservoir, may be all that is practicable.

In this paper we examine the role of a putative new reservoir host in a complex system of a generalist pathogen with a non-specific vector, the louping-ill virus and the sheep tick (*Ixodes ricinus* L.). This virus can infect a range of domestic and wild vertebrate hosts such as sheep, cattle, pigs, red grouse (*Lagopus lagopus scoticus* Lath), red deer (*Cervus elaphus* L.), mountain hares (*Lepus timidus* L.), roe deer (*Capreolus capreolus* L.), short tailed voles (*Microtus agrestis* L.) and humans. The virus is transmitted through the tick vector and causes encephalomyelitis in susceptible hosts. In upland Britain, only two numerous vertebrate hosts, sheep and red grouse, exhibit a post-infection viraemia sufficient for feeding tick instars to acquire the virus and thus allow vector–host–vector transmission (Beasley, Campbell & Reid 1978; Reid 1978). Red grouse were not thought to be a reservoir host for two reasons (Reid 1975; Reid 1978; Hudson 1992). First, it was argued first that high grouse mortality rates would lead to a decline in red grouse productivity and therefore reduce the population below the critical community size for viral persistence. Second, adult ticks rarely feed on birds and so grouse could not support the vector population. Louping-ill virus persistence has therefore traditionally been considered to be largely dependent on sheep (Reid 1978), which could sustain both tick and virus populations. Thus the removal of sheep, or their effective removal through the use of acaracides and vaccination against louping-ill virus, is predicted to lead ultimately to louping-ill eradication.

Despite the vaccination of adult sheep for over 30 years, the disease is poorly controlled in red grouse

in certain regions of the Scottish Highlands (Hudson *et al.* 1995). Several hypotheses could account for this observation (Hudson *et al.* 1995). For example, although modelling suggests that lambs that are infected between the waning of maternal immunity and vaccination could account for viral persistence, the use of acaracide and short grazing seasons means that the necessary conditions are rarely met in most farming systems (Laurenson *et al.* 2000). Alternatively, another host species could be a reservoir and recent laboratory studies have shown that ticks feeding on mountain hares, which do not become highly viraemic after infection, can acquire virus from co-feeding with infected ticks (Jones *et al.* 1997). A mathematical model of this system suggested that hares alone might allow tick and virus persistence (Gilbert *et al.* 2001). Thus we propose that mountain hares are a reservoir species and crucial epidemiological component for this virus–vector system.

In this paper we test this hypothesis empirically in a large-scale field quasi-experiment and use a theoretical model to test our assumptions of this system. First we examined the importance of hares as tick hosts and tested whether a reduction in hare densities leads to a decline in tick and louping-ill virus populations. We then examined the consequences of a reduction in louping-ill virus prevalence in red grouse populations. Lastly we examined our assumptions of this system, through mathematical modelling, by assessing the significance of the biological processes involved in this manipulation.

## Methods

### STUDY SITES

The study was conducted between 1993 and 2001 in the Scottish Highlands, with the manipulated treatment site in Morayshire (12 775 hectares) where 1-year-old sheep were vaccinated louping-ill virus and all sheep were treated with acaracide three times yearly. At control site 1, in Perthshire (11 500 hectares), used for comparisons of ticks and virus abundance sheep were similarly managed. Control site 2 in Inverness-shire (12 800 acres), where climatic conditions were similar to the nearby treatment site, provided the most appropriate comparison of red grouse production (number of young grouse per adult female) and density after louping-ill declined at the treatment site, as these factors are determined by a range of factors including weather conditions (Hudson 1992). No serological evidence of louping-ill virus was found in grouse or hares at this site.

All sites were located on private sporting estates that were managed to maximize red grouse harvests and vegetation consisted predominantly of heather moorland. Sheep, mountain hares and red grouse were found on all sites, with a low density of roe deer at the moorland/woodland edge. Some red deer also grazed

seasonally on control site 1 and up to 60 feral goats intermittently grazed the higher ground at the treatment and control sites 2.

#### ESTIMATION OF TICK BURDENS

The number of adult, nymphal and larval ticks were counted on the head and neck of mountain hares, obtained from culling programmes or during commercial shoots. Samples were put in plastic bags immediately after being shot and ticks subsequently counted. Tick infestations were measured by obtaining at least 10 samples of hares from April to October each week from 1993 to 1995 and fortnightly in 1996. Ticks were counted on live grouse chicks during June from 1993 to 2001 after locating broods with pointer dogs.

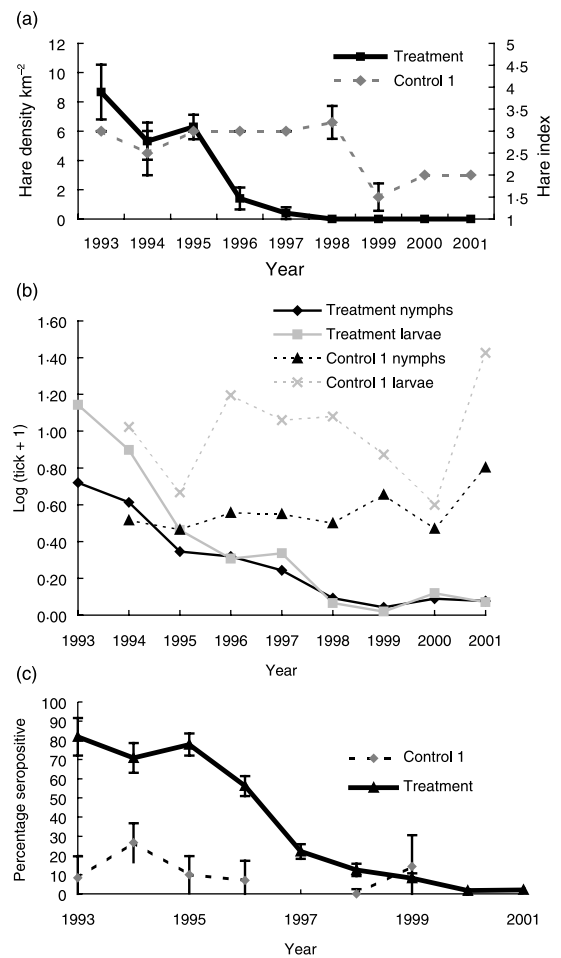
Tick burdens were estimated on  $\geq 30$  sheep per site during June/July of 1993 and 1999. Adult female ticks were counted on the head, neck, and axilla and inguinal regions; these areas support over 90% of adult females feeding on each ewe (Ogden, Hailes & Nuttal 1998). Observed tick burdens were therefore corrected accordingly. The ratio of adult to nymphs and larvae was calculated at 1 : 5 : 8, respectively, following data presented in Ogden *et al.* (1998). Tick burdens on culled red deer in the summer months were estimated by counting adult female ticks on the head, neck, and axilla and inguinal regions, but reliable data are not available on the ratio of nymphs and larvae to adult female ticks.

#### HOST DENSITIES

Red grouse, hare and red deer densities were estimated by counting the number of animals flushed by pointing dogs in a 1-km<sup>2</sup> area. Areas were selected by their topographical suitability (flatness and/or ease of visibility) for counting with dogs and to be representative of the habitat on the study sites. The average value for April and July was used for mountain hares and red deer, whereas only July counts were used for red grouse. One area was counted at control site 1 whereas at the treatment site and control site 2, the same five areas were counted each year and the mean value used. Sheep density was calculated from flock size and the area they grazed on the study sites. Grouse production at the treatment site and control site 2 was estimated by recording the number of young grouse counted for each hen during July on all five areas.

#### CALCULATIONS OF RELATIVE IMPORTANCE OF SPECIES AS TICK HOSTS

The relative importance of different species as tick hosts was estimated using observed tick intensities and host densities and incorporated data from a moor adjacent to control site 1 in 1993, where only a single acaricide treatment on sheep was applied. Mean



**Fig. 1.** Changes of (a) mountain hare densities, (b) tick burdens on red grouse chicks and (c) louping-ill virus antibody prevalence in young red grouse, between 1993 and 2001 on treatment and control site 1 in the Scottish Highlands. Bars represent standard errors, with seroprevalence data transformed using the angular transformation.

intensities for each tick stage on each host species in June/July were multiplied by the summer density of each species. Larvae, nymphs and adults were then summed separately and the relative importance of each host for each stage calculated as a percentage of this total. Data were not transformed for this calculation.

#### REDUCTION IN HARE DENSITY

Hare density was manipulated by trained personnel of the sporting estate at the treatment site, through intensive hare culling by shooting and snaring. Preliminary efforts to reduce hare densities commenced in the late 1980s, but became concerted in 1992/3. It is likely that hare densities prior to 1993, when intensive monitoring commenced, were greater than reported here (Fig. 1a) and probably in excess of 20 km<sup>-2</sup> (S. McConnachie and D. Taylor personal communication). Summer hare densities were reduced from over 8 km<sup>-2</sup> in 1993 to 0 by 1998 (Fig. 1a). No changes in the intensity of hare culling took place at the control site until 1999.

## SEROLOGY

In August/September, 0.08 mL of blood was collected from freshly shot red grouse and tested for haemagglutination-inhibiting antibody (HIA) (Clarke & Casals 1958). Reciprocal titres of  $\geq 40$  were regarded as seropositive.

## ANALYSES

Tick burdens on grouse chicks were log transformed and then analysed using a standardized analysis of variance, using a brood identification number as a factor to remove pseudoreplication. Louping-ill virus seroprevalence was analysed using logistic regression, with a model incorporating the year and place of sampling and the interaction of these effects. Data were weighted according to the number of samples taken each year.

The effect of louping-ill virus on grouse production was examined by comparing the difference in the ratio of young to adult female grouse at the treatment and control site between 1993 and 2001. Changes in the magnitude of these differences after louping-ill prevalence declined was analysed using a *t*-test by comparing the differences between 1993 and 1998, when louping-ill virus prevalence was high, with those after 1998, when prevalence had declined. Mean grouse densities from five sites at the control and treatment moors were analysed in a similar way.

## ECOLOGICAL SYSTEM MODEL DESCRIPTION

A standard susceptible-infectious-recovered (SIR)-type model was constructed of this two host-tick-virus system to examine the hypothesis that mountain hares could be a reservoir in this system and the relative importance of hares as tick or virus hosts. The model is fully described in Gilbert *et al.* (2001). However we state the models equations here for convenience:

$$\frac{dG_s}{dt} = A + (a_g - s_g G)G - b_g G_s - \beta_1 T_i G_s$$

$$\frac{dG_i}{dt} = \beta_1 T_i G_s - (\alpha + b_g + \gamma)G_i$$

$$\frac{dG_z}{dt} = \gamma G_i - b_g G_z$$

$$\frac{dT_s}{dt} = (a_T - s_T T)T \beta_3 D - b_T T_s - \theta T_i T_s H - \beta_2 T_s G_i - \beta_3 T_s D$$

$$\frac{dT_i}{dt} = \theta H T_i T_s + \beta_2 T_s G_i - b_T T_i - \beta_3 T_i D$$

where  $G_s$ ,  $G_i$ ,  $G_z$ ,  $T_i$  and  $T_s$  are susceptible, infective and immune grouse densities and infected and susceptible tick densities, respectively.  $G$  is the total grouse population,  $T$  is the total tick population and  $D$  and  $H$  are the hare densities, assumed constant and with  $D = H$  in simulations where hares are allowed to support vector-host-vector transmission (see below). Parameters

**Table 1.** Model parameter definitions and value estimates. For  $s_T$ ,  $\beta$  and  $\theta$ , first values are used when there is no vector-host-vector transmission (nvht) by hares through co-feeding and the second value is used when  $\theta \neq 0$ . Carrying capacity of grouse and ticks were set at 80 km<sup>-2</sup> and 40 000 km<sup>-2</sup>, respectively, in this simulation to establish parameters estimated from equilibrium thresholds (see Gilbert *et al.* 2001)

Symbol	Definition	Value (per month)	Origin of estimates
$a_g$	Per capita grouse birth rate	0.17	4 per pair per year (Hudson 1992)
$b_g$	Per capita grouse death rate	0.087	65% mortality per year (Jenkins <i>et al.</i> 1963; Hudson 1992)
$s_g$	Measure of density dependent constraints on grouse	0.0013	Estimated from equilibrium and carrying capacity data
$A$	Grouse immigration rate	1.9	Estimated from equilibrium and carrying capacity data
$\alpha$	Per capita grouse death rate due to disease	5	Mean 6 days of viraemia (Reid 1975; Reid <i>et al.</i> 1978)
$\gamma$	Per capita grouse recovery rate from disease	1.25	Estimated from 80% mortality rates in experimentally infected grouse (Reid 1978)
$a_T$	Per capita tick birth rate	83.3	1000 per tick per year
$b_T$	Per capita tick death rate	0.083	Each tick stage lives for 1 years
$s_T$	Measure of density dependent constraints acting on ticks	0.00194 (without nvht) or 0.00135 (with nvht)	Estimated from equilibrium and carrying capacity data
$\beta_1$	Transmission rate from infected ticks to grouse	$\beta_1 = \beta$	Biting rate of infected nymphs on susceptible grouse
$\beta_2$	Transmission rate from infective grouse to ticks	$\beta_2 = 9.75\beta$	Biting rate of susceptible larvae and nymphs on infected grouse
$\beta_3$	Rate at which adult ticks bite hares and reproduce	$\beta_3 = 1.84\beta$	
$\beta$	Transmission constant	0.00115 (without nvht) or 0.0002 (with nvht)	Estimated from field and equilibrium data (Gilbert <i>et al.</i> 2001)
$\theta$	Non viraemic transmission coefficient	0 (without nvht) or $3.78 \times 10^{-7}$ (with nvht)	Estimated from equilibrium data

**Table 2.** Mean (SE) baseline summer tick burdens on host communities at two study sites. Overall sheep density at the treatment site declined when a shepherd on one heft retired in early 1999. Given that significant reductions had occurred in tick and virus prevalence by 1998, and considering the length of the tick life cycle, we do not believe that this could have affected these results

	Summer density (km <sup>-2</sup> )		Larvae		Nymphs		Adult females	
	1993	1999	1993	1999	1993	1999	1993	1999
(a) Treatment site								
Mountain hare	8 (1.9)	0 (0)	85.8 (7.2)		52.0 (4.5)		12.1 (0.8)	
Red grouse	25 (10.4)	92 (11.5)	25.8 (2.7)	0.05 (0.01)	7.0 (0.7)	0.1 (0.01)	0.02 (0.02)	0
Sheep	11	6	6.5 (1.1)	0.04 (0.04)	4.3 (0.7)	0.03 (0.03)	0.8 (0.1)	0.05 (0.01)
	Summer density (km <sup>-2</sup> )		Larvae		Nymphs		Adult females	
	1993		1993		1993		1993	
(b) Site adjacent to control 1, 1993								
Mountain hare	7		44.0 (14.5)		22.1 (5.4)		6.7 (1.5)	
Red grouse	26		14.6 (2.3)		2.9 (0.6)		0 (0.02)	
Sheep	13		76.2 (11.8)		49.9 (7.7)		9.7 (1.5)	
Rabbit	10		2.6 (0.7)		1.1 (0.4)		0	
Red deer	1						11.5 (2.5)	

values are given in Table 1. We assume that the experimental system was at equilibrium in 1993 and use these measured equilibrium values to estimate immigration and transmission parameters. Because insufficient data were available to estimate the three systemic transmission parameters individually, these were assumed to be related by a constant factor, so we only estimated one parameter. Data on the tick biting rates on grouse and hares of each tick stage were used to estimate this relationship (Table 1).

The system was simulated by running the model from the 1993 equilibrium value for 1 year with the 1994 hare density to predict tick and louping-ill virus prevalence during 1994. The model was then stopped and run from those values given by the model at the end of 1994 with the 1995 hare density. This process was repeated for each subsequent year. The model run under three different scenarios where (i) hares could transmit virus and are hosts of the tick ( $H = D$ ), (ii) hares could not transmit virus and were simply tick hosts ( $\theta = 0$ ), and (iii) hares could transmit virus, but did not affect the tick density ( $D$  constant and  $H \neq D$ ) (see Table 1 for parameters).

## Results

### THE ROLE OF MOUNTAIN HARES AND OTHER MOORLAND HOSTS IN MAINTAINING TICK POPULATIONS

At the treatment, site mountain hares had greatest burdens of all stages of *Ixodes ricinus* in 1993 (Table 2a). Hares hosted approximately 12 times more ticks of all stages than sheep, which were treated with acaricide, and 3–7 times more larvae and nymphs than red grouse. At the site adjacent to control site 1, although host densities were similar, rabbits and red deer also occurred but the acaricide regime for sheep was poor. Here adult female tick burdens were very similar on

**Table 3.** Percent of tick populations fed by each host species

	Larvae		Nymphs		Adult females	
	1993	1999	1993	1999	1993	1999
(a) Treatment site						
Mountain hare	83.5	0	65.2	0	91.2	0
Red grouse	7.8	94.4	27.4	98.2	0.5	0
Sheep	8.7	5.6	7.4	1.8	8.3	100
	Larvae		Nymphs		Adult females	
	1993	1999	1993	1999	1993	1999
(b) Site adjacent to control 1						
Mountain hare	18.1		17.4		25.4	
Red grouse	22.3		8.5		0	
Sheep	58.1		72.9		68.3	
Rabbit	1.5		1.2		0	
Red deer					6.2	

sheep and red deer (Table 2b) although hares also carried substantial tick burdens.

The relative importance of the three species studied for the tick population varied between sites with host density and acaricide regime (Table 3). At the treatment site, mountain hares were responsible for feeding the majority of adult female ticks (91.2%), nymphs (65.2%) and larva (83.5%) in 1993, although red grouse fed a substantial proportion of nymphs (27%). At the other site, sheep were the most important tick host, although mountain hares fed between 17% and 25% of the tick population and red grouse were important for larvae.

### THE EFFECT OF REDUCING HARE DENSITY ON TICK AND LOUPING-ILL VIRUS PERSISTENCE

#### Effect on tick population

Tick numbers on grouse chicks decreased significantly on the treatment site between 1992 and 2001 in

comparison to the control site (Fig. 1b. ANOVA; Larvae: Interaction site  $\times$  year,  $F_{7,1540} = 39.4$ ,  $P < 0.001$ ; Nymphs,  $F_{7,1540} = 24.7$ ,  $P < 0.001$ ). Chick age, site and year also had a significant effect on tick burdens (Larvae; Moor  $F_{1,1540} = 149.2$ , Year  $F_{9,1540} = 149.2$ , Age  $F_{1,1540} = 149.2$ , all  $P < 0.001$ ; Nymphs; Moor  $F_{1,1540} = 645.1$ , Year  $F_{9,1540} = 152.1$ , both  $P < 0.001$ , Age  $F_{1,1540} = 5.25$ ,  $P < 0.05$ ). The size of the tick population over the treatment period declined such that by 1999 there were just 0.6%, 0.02% and 0.03%, respectively, of the 1993 larva, nymph and adult female tick populations, with grouse feeding most larvae and nymphs.

*Effect on louping-ill virus prevalence*

There was a significant reduction of louping-ill virus prevalence at the treatment site in comparison to the control site during the study (Fig. 1c. Logistic regression: Interaction site  $\times$  year;  $\chi^2 = 18.1$ , d.f. = 1,  $P < 0.001$ ; Site,  $\chi^2 = 15.4$ , d.f. = 1,  $P < 0.001$ ; year  $\chi^2 = 14096.5$ , d.f. = 1,  $P < 0.001$ ).

EFFECT ON RED GROUSE PRODUCTION AND DENSITY

The number of young produced per adult female grouse at the treatment site increased relative to control site 2 once the prevalence of louping-ill virus had declined to low levels after 1998 (Fig. 2a;  $t$ -test,  $t = -2.41$ , d.f. = 7,  $P < 0.05$ ), but there was no significant change in relative grouse density (Fig. 2b;  $t = 0.32$ , d.f. = 7, NS).

THEORETICAL EXPLORATION OF THE ROLE OF MOUNTAIN HARES IN TICK AND VIRUS DYNAMICS

Simulations of the model of this ecological system suggested that louping-ill virus could persist with hares as the sole host, if their density exceeded  $6.5 \text{ km}^{-2}$  (see Fig. 3, Gilbert *et al.* 2001). However, grouse alone or with low densities of hares did not allow viral persistence.

This model predicted a slight decline in louping-ill virus prevalence due to a reduction in tick numbers after hare densities were reduced (Fig. 3). A large decline was predicted when hares were included as vector–host–vector transmitters, suggesting that this mode of viral transmission has epidemiological significance. However, the relative importance of hares as tick hosts and virus transmitters changed when tick densities were increased by an order or magnitude in the model, suggesting that the relative importance of these roles may depend on the specific situation. Under all scenarios, the inclusion of hares with both these roles gave the best fit (Fig. 3). Preliminary sensitivity analyses revealed that that the qualitative behaviour of the model is reasonably resilient to changes in the parameter values.

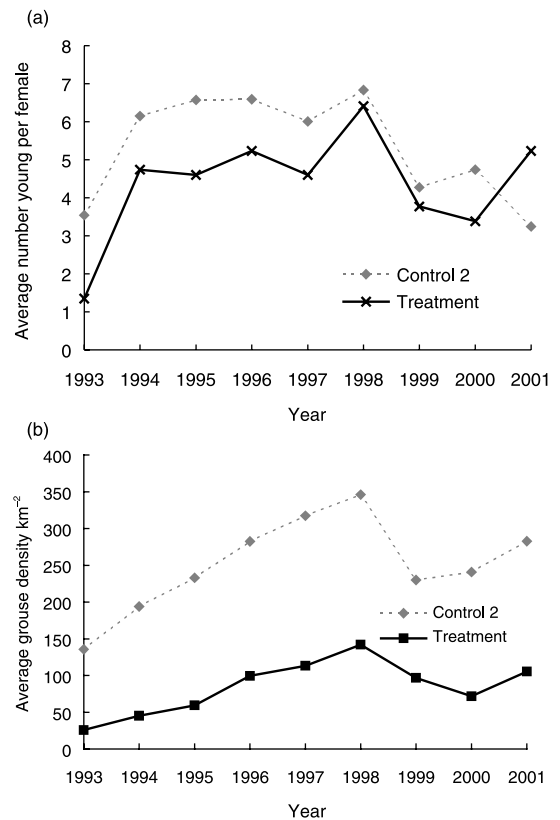


Fig. 2. Changes over time in the (a) number of young grouse per adult female and (b) summer grouse densities on treatment and control site 2 in the Scottish Highlands.

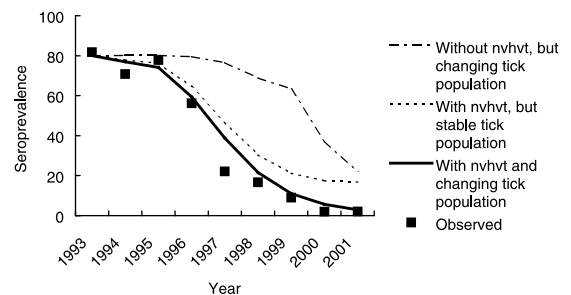


Fig. 3. Observed and predicted decline in seroprevalence in grouse with and without non-viraemic–host–vector transmission (nvhvt) and with a constant tick population.

Discussion

MOUNTAIN HARES AS RESERVOIR HOSTS FOR TICKS AND LOUPING-ILL VIRUS IN RED GROUSE

Isolation of a target population from any external pathogen transmission, in this case isolating red grouse and/or sheep from mountain hares, by removing hares, provides the ultimate proof of the existence of a reservoir (Haydon *et al.* 2002). This study provides the first powerful evidence, through a large scale field manipulation, that mountain hares can be a reservoir host for the louping-ill virus/*Ixodes ricinus*/red grouse system. Four strands of evidence support this conclusion.

First, even when hare densities were relatively low compared to other hosts, hares are an important host for ticks of all stages and, depending on host community composition, can be the most important host for maintaining tick populations on moorland. Second, when hare numbers were reduced, tick infestations on other hosts and thus the overall size of the tick population, declined to very low levels. Third, the prevalence of louping-ill virus declined dramatically when hare densities were reduced. Last, a model of the system suggested that the virus may persist if only hares are present (Gilbert *et al.* 2001).

Undertaking experiments at the correct scale is important because small-scale experiments do not always reflect processes at a larger scale and can lead to misunderstandings of the processes involved (May 1999). However the trade-off in undertaking large-scale rather than small-scale experiments is that replication, randomization and obtaining sufficient controls are often difficult. Indeed we can think of few examples in host–pathogen systems, with experiments involving *T. tenuis* parasites of red grouse, parasites of snowshoe hares and current work on badgers and bovine tuberculosis perhaps being the only notable exceptions (Murray, Cary & Keith 1997; Hudson, Dobson & Newborn 1998; Krebs *et al.* 1998). However, large scale but unreplicated manipulations of host–pathogen systems have yielded a wealth of information on the dynamics and reservoirs of these systems. Examples from wildlife include the wide-scale vaccination of foxes and racoons against rabies in Europe and North America (Roscoe *et al.* 1998; Pastoret & Brochier 1999; MacInnes *et al.* 2001) and possum control for bovine tuberculosis in New Zealand (Caley *et al.* 1999).

In this tick and louping-ill system, management and logistical constraints unfortunately precluded us conducting a rigorous experiment with replication and randomization. Sporting estates are business enterprises and thus managers were unwilling to follow management practices that might reduce their red grouse harvests. Additionally, study sites with similar host and pathogen suites were not available. Thus we had to conduct either small-scale treatments with pseudo-replication or a single large-scale treatment. Given the large home range size of hares, the considerable environmental variability in tick habitat and the importance of scale (May 1999), we considered it most appropriate to combine our results from different areas within individual management units.

#### OTHER RESERVOIRS FOR LOUPING-ILL VIRUS AND *IXODES RICINUS*

In most situations in the British Isles, sheep are the primary reservoir for this pathogen–vector system (Reid 1978; Laurenson *et al.* 2000). Models of the system suggest that the virus cannot persist when only red grouse are present (Gilbert *et al.* 2001) and empirical studies in the Bowland Fells in north-west England and

at our treatment site in Scotland will clarify this issue. If, however, another host for adult ticks such as red deer is present, then grouse and that species can be a tick–virus reservoir (Gilbert *et al.* 2001). We consider it unlikely, however, that any single potential host other than mountain hares on moorland sites can act as a reservoir in this system for one or more reasons. Some hosts do not support vector–host–vector transmission of the virus (red deer and rabbits), others do not occur in sufficiently dense or large populations (roe deer, short-tailed voles and feral goats), whilst others do not feed sufficient quantities of adult female ticks (birds). However, these species could contribute to a louping-ill virus reservoir in conjunction with other species.

#### IS PARASITE-MEDIATED COMPETITION OCCURRING?

Louping-ill virus undoubtedly causes substantial mortality in red grouse and reduced the number of young grouse produced per female, and thus could be a limiting factor for grouse populations. In this scenario, it would seem likely that parasite-mediated competition is occurring between mountain hares and red grouse (see also Gilbert *et al.* 2001) but we cannot yet demonstrate this phenomenon empirically. The factors influencing the density of red grouse populations are varied, complex and only partially understood (Hudson 1992; Hudson *et al.* 1998; Thirgood *et al.* 2000a; Moss & Watson 2001; Smith *et al.* 2001). More time series data is clearly required to ascertain whether the troughs and peaks of grouse densities are consistently altered as a result of the reduced density of mountain hares.

#### IMPLICATIONS FOR MANAGEMENT AND CONSERVATION

Good sheep management through vaccination and tick control is still fundamental to the control of louping-ill virus and its tick vector, whether or not hares and red grouse are present. Tick control is particularly crucial when wild hosts are unavailable for vaccination and thus the number of susceptible hosts is difficult to reduce; the only way to further reduce  $R_0$  in this scenario is to reduce vector density and thus transmission. However, when sheep are well managed, red deer are absent and mountain hare, tick and louping-ill virus densities are high, it may not be possible to take large harvests of red grouse. In this situation, a reduction in hare density may be the only method of improving red grouse harvests.

Disease transmission between domestic animals and wildlife can be a source of human–wildlife conflict either when transmission occurs from a wildlife reservoir to livestock or from domestic animals to endangered wildlife (Cleaveland *et al.* 2001). The discovery that mountain hares can be a reservoir for louping-ill virus could present another conservation conflict.

Mountain hares, although not endangered and found through much of the Palearctic, are listed on Annex V of the EU habitats' directive due to the status of some Alpine populations (Corbet & Harris 1991). If red grouse harvests are not compatible with high densities of hares, ticks and louping-ill virus, then the management of such heather moorland areas for red grouse may be uneconomic. If land use alters as a consequence, the conservation benefits of managed heather moorland will be lost for hares themselves (Corbet & Harris 1991) and other species (Thompson *et al.* 1995; Thirgood *et al.* 2000b). These complex consequences must therefore be weighed against the undesirability of a local reduction in mountain hare densities to control disease. Luckily this situation of potential conflict is rare and probably primarily limited to parts of Morayshire. Overall, the distribution and population size of mountain hares in the UK should be essentially unchanged whatever management decision is adopted.

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