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Disease dynamics in cyclic populations of field voles (*Microtus agrestis*): cowpox virus and vole tuberculosis (*Mycobacterium microti*)

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The possible role of pathogens in rodent population cycles has been largely neglected since Elton's 'epidemic hypothesis' of 1931. To revisit this question, 12 adjacent, cyclic but out-of-phase populations of field voles (*Microtus agrestis*) in North East England were studied and the initial results are presented here. The prevalences of antibodies to cowpox virus and of clinical signs of *Mycobacterium microti* infection (vole tuberculosis) showed delayed (not direct) density dependence (with a lag of three to six months). This did not result from changes in population structure, even though there were such changes associated with the different phases of the cycle. The prevalences rose as vole numbers rose, and peaked as numbers declined. The apparent lag in the numerical response of infection prevalence to changes in host abundance is consistent with the hypothesis that diseases, singly or in combination, play a hitherto underestimated role in the dynamics of cyclic populations.

Keywords: population cycles; disease transmission; time delays; host–pathogen dynamics; numerical response

1. INTRODUCTION

Cyclic rodent populations have long been a focus of ecological enquiry, but, while phenomenological descriptions of such cycles abound, characterizations of the underlying delayed density-dependent mechanisms remain speculative. Specialist predator–prey interactions are usually invoked, although it is acknowledged that other processes may be responsible (e.g. Stenseth 1999; Turchin & Hanski 2001). Mechanisms relying on variation in social and other intrinsic factors alone have not found empirical support (e.g. Ergon *et al.* 2001*a*), and modelling suggests that herbivore–food interactions may be responsible for the cyclic dynamics of herbivores only under restricted conditions (Turchin & Batzli 2001). Pathogens are clearly capable of regulating host populations (Anderson & May 1978, 1979), but empirical evidence regarding their potential roles in cycling populations is largely restricted to macro-parasites (nematodes) in birds (Hudson 1986; Moss *et al.* 1993, 1996; Hudson *et al.* 1998). The role of microparasites in the dynamics of natural populations is essentially unexplored, despite suggestions of a potential role (Elton 1924; Elton *et al.* 1931; Mihok *et al.* 1985; Descoteaux & Mihok 1986; Soveri *et al.* 2000). In addition, the majority of the few studies of pathogens in wild populations (see Hudson *et al.* (2002) for a general review) have been of epidemics of introduced pathogens (e.g. phocine distemper virus (Swinton *et al.* 1998) and rabbit haemorrhagic disease (Saunders *et al.* 1999)), but most pathogens in wild populations are naturally occurring endemics that persist in populations for long periods (Anderson & May 1979). Here, we report the first results from a research programme aimed at re-examining the role of endemic pathogens in a cyclic rodent population.

Historically, Elton formulated the 'epidemic hypothesis' (that crashes in wildlife population cycles were the result of recurrent epidemics of infectious disease) following high mortality from toxoplasmosis among field voles (*Microtus agrestis*) taken into captivity (Findlay & Middleton 1934; Elton *et al.* 1935). However, the absence of obvious toxoplasmosis during a subsequent decline seriously undermined the hypothesis (Elton 1942). Further related work led to the discovery of vole tuberculosis (TB) (Wells & Oxon 1937), but, as with *Toxoplasma*, while TB was sometimes associated with a decline in field vole numbers, it was also found at high prevalence in some populations that did not decline, and at low prevalence in others that did (Chitty 1938). Hence Chitty (1954) generalized that disease was irrelevant to host dynamics, a

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statement largely accepted by most in the field (Stenseth & Ims 1993).

Data on macroparasite (helminth) prevalence in cyclic vole populations have left this view unchallenged (Haukisalmi *et al.* 1988; Haukisalmi & Henttonen 1990; Laakkonen *et al.* 1998). A wide range of pathogens were found to be prevalent in declining populations of field and bank voles, suggesting a contribution of opportunistic pathogens to declines (Soveri *et al.* 2000). In a study of *Puumala* virus antibodies in a cyclic population of bank voles, *Clethrionomys glareolus*, in Sweden, sampled twice per year, Niklasson *et al.* (1995) detected direct density dependence of antibody prevalence on a vole trapping index in the autumn samples, and significant delayed density dependence at both six months and 1 year in the spring samples. Singleton *et al.* (2000) monitored the prevalences of three viral antibodies in relation to a large population fluctuation of house mice (*Mus domesticus*).

Here, prevalences of two microparasites (expressed as vole TB clinical signs and cowpox virus antibodies) are reported, in relation to host density in field vole populations in northern England that show multi-annual cyclic dynamics similar in most respects to those that prevailed in mid-Fennoscandia until the mid-1980s, but with relatively high vole densities in the low phase (Lambin *et al.* 2000) and marked spatial asynchrony over short spatial scales, resembling a travelling wave (Lambin *et al.* 1998). Specialist predation by weasels has been tested and rejected as a cause of vole cyclicity in this area (Graham & Lambin 2002). We consider whether patterns of pathogen prevalence are density dependent, and, if so, whether this is direct or delayed, whether such patterns themselves vary with season or host functional group and whether any such association is with density *per se* or with another factor that itself varies with density.

The causative agent of vole TB, *Mycobacterium microti*, is a member of the *M. tuberculosis* complex (Wayne & Kubica 1986). Very few studies of vole TB have been undertaken since it was first described more than 60 years ago; thus little is understood of its pathology. However, the infection is known to cause clinical tuberculous signs in rodents, internally and in some cases externally (Wells 1946; Cavanagh *et al.* 2002), and can also cause pulmonary tuberculosis in humans and other species including badgers and cattle (van Soolingen *et al.* 1998). Cowpox virus is a member of the genus *Orthopoxviru*s, endemic in Europe and some western states of the former Soviet Union (Baxby & Bennett 1999). Despite its name, it rarely infects cattle, and the disease is most often diagnosed in domestic cats. It is also a zoonosis although human cases are rare (Baxby *et al.* 1994). In Great Britain the highest cowpox virus antibody seroprevalence is found in bank voles, wood mice and field voles, and they are believed to be the reservoir hosts (Chantrey *et al.* 1999). Under experimental conditions, cowpox virus infection delays the time to production of the first litter in bank voles and wood mice by approximately one month (Feore *et al.* 1997). In the field, cowpox virus infection may either increase or decrease survival in these species, depending on the time of year, though the former effect may be a consequence of the reproductive restraint (Telfer *et al.* 2002).

2. MATERIAL AND METHODS

(a) *Field data*

The study was carried out in Kielder Forest (55°13′ N, 2°33′ W) and adjacent Kershope and Redesdale Forests, in grass-dominated clear-cut sites (5–12 hectares (ha), where 1 ha = 10^4 m^2) within a large man-made spruce forest. At four sites in Kielder Forest (sites 1–4), monitored as part of a long-term study (MacKinnon *et al.* 2001; Graham & Lambin 2002), field voles were trapped monthly during March to October 1998, March to October 1999 (inclusive) and in March and April 2000. The populations at all four sites were in the increase phase in 1998, reached peak densities (250–400 ha^{-1}) by late summer or autumn 1999 and began to crash in 2000 (figure 1). Voles were live-trapped monthly in grids (0.3 ha) consisting of 100 Ugglan Special traps set at 5 m intervals (details of the trapping regime are given in Graham & Lambin (2002)).

Tail-tip blood samples for cowpox virus serology were taken during spring (March), summer (July) and autumn (October) 1999 and in spring (April) 2000. Animals were recorded as having clinical tuberculosis if they had characteristic skin lesions or obvious lymphadenitis (Wells & Oxon 1937; Cavanagh *et al.* 2002).

Information was also recorded on visible signs of tuberculosis in animals trapped at the same times from an additional two sites where similar host data were available (sites 13 and 14), although there were no cowpox data from these sites. In addition, data were recorded on weight, sex, reproductive status and presence of biting wounds (resulting from fighting) from all animals trapped at these six sites.

A further eight sites were selected on the basis of having experienced different past densities (i.e. they are in different phases of the cycle): five sites in Redesdale Forest 10–15 km to the east of Kielder (sites 5–9) and three in Kershope Forest 10–15 km to the west (sites 10–12). Vole populations in Redesdale had declined from a peak density during summer 1998 and were experiencing an 'extended low' phase in spring 1999 when sampling took place (figure 1). Numbers were rising in Kershope Forest, which had fairly high vole densities (150–200 ha^{-1}), although the sites were not fully synchronized (figure 1). At each of these sites, trapping took place in April and July 1999, using a grid of 50 Longworth live-traps set at 5 m intervals, baited with wheat and carrots. Traps were pre-baited 2–3 days before each session, set at *ca.* 18.00 and then checked three times at roughly 12 h intervals at dawn and dusk. Individuals at these sites were not tagged, and TB clinical signs were not monitored, but data were recorded on weight, sex and reproductive status, and blood samples were collected. Vole-abundance estimates were obtained using vole sign index (VSI) surveys. This index is based on the presence of fresh grass clippings (a sign of recent vole activity), and is defined and calibrated in Lambin *et al.* (2000).

Blood samples were obtained from all animals captured at sites 5–12, and on an opportunistic basis from sites 1–4: in some cases, high numbers made it impossible to sample every individual. To determine the number of animals with cowpox virus antibodies and the 'seroprevalence' (the percentage positive), sera were tested by immunofluorescence assays (Crouch *et al.* 1995). Antibody-positive hosts may either be currently infected with cowpox virus or have recovered from an infection. Seroconversion typically occurs about two weeks after initial infection, and infection itself probably lasts for around four weeks (Chantrey *et al.* 1999), but animals then remain seropositive,

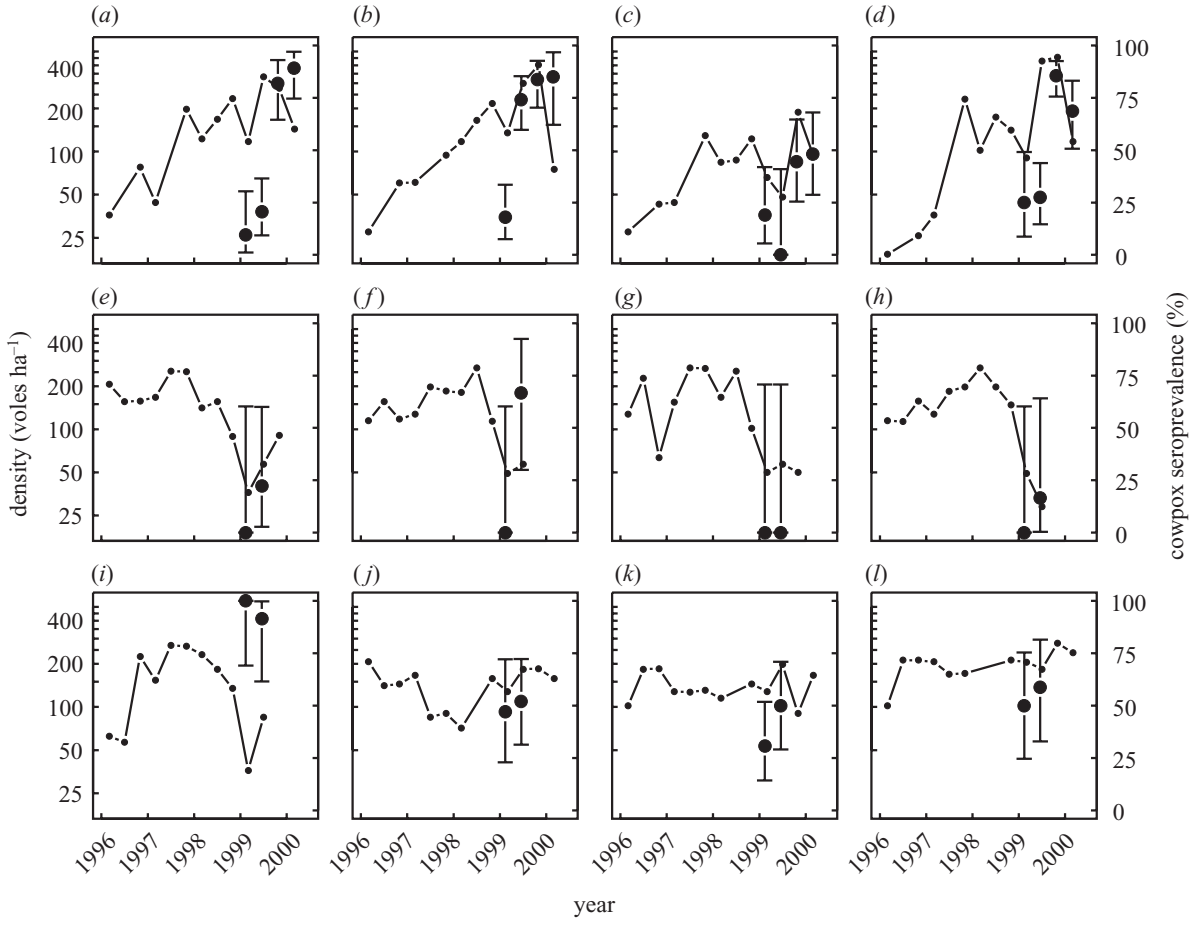

Figure 1. Observed cowpox seroprevalences (large dots, with 95% confidence limits) at the 12 sites in Kielder ((*a*) site 1, total sample size of 130; (*b*), site 2, total sample size of 158; (*c*) site 3, total sample size of 82; and (*d*) site 4, total sample size of 171), Redesdale ((*e*) site 5, total sample size of 13; (*f*) site 6, total sample size of 13; (*g*) site 7, total sample size of six; (*h*) site 8, total sample size of 10; and (*i*) site 9, total sample size of 22) and Kershope Forest ((*j*) site 10, total sample size of 42; (*k*) site 11, total sample size of 50; and (*l*) site 12, total sample size of 33), along with estimated vole densities on a log scale (solid lines, small dots) during and preceding the monitoring period.

effectively, for the rest of their lives. Thus, for example, a high seroprevalence may reflect a high prevalence of infection at present and/or in the past.

(b) *Statistical analysis*

Seroprevalence of cowpox virus antibodies and the prevalence of clinical signs of vole TB were modelled, separately, according to individual-level and population-level covariates. Animals were assigned to age and reproductive classes on the basis of the external appearance of the reproductive organs and coat colour. Juveniles had juvenile or transitory (juvenile–adult) coats. Sub-adults had an adult coat and a non-perforate vulva/vagina or semi-abdominal testes; however, non-perforate females with an adult coat and an open pubic symphysis were classed as mature adults because they had bred previously. Mature adults also included animals with adult coats and a perforate vulva/vagina or scrotal testes. Voles were also allocated to one of four body-mass categories (less than 17 g; 17–24 g; 25–39 g and 40 g or more), as size is a better proxy variable for age than is sexual maturity. Preliminary analysis had suggested size-related variation in seroprevalence, and chronic infection (or seropositivity) ought to be more apparent among older individuals.

Estimates of present and past vole densities in the populations (clear-cuts) were calculated for lags of no, three and six months. Estimates for the Kielder sites were derived from capture–recapture analyses of the monthly trapping data. Because the Redesdale and Kershope sites were trapped infrequently (see § 2a), vole densities were estimated using VSI surveys (seasonally rather than monthly) both before and during this study period. The r^2 of the VSI linear calibration relationship (Lambin *et al.* 2000) ranged between 0.62 and 0.72 according to season. However, on occasions where trapping and VSI data were available, the qualitative changes in vole numbers were well reflected by both measures. Where VSI data were unavailable for a given lag, linear interpolation between earlier and later months was used. Changes in population density over the three and six months preceding sampling, calculated as ratios of present to past densities, were also entered as covariates. A categorical variable coding for season of sampling—spring (March and April), summer (May to August) and autumn (September and October)—was also included.

As is often the case with parasitological data, our sampling design may have resulted in a degree of correlation between observations because groups of samples were taken from the same geographical location (site) or were taken at different time points from the same individuals. To control for these potential sources of pseudoreplication in our analyses, we used generalized linear mixed models (GLMM) where the errors within groups are correlated with each other, with covariances that specify the correlation between the errors associated with pairs

of observations (see recent review in Paterson & Lello (2003)). Models with a logit link and a binomial error were constructed, as cowpox virus seropositivity and the presence of clinical signs of TB are binary measures. Vole-density estimates were shared by voles caught in the same clear-cut on the same occasion. Accordingly, the interaction term 'site × sampling occasion' was included as a random effect. GLMMs were implemented using the GLIMMIX macro in SAS (Littell *et al.* 1996). In the absence of model-selection criteria, such as the Aikake Information Criterion, appropriate for comparing mixed models with different fixed effects (Burnham & Anderson 2002), we constructed models using a two-stage stepwise selection procedure. First, to investigate population-level patterns, density dependence was modelled by including vole density at lags of no, three and six months, population growth rates over these three lags, season, and the interactions between season and these six density-related covariables. Following a step-down procedure, we retained only terms significant at the 1% level, using SAS type III *F*-tests (SAS/STAT 1992) and denominator degrees of freedom calculated using Satterthwaite's formula (Littell *et al.* 1996). Second, to investigate whether the density dependence of disease prevalence could be caused by changes in the population structure (especially with respect to size (age) and maturity), individual-level covariates and their two-way interactions were included in the models. Again, non-significant terms were dropped sequentially. Finally, parameters were estimated using restricted maximum-likelihood procedures in SAS.

The analysis of cowpox seroprevalence was based on samples taken two or three times per year. As the same individuals did not occur in more than one of the samples, repeated samples within sites were treated as independent, and only random variation between sampling sites and sampling periods was included in the GLMMs. TB, however, was recorded in permanent live-trapping grids monitored at one-month intervals, and the same individuals were registered repeatedly. A first-order autoregressive term accounting for the correlation between repeated samples within sites was therefore included when fitting GLMMs. However, owing to the low prevalences of TB symptoms, it was not possible to fit both the random site variation and the autocorrelation in the same models. As density varied more within sites over time than between sites, there would be a larger concern about biased estimates of density dependence (and incorrect estimates of precision owing to pseudo-replication) if the autocorrelation was not accounted for than if the between-site variance was excluded from the model. We therefore used a model with a first-order autoregressive temporal variance (see Paterson & Lello 2003), but with no between-site variance, when analysing the TB data. There was evidence of mild under-dispersion in the TB data (estimated dispersion parameter of 0.8), probably owing to low prevalence, but the dispersion parameter was fixed to 1 when fitting the models.

3. RESULTS

(a) *Cowpox antibody seroprevalence*

Figure 1 shows cowpox virus seroprevalences at the 12 sites, along with estimated vole densities during and preceding the monitoring period. In Kielder Forest (sites 1–4), where populations moved through a peak summer to a crash, cowpox seroprevalences rose through 1999 and were still high in spring 2000. Thus, because seroprevalence reflects both past and present infection, the prevalence of cowpox infection appears to have been much higher in 1999 than in 1998. In Redesdale Forest (sites 5–9), seroprevalences in spring and summer 1999 were highly variable. In spite of the large confidence intervals (CIs) in this forest (low densities, small samples), CIs at site 9, where prevalences were high, for example, failed in several cases to overlap those at other Redesdale sites. Thus, there appear to have been both high and negligible prevalences of infection at low densities in these populations during their 'extended low' phase (more than 12 months since the populations crashed). Finally, in Kershope Forest (sites 10–12), seroprevalences in spring and summer 1999 were typically lower than those in Kielder Forest and the higher Redesdale sites, but higher than those in the lower Redesdale sites. There appear to have been consistently moderate rates of infection at these sites in 1998, during the 'increase' phase of the cycle.

Investigating the presence and nature of density dependence in these patterns at the level of the whole population yielded two alternative models, both providing clear evidence of delayed density dependence in the prevalence of cowpox virus antibodies. Cowpox could be modelled as varying either solely with density three months ago ($F_{1,219} = 8.77$, $p < 0.008$), or with density six months ago and with a higher intercept in autumn than in spring or summer (density: $F_{1,21} = 10.36$, $p < 0.004$; season: $F_{2,19.6} = 6.24$, $p < 0.008$). The estimated slopes of density dependence for these models were very similar (three-month delay: 0.0074, s.e. = 0.0025 on logit scale; six-month delay: 0.0106, s.e. = 0.0033, yielding odds ratios per 100 voles ha^{-1} (95% CI) of, for three months' delay, 2.10 (1.27, 3.46), and, for six months' delay, 2.89 (1.49, 5.60)). Predicted values for a six-month lag are shown in figure 2, where it is apparent, as previously noted, that seroprevalence (at any given 'six-months-ago density') tended to increase between spring and autumn. Note, however, that the effect of past density on prevalence is poorly estimated for autumn where there were data for only four sites, and the ranges of values were correspondingly small.

This apparent increase in seroprevalence with increasing past density could, however, be a consequence simply of population structure changing with density, either in the population itself, or in the samples because the time of sampling varied relative to the time of sexual maturation in spring. Then, the pattern could be an artefact arising because different subgroups of the population exhibited different prevalences, independent of density. Indeed, the proportions of sexually mature voles *were* much greater in the spring samples taken in April 2000 from crash populations at sites 1–4 (100% ($n = 37$), 95% ($n = 20$), 100% ($n = 27$) and 88% ($n = 35$), respectively) than in the March 1999 (spring peak population) samples from the same sites (33% ($n = 21$), 64% ($n = 39$), 62% ($n = 13$) and 85% ($n = 20$), respectively). Nonetheless, even after including individual-level covariates and controlling for population structure (variation in the seroprevalences of different maturity classes; table 1), the effect of past density (at three or six months' lag) remains significant. Seroprevalence was, however, consistently lower in juveniles than in subadult and mature individuals, and it was not density dependent among juveniles whereas it clearly was among subadult and mature voles.

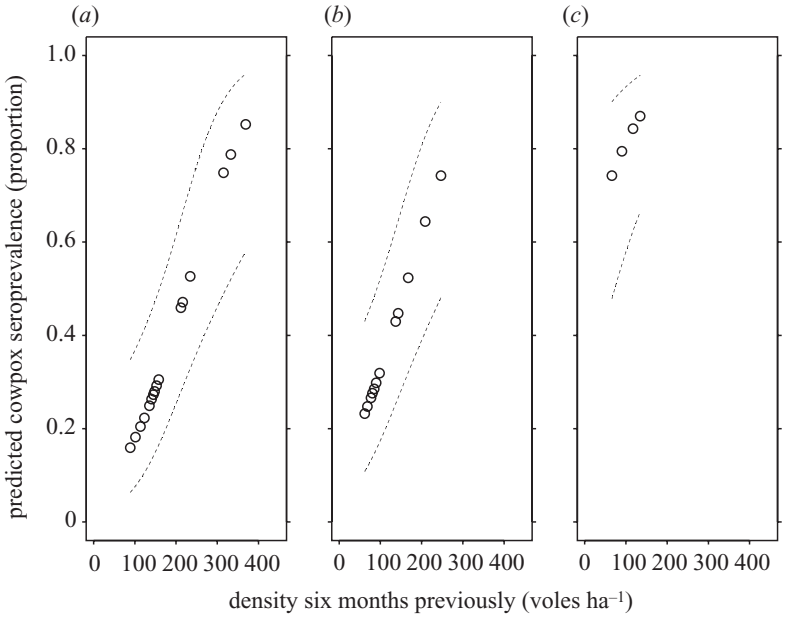

Figure 2. Predicted cowpox seroprevalences in relation to vole density with a six-month time-lag from the model with additive effects of lagged density and season: (*a*) spring; (*b*) summer; and (*c*) autumn. Dashed lines are 95% confidence limits on the fitted mean predictions (i.e. uncertainty in the mean value not including the random 'site × sampling occasion' variation). The fitted variance component of the 'site × sampling occasions' interaction, expressed as a standard deviation on a logit scale, was 1.1 (95% CI: 0.80,1.78).

Table 1. Parameter estimates (logit scale) for a model of cowpox seroprevalence selected in a two-stage stepwise procedure starting with a backward selection of population-level covariates, followed by a backward selection of individual-level covariates. (Intercept (reference) represents mature males with a 'past density' of 0.)

effect	standard estimate	error	d.f.	*t*-value	Pr > |*t*|	odds ratio
intercept	−1.6347	0.5283	—	—	—	—
past density (three months)	0.01172	0.003091	35.9	3.795	0.0006	1.0118
juvenile	−0.3762	0.5316	724	−0.71	0.4794	0.6865
subadult	0.007782	0.5815	724	0.13	0.8936	1.0078
mature	0	—	—	—	—	—
interactions with past density (three months)						
past density × juvenile	−0.01008	0.002813	679	−3.58	0.0004	0.99
past density × subadult	−0.00396	0.002803	711	−1.41	0.1578	0.996
past density × mature	0	—	—	—	—	—

(b) *Prevalence of clinical signs of* Mycobacterium microti

The prevalence, from March 1998 to April 2000, of TB-positive characteristic lesions or obvious lymph-node swellings was 2% overall for the six Kielder sites combined. This prevalence was higher among larger (and older) individuals. Among captured voles with body masses of greater than 39 g, 4.1% (95% CI: 2.7, 6.2%) had external signs of TB. The prevalence of external TB signs in the weight group 25–39 g was 3.5% (2.8, 4.5%), whereas only 0.60% (0.34, 1.1%) of captured voles weighing 17–24 g had TB signs ($p < 0.0001$). No vole lighter than 17 g (juveniles) had developed signs of TB. Thus, juveniles were excluded from the subsequent analysis. In total 2217 individuals above 17 g were captured at least once in the study (4244 captures altogether). Out of these, 78 individuals were recorded to have TB lesions on one or more capture occasions (99 captures in total).

Twenty-six out of 939 females (2.8%, 95% CI: 1.9, 4.0%) and 52 out of 1278 males (4.1%, 95% CI: 3.1, 5.3%) were observed with TB on at least one capture occasion (test of sex difference: $p = 0.1$). It also appeared that animals with biting wounds had a higher prevalence of TB: out of those with wounds (418 captures) 10.0% (7.5, 13.3%) had visible TB lesions, compared with only 1.5% (1.2, 1.9%) among unwounded animals ($p < 0.0001$).

Prevalence rose from 1998 (0.63%, $n = 1919$) to 1999 (2.66%, $n = 2482$), and this increase appeared, from the two months sampled, to have been continued in 2000 ($n = 410$), reaching 8% in April 2000, compared with 5% in May 1999 (figure 3). This coincided with the increase, peak and decline in vole numbers at these sites. There was strong evidence that TB prevalence changed in a delayed density-dependent manner (figure 4), and a six-month delay explained the variation better than a three-month delay. The estimated slope of the six-month-delayed density dependence was 0.0051 per units of voles ha^{-1} (s.e. = 0.0016) on a logit scale. This means that, if past densities increase by 100 voles ha^{-1}, the odds of TB

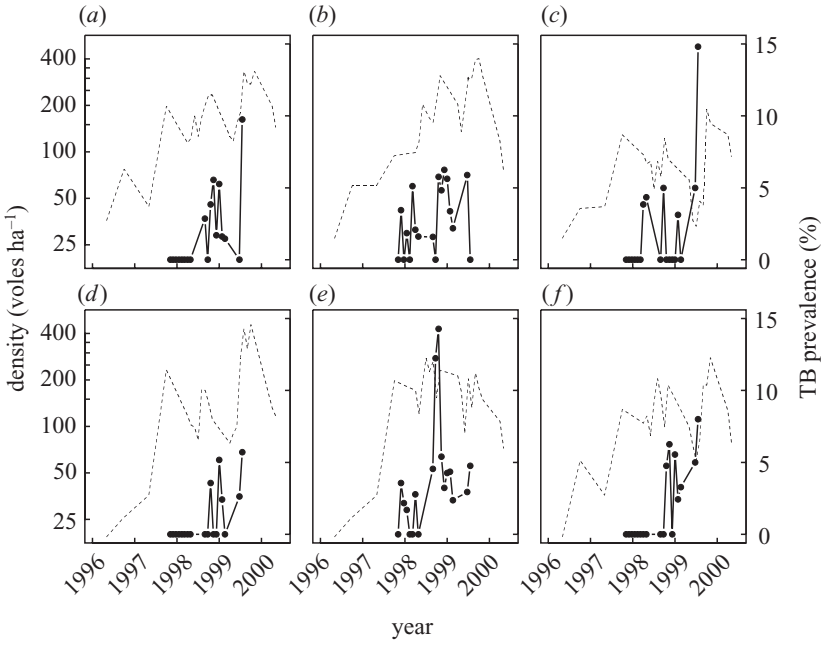

Figure 3. Observed prevalences of characteristic TB lesions or obvious lymph-node swellings (filled circles, solid line) at six sites in Kielder ((*a*) site 1, total sample size of 776; (*b*) site 2, total sample size of 933; (*c*) site 3, total sample size of 381; (*d*) site 4, total sample size of 749; (*e*) site 13, total sample size of 803; and (*f*) site 14, total sample size of 602), along with estimated vole densities on a log scale (dashed lines) during and preceding the monitoring period.

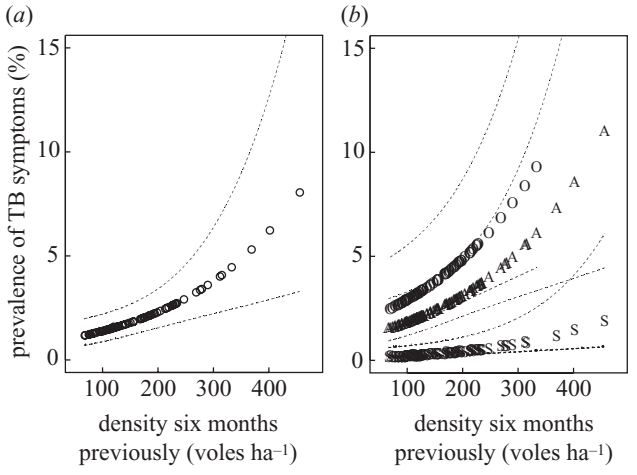

Figure 4. Fitted delayed density dependence in TB symptoms. (*a*) Population-level predictions; and (*b*) predictions from the model with an additive 'size' effect (S, 'subadults' (17–24 g); A, 'adults' (24–39 g); O, 'old' (40 g or more). Stippled lines show 95% confidence limits of the mean predictions. The standard deviation of the random variation between sampling months within sites was estimated to be 0.68 (95% CI: 0.45,1.37) on a logit scale, and the estimated first-order autocorrelation between sampling occasions within sites was 0.74 (95% CI: 0.46,1.0).

infection increase by 67% (95% CI: 23, 127%). The predictions from this model are plotted in figure 4*a*. Additive effects of season and present density were not significant ($p > 0.7$).

In vole populations, the size and age structures typically change with the density fluctuations (e.g. Chitty 1960; Krebs & Myers 1974). In our data, the proportion of voles above 24 g, for example, varied between different spring samples (March 1998: 71.8% ($n = 174$); March 1999: 26.4% ($n = 242$); March 2000: 42.8% ($n = 238$)). Because the probability of TB infection varied greatly between size groups (see above), variation in the size structure of the population may confound the density-dependent effects. However, when 'size' was included as a factor in the models, the additive effect of past density remained virtually the same (slope = 0.0051, s.e. = 0.0017, representing an increase in the odds of 67% (CI: 20, 132%) per 100 voles ha^{-1}). This clearly indicates that the delayed density-dependent pattern in prevalence of TB was not the result of changes in the size structure of the population. The predictions from this model are presented in figure 4*b*.

4. DISCUSSION

The main results here are certainly robust and important, namely that the prevalences of both cowpox antibodies and TB clinical signs varied in a delayed not a direct density-dependent fashion. Inferring causation, however, must remain tentative at this stage. Nonetheless, to the best of our knowledge, this is the first clear demonstration of microparasite prevalences fluctuating with a time delay relative to host density (of around three to six months) in a cyclic population. The delayed density dependence is robust in that it did not result, as it might well have done, from changes in population structure, even though there were such changes associated with the different phases of the cycle.

For a predator or parasite to be instrumental in generating multi-annual cycles in a host population, it must give rise to a time delay between host density and host population growth rate (May 1976), i.e. host density must decline most rapidly after the peak in host density has been passed, and so on. For a parasite that reduces host population growth rate (by decreasing survival or fecundity), this translates into a time delay between host

density and pathogen prevalence. It is crucial to ask, therefore: to what extent do the delayed density dependences in prevalence observed here simply reflect the cumulative nature of the characters measured? Do they reflect genuine numerical responses of the pathogens, similar to any other predator response to prey density? Also, to what extent might any response by the pathogens feed back, in turn, to the population dynamics of the host?

Both cowpox virus seroprevalence and the prevalence of clinical signs of TB reflect acquisition of infection at some undefined time in the past. Cowpox virus antibodies are first detectable around two weeks after initial infection, and remain present, essentially, throughout the remainder of the animal's life (Chantrey *et al.* 1999). The seroprevalence signifies the proportion of a population that 'have had' rather than 'have' cowpox virus infection. However, because most animals live no more than a matter of months, and very few live for longer than 1 year (Ergon *et al.* 2001*b*; Graham & Lambin 2002), the influence of past infection rates on cowpox virus seroprevalence declines as the past becomes more distant.

For TB, external lesions are late-stage signs of what began as an internal pathology. Hence, the external clinical signs are accurate reflections of current late-stage infection but underestimate current infection overall. To illustrate this point, dissection of 180 voles, of which 7% had external clinical signs of TB on capture, revealed that 14% had internal tuberculous lesions (Cavanagh *et al.* 2002). In any case, although the prevalence of clinical signs is low at certain times during the study, it is well established, in principle, that pathogens can regulate host populations even when prevalences are low. For example, Anderson (1995) presents a worked example of a fictitious respiratory infection in foxes with a case mortality rate of 50%, where a prevalence of just 0.18% is required for regulation of the host population. The prevalence of external TB signs is relatively little influenced by both the distant past (too few survivors) and the recent past (insufficient time for symptom development).

For both pathogens, at least one aspect of the data is an inevitable consequence of these cumulative effects: cowpox virus seroprevalences were lowest in the youngest animals (juveniles), which had been exposed to infection for the shortest time; and TB sign prevalences increased with body-weight class, reflecting variations in both the period of exposure to infection and the period over which symptoms could become apparent.

More generally, the cumulative nature of seroprevalence will itself play a part in the delayed density dependence detected in cowpox virus seroprevalence, because, if infection (rather than antibody) prevalence tended to be higher at higher host densities (as would be expected; Begon *et al.* 1998, 1999) then seroprevalence would inevitably tend to be high when density *had been* high. The data, however, clearly suggest that this is not the only reason for the delayed density dependence. The prevalence of cowpox virus *infection* must have been higher in peak (1999) than in pre-peak (1998) populations in Kielder Forest (figure 1); but it is also apparent (table 1) that delays between increases in density and increases in infection prevalence during the peak year occurred not only in adults but also in subadults, where there was little chance of accumulation. A numerical response (spread of infection from an initially small inoculum: basic reproductive rate $R_0 > 1$) therefore seems likely to have contributed to the lag—that is, the lag appears to have been genuine rather than artefactual. Furthermore, prevalence of infection remained high during the 'extended low' phase following a crash in at least some of the Redesdale sites. Again, the lag, this time between the crash in host density and the eventual crash in prevalence, can convincingly be attributed to a numerical response (failure of the pathogen to sustain itself: $R_0 < 1$).

It is particularly noteworthy that prevalences of cowpox virus infection were apparently high following population crashes and even (at least sometimes) into the 'extended low' phase of the cycle. One of the key features of microtine cycles, demanding a cause if cycles are to be properly understood, is the poor condition and low fitness of hosts some time after a cycle crash (Boonstra *et al.* 1998). Infection may provide an explanation.

With TB, there are insufficient data to infer whether the prevalence of *newly acquired* (as opposed to patent) infection followed changes in density with or without a delay: the high prevalences of clinical signs following the crashes may simply reflect infections acquired during the peaks. Here, though, prevalence, while cumulative, is also biologically most pertinent at the time it is measured. The prevalence of late-stage patent infection was demonstrably highest after host density had crashed, and if these infections have the greatest effect on host fitness (see below), then the patterns observed are capable of generating the time delay between host density and host population growth rate necessary to generate cyclic dynamics.

Notwithstanding the above complexities, moreover, the patterns revealed in this study stand in stark contrast to the pattern emerging from studies of other vole pathogens for which no clear time delay has been detected. For example, the prevalence of common helminths in bank voles (*C. glareolus*) followed that of their hosts with no delay (Haukisalmi *et al.* 1988; Haukisalmi & Henttonen 1990). Fluctuations in the prevalences of rare helminths were erratic, with extinctions during the cyclic lows in host density (Haukisalmi & Henttonen 1990). Laakkonen *et al.* (1998) reported that the prevalence of *Eimeria* infections remained low before and during population crashes of field and bank voles.

Analysis further suggests that wounding is strongly associated with the prevalence of TB clinical signs. Because these wounds were recent, whereas clinical signs of TB reflect an infection acquired several months previously, there are two possible explanations. First, those animals with TB may have more chance of getting wounded. This suggests an effect of TB on behaviour that would inevitably decrease the survival rate. Second, certain individual animals—perhaps those that are more aggressive—may be more likely both to get wounded and to become infected with TB. This might suggest a significant role for agonistic encounters in the transmission of TB.

Of particular interest is the question of whether these pathogens, with their delayed density-dependent patterns of prevalence, have delayed density-dependent effects on host dynamics. The known impacts of cowpox on demography include delayed reproduction by subadult bank voles and wood mice under laboratory conditions (Feore *et al.* 1997) and in the field (S. Telfer, unpublished

data), and an increase in survival in both these species in the breeding season (perhaps as a result of suppressed reproduction) but a decrease in the winter (Telfer *et al.* 2002). It is striking that related traits, such as time of production of the first litter of overwintering animals in the spring (Ergon *et al.* 2001*a*,*b*) and the proportion of summer-born animals maturing before the winter (Ergon *et al.* 2001*b*), also vary profoundly between different phases in cyclic field vole populations in Kielder Forest. It is also striking that the seroprevalences in the present study were much higher than those for which the effects on bank vole and wood mouse survival and reproduction were observed (Telfer *et al.* 2002). Hence, this study has established the potential for cowpox virus to have a delayed density-dependent effect on host dynamics, but further work (see below) will be required to determine whether this potential is realized.

TB is a progressive disease, and there is no evidence of voles recovering from the lesions once established (Wells 1946). Thus, as noted above, TB has the potential to have its strongest demographic impact some time after peak density, when the proportion infected is highest and the disease has progressed to the extent that survival of the voles is affected. Better diagnostic techniques are required, however, to assess the proportion of the population affected by TB, especially in its early stages; for example, PCR techniques for detecting *M. microti* in faeces, urine or oral swabs as discussed by Cavanagh *et al.* (2002). Also, for both pathogens, longitudinal data, in which marked individuals are monitored repeatedly throughout their lives, would allow us to determine better when infection is acquired. This would help to separate cause and effect (e.g. does infection precede or follow changes in individual fitness?) and would facilitate estimates of the effects of infection on survival, fecundity and so on (e.g. Telfer *et al.* 2002). Such work is currently being undertaken.

Although falling well short of demonstrating that micropathogens are responsible for population cycles in Kielder Forest, the pattern of delayed density dependence uncovered here is consistent with such a role. The difference between cycles observed at Kielder and in Fennoscandia (higher density in the low phase and asynchrony) as well as the refutation of the specialist-predator hypothesis in this area (Graham & Lambin 2002) make a role for pathogens plausible. However, the data are also consistent with the alternative that animals in a cycle decline may be disproportionately affected by any of a range of pathogens, as hinted at in the study by Soveri *et al.* (2000). Laboratory studies of co-infection show that the interaction of pathogens can have positive or negative effects on the host (e.g. Cowley *et al.* 1997), which in turn could affect population demographics. Whether a single pathogen is dominant at one site over time, or whether members of a community of pathogens may interact to influence vole demography in a manner causing cycles is another key question that is currently being investigated.

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