Published in final edited form as:
J Anim Ecol. 2008 January ; 77(1): 110–119. doi:10.1111/j.1365-2656.2007.01302.x.

Cowpox virus infection in natural field vole *Microtus agrestis* populations: significant negative impacts on survival

Sarah Burthe[*,†], **Sandra Telfer**[*,†], **Michael Begon**[*], **Malcolm Bennett**[†], **Andrew Smith**[*,†], and **Xavier Lambin**[‡]

[*]Population Ecology and Evolution Group, Biological Sciences, University of Liverpool Biosciences Building, Crown Street, Liverpool L69 7ZB, UK

[†]Department of Veterinary Pathology, University of Liverpool, Liverpool, UK

[‡]School of Biological Sciences, University of Aberdeen, UK

Summary

1. Cowpox virus is an endemic virus circulating in populations of wild rodents. It has been implicated as a potential cause of population cycles in field voles *Microtus agrestis* L., in Britain, owing to a delayed density-dependent pattern in prevalence, but its impact on field vole demographic parameters is unknown. This study tests the hypothesis that wild field voles infected with cowpox virus have a lower probability of survival than uninfected individuals.

2. The effect of cowpox virus infection on the probability of an individual surviving to the next month was investigated using longitudinal data collected over 2 years from four grassland sites in Kielder Forest, UK. This effect was also investigated at the population level, by examining whether infection prevalence explained temporal variation in survival rates, once other factors influencing survival had been controlled for.

3. Individuals with a probability of infection, P(I), of 1 at a time when base survival rate was at median levels had a 22·4% lower estimated probability of survival than uninfected individuals, whereas those with a P(I) of 0·5 had a 10·4% lower survival.

4. At the population level, survival rates also decreased with increasing cowpox prevalence, with lower survival rates in months of higher cowpox prevalence.

5. Simple matrix projection models with 28 day time steps and two stages, with 71% of voles experiencing cowpox infection in their second month of life (the average observed seroprevalence at the end of the breeding season) predict a reduction in 28-day population growth rate during the breeding season from $\lambda = 1{\cdot}62$ to 1·53 for populations with no cowpox infection compared with infected populations.

6. This negative correlation between cowpox virus infection and field vole survival, with its potentially significant effect on population growth rate, is the first for an endemic pathogen in a cyclic population of wild rodents.

Correspondence: Michael Begon, School of Biological Sciences, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK. mbegon@liverpool.ac.uk.

Keywords

capture–mark–recapture; host–parasite dynamics; host–pathogen interactions; MARK; wildlife disease

Introduction

The conclusion of general parasite host models that parasites may regulate the abundance of hosts in some circumstances (e.g. Anderson & May 1978) continues to motivate empirical research (Grenfell & Dobson 1995). Only a few studies, however, have provided empirical support for this hypothesis for wildlife species, and the majority have focused on epidemic pathogens causing high levels of mortality. This is in spite of the fact that most pathogens are endemic, persist in host populations and show relatively small fluctuations in prevalence (Anderson & May 1979). To a large extent, the effect of endemic pathogens on host survival in the wild remains unknown (but see Telfer *et al*. 2002).

This study focuses on endemic cowpox virus in cyclic populations of one of its reservoir hosts, the field vole *Microtus agrestis* L., in Kielder Forest, northern England. Cowpox virus is an orthopoxvirus endemic in rodent populations throughout Europe and western Asia (Baxby & Bennett 1999). In the UK, the highest seroprevalence occurs in bank voles *Clethrionomys glareolus* Schreber, wood mice *Apodemus sylvaticus* L. and field voles, and these species are accepted as being the reservoir hosts (Chantrey *et al*. 1999). Cowpox does not cause any obvious pathology or clinical signs either in the laboratory or in the field, and is an acute infection with a 4-week infectious period (Bennett *et al*. 1997; Chantrey 1999). Following Cavanagh *et al*. (2004), Burthe *et al*. (2006) established that the risk of Kielder field voles becoming infected with cowpox virus increased with host density 3 months previously, and also that its prevalence in field voles was higher than that reported previously for either bank voles or wood mice. Crucially, a previous study on noncyclic populations of bank voles and wood mice demonstrated that cowpox indirectly increased the probability of host survival in the wild in summer, and decreased it in winter, possibly by modulating reproduction and its associated energy costs, as the virus also substantially delayed the onset of reproduction in these species both in the laboratory and in the field (Feore *et al*. 1997; Telfer *et al*. 2002, 2005).

Pathogens may potentially have an important role in affecting cyclic host dynamics (Mihok, Turner & Iverson 1985; Soveri *et al*. 2000). Indeed, pathogen effects may provide an alternative hypothesis to the specialist predation hypothesis (Anderson & Erlinge 1977; Hanski *et al*. 2001) as an explanation for multiannual cycles. The latter is not supported as a cause of field vole density cycles in Kielder Forest on the grounds that variation in common weasel *Mustela nivalis* L. abundance only accounted for a small fraction of variation in vole survival and that weasels did not show a numerical response to vole abundance (Graham 2001; Graham & Lambin 2002).

Parasites with the potential to drive host density cycles must adversely affect host survival or reproduction, and, as an emergent property of transmission or owing to the existence of arrested stages, also exhibit delayed-density dependence. Their demographic impact could be a direct pathological effect or an indirect effect, mediated by, for example, increasing host susceptibility to predation or to other diseases (Scott 1988). The present study, then, used capture–mark–recapture (CMR) data to investigate whether field voles infected with cowpox virus had lower survival probabilities than uninfected individuals, and whether these individual level effects translated into reductions in population-level average survival at periods of high

cowpox prevalence such that variation in cowpox prevalence might translate into variation in population growth rate.

Materials and methods

STUDY AREA AND TRAPPING DESIGN

The study took place in Kielder Forest, a man-made spruce forest occupying 620 km^2, situated on the English–Scottish border (55°13′ N, 2°33′ W). Field voles inhabit grassy clear-cuts that represent 16–17% of the total area, but are completely absent from forested areas. Clear-cuts range in size from 5 to 100 ha. Field vole populations at Kielder fluctuate cyclically within a 3–4-year period (Lambin, Petty & McKinnon 2000). Populations situated close together fluctuate in synchrony, but populations further apart are out of synchrony (Lambin *et al*. 1998; Bierman *et al*. 2006). Voles were trapped in four similar-sized clear-cuts, in two areas of the forest approximately 12 km apart, between May 2001 and July 2003. Kielder Site (KCS) and Plashett's Jetty (PLJ) were situated 4 km apart, with vole populations at low to increasing density during the study. Black Blake Hope (BHP) and Rob's Wood (ROB) were 3·5 km apart, with voles at increasing and peak density.

Populations were trapped in primary sessions every 28 days from March to November, and every 56 days from November to March. Each site had a permanent 0·3 ha live-trapping grid consisting of 100 Ugglan Special Mousetraps (Grahnab, Marieholm, Sweden), in optimal habitat dominated by *Deschampsia caespitosa* Beauv., *Agrostis tenuis* Sibth. and *Juncus effusus* L. Traps were set at 5-m intervals and baited with wheat and carrots. Traps were pre-baited with a slice of carrot and a few grams of oats 3 days before each trapping session, set at approximately 18.00 h on the first day and checked five times ('secondary sessions') at roughly 12 h intervals at dawn and dusk.

Individual animals were identified using subcutaneous microchip transponders (AVID plc, East Sussex, UK) injected into the skin at the back of the neck. Mass, sex and reproductive status were recorded at the time of first capture in each primary session. Animals with juvenile fur or in their first moult were classed as juveniles (Graham & Lambin 2002). A multiple-regression analysis was used to identify a mass threshold for assigning animals with adult coats to juvenile or adult categories, using monthly growth rates estimated from field data, in conjunction with laboratory information on mass at 2 weeks of age (Burthe 2005). Animals were assigned to reproductive classes according to the external appearance of reproductive organs. A 20–30 μL blood sample was taken from the tail tip of each individual each primary session. Antibody to cowpox virus was detected in sera by immunofluorescence (IF) assay (Crouch *et al*. 1995), allowing individuals in each primary session to be classified as seropositive (antibody present) or seronegative. Vole density estimates for each primary session were calculated via the closed population model M_{TH} in the program CAPTURE (Otis *et al*. 1978) and using the estimator of Chao & Lee (1991).

DATA SET USED TO MODEL RECAPTURE AND SURVIVAL

The first captures of all individuals were removed (1514 in total) in order to overcome biases caused by individuals only caught in a single primary session (transients). This reduced the risk of confounding emigration and mortality as a cause of disappearance, but precluded the inclusion of age (juvenile/adult) as a parameter in model selection (below), as individuals only had a juvenile coat for a single trap session. The reduced data set of 1275 individual capture histories yielded a more robust analysis and was used to estimate the best 'base' recapture and survival model, with which possible effects of cowpox virus infection could be investigated.

GOODNESS OF FIT TESTS

First, though, the goodness of fit (GOF) of a 'global' (most fully parameritized) model was assessed, as the CMR models used assume that: (1) every marked animal in the population immediately after time (i) has the same probability of surviving to time ($i + 1$), and (2) every marked animal present in the population at time (i) has the same probability of recapture (p_i). Individual capture histories were classified by sex and site. GOF was assessed using tests implemented in the program RELEASE (Burnham *et al*. 1987) using a standard Cormack–Jolly–Seber model applied to the each group (Table 1). 'Test 2' in RELEASE tests for violation of assumption (2), and 'Test-3' tests for violation of assumption (1).

OUTLINE OF THE MODELLING APPROACH

The analysis was undertaken in steps following Lebreton *et al*. (1992) using program MARK (White & Burnham 1999). First, recapture was modelled in three stages (see Table 1 for a list of covariates). The first examined whether recapture rate varied with time, site or sex. The second investigated whether the temporal component of the recapture model could be adequately described by month, season or year. Effects of average rainfall and temperature over the 3-day trapping period, and of reduced trapping effort in some primary sessions (caused by inclement weather) were also investigated. Weather data were recorded at the Kielder Castle weather station (59°35′ N, 36°32′ E; 201 m above mean sea level). The third stage then examined individual level covariates such as trap dependency and 'edge'. An edge animal had ≥ 75% of its captures at the edge of the trapping grid. Trap dependency was a time-dependent individual covariate determined by whether or not an individual had been caught in the preceding primary session.

The fits of the models were assessed by Akaike's Information Criterion corrected for small samples (AICc) (Hurvich & Tsai 1989). This selects the most parsimonious model for the data by penalizing the better fit of more complex models according to the number of parameters included in the model. Models with a difference of AICc (ΔAICc) of less than 2 may be considered similar in their ability to account for the data (Sakamoto, Ishiguro & Kitagawa 1986). According to the principal of parsimony, if two alternative models had indistinguishable AICc values (ΔAICc < 2), the model with fewer parameters was selected.

Survival was then modelled similarly using the best recapture model. To investigate the effect of climatic variables on survival probabilities, the average daily temperature or the average daily rainfall over the entire 28-day period were included as weather variables. Finally, this best model of recapture and survival (the 'base' model) was used to investigate whether cowpox virus infection affected host survival and recapture probabilities. Model notation is based on Lebreton *et al*. (1992), with subscripts denoting the parameters included within the model. Additive effects are denoted by a plus (+) sign, and interaction terms by an asterisk (*). The subscripts for model parameters used are listed in Table 1.

INVESTIGATING THE EFFECT OF COWPOX ON SURVIVAL : DATA USED IN ANALYSIS

Infection with cowpox virus results in long-term antibody production (Chantrey 1999) and therefore any negative results succeeding a positive result were considered to be false negatives resulting from low serum titres and assumed to be positive (193 individual capture histories had negative results succeeding positive ones out of a total of 1808 individuals that had > 1 serological results). Animals infected with cowpox virus develop an antibody response after approximately 2 weeks, and remain infected for a period of approximately 4 weeks (Bennett *et al*. 1997; Chantrey 1999). Therefore, in a time series of antibody results, we assumed that an animal became infected with uniform probability between a time 14 days prior to its last negative result, and 14 days prior to its first positive result.

Time series of serological results were used to calculate probabilities that individual animals were infected with cowpox virus (P(I)) for each trapping session. Telfer *et al*. (2002) provide a detailed description but to take an example: an individual caught negative at trap sessions $t-2$ and $t-1$, and positive at t and $t+1$ would have a 0·5 probability of being infected at $t-1$ and a 0·5 probability of being infected at t. Individual P(I) values were then summed for each trap session to estimate the total number of individuals infected (I_t) at trap session t. Similarly, individual and summed probabilities of being susceptible, P(S), or recovered, P(R), could be calculated, respectively, for animals before (or in the absence of) and after infection.

Individuals that were recorded seropositive at their first capture, or seronegative at their last capture, could not be confidently assigned a P(I) for that trap session. Further, individuals that were juvenile and seropositive at first capture could have been positive due to the presence of maternal antibodies, and not because of infection with cowpox virus (see Burthe *et al*. 2006). Although such first captures were removed from the capture histories, they needed to be characterized to determine the P(I) for subsequent capture events. Specifically, individuals first caught at a mass of ≤ 14 g were assumed to have been noninfected (functionally seronegative) at first capture because they had insufficient time to have been infected, and this was used to determine their P(I) value subsequently. This assumption neglects the possible but unlikely scenario of mothers becoming infected around the time of birth, and passing virus but not maternal antibody to their pups.

EFFECT OF COWPOX ON SURVIVAL AT THE INDIVIDUAL LEVEL

Cowpox is most likely to affect an individual's survival during the period of infection. However, infection could not be detected until 2 weeks after initial infection, when animals seroconverted. Therefore the effect of cowpox on survival was modelled using a time-dependent individual covariate that allowed survival immediately after seroconversion to depend on that individual's P(I) at the time of seroconversion. Some of the individuals that were seronegative at their final capture, and hence could not confidently be assigned a P(I) (above), must none the less have been infected with cowpox virus. Thus, any effect of cowpox on survival at the individual level must have been underestimated. Note also that a P(I) of 0·5 represented the highest possible individual P(I) included in the data. Approximately 50% of individuals with a P(I) of 0·5 would have been infected, and the other 50% would have recovered from infection.

EFFECT OF COWPOX ON SURVIVAL AT THE POPULATION LEVEL

The effect of cowpox on survival at the population level was investigated by including population level cowpox prevalence ($\Sigma P(I)/(\Sigma P(S) + \Sigma P(I) + \Sigma P(R))$) as a time-varying covariate in capture–recapture models. At the individual level it was important to include only those individuals who could have a P(I) assigned to them with confidence. At the population level, it was essential to compute the most accurate estimate of infection prevalence. Voles were therefore classified into three categories that were combined to derive this estimate. The first was simply adults that seroconverted during the study period, and hence had a known P(I).

The second category was individuals recorded as seropositive on first capture. These had their probability of infection calculated according to their age at first capture. Juveniles (less than 6 weeks old) were assumed to have been seronegative 4 weeks previously (because they would not have been active outside the nest at this time), and were therefore assigned a P(I) of 0·5 for that session (n = 515 out of total of 2706 individuals), and a P(I) of 0 thereafter. Adults first caught positive (n = 884) could have been infected at any time prior to capture, but it was necessary to assign probabilities of infection to them, because the overall prevalence of cowpox would otherwise have been underestimated. To do so, factors influencing their probability of

infection were investigated, utilizing the seropositive captures of animals first captured as adults with known P(I)s (above). Generalized linear models with a Poisson error distribution and an identity link function were fitted to the data. Sex, mass (as a proxy for age of adults), site and site-pair were considered as predictive variables, as was temporal variation investigated as either month or season (Spring = March–May, Summer = June–August, Autumn = September–November, Winter = December–February).

The third category was individuals (adults and juveniles) recorded as seronegative at last capture ($n = 629$). As with the adults above, to estimate the probability that such animals might have been infected, factors influencing the probability of infection were investigated for all seronegative captures with subsequent captures [and hence known P(I)s]. The same explanatory variables were considered.

For both the individual level and the population level analysis, the effect of cowpox on survival was examined to see whether it varied between sexes or sites, or temporally. Two-way interactions were included.

MATRIX PROJECTION MODELS

In order to investigate the potential impact of differences in individual survival rates due to cowpox virus infection on population growth during the breeding season, we constructed a simple two-stage (subadults and adults) matrix model with post-breeding surveys similar to those used by Lambin & Yoccoz (2001) and Graham & Lambin (2002). There were 2·5 female offspring per litter (Innes & Millar 1994), and these had a 0·61 probability of survival in the first month. Survival over the transition from subadult to adults was affected by cowpox: survival probabilities for subadults in uninfected populations (P(I) = 0) were reduced appropriately for the minimum (0·46), average (0·71) and the maximum (0·89) proportions infected, respectively.

Results

In all, 1514 individuals were caught for one primary trapping session only, and hence removed from the data set used for the MARK analysis. The remaining data consisted of 3174 capture records from 1277 individuals caught over 23 primary sessions. The mean number of primary captures per individual was 2·14, and the maximum was 14. Overall, 858 animals were recorded as seroconverting.

There were 213 individuals caught seronegative at last capture, which could not therefore be assigned a P(I) for that primary session. The data set used to investigate the effect of cowpox on survival thus consisted of 2885 capture records from 1064 individuals.

Population size increased on all four grids throughout the study period, reaching peak densities during 2003 and subsequently crashing to low levels in 2004, after the completion of this study. The density of voles ranged from 33 voles ha^{-1} to 662 voles ha^{-1}. Clear seasonal patterns in density fluctuation were overlaid on the multiannual fluctuation, with summer peaks and over-winter declines in density (see Bierman *et al*. 2006; Burthe *et al*. 2006).

GOODNESS OF FIT TESTS

The overall GOF test for the whole data set, split by sex and site, suggested that the fit of the global model was acceptable (Test 2 and 3, RELEASE: $\chi^2 = 197{\cdot}32$, d.f. = 275, $P > 0{\cdot}99$). However, although Test 3 was not significant ($\chi^2 = 111{\cdot}56$, d.f. = 217, $P \geq 0{\cdot}99$), Test 2 was significant ($\chi^2 = 85{\cdot}75$, d.f. = 58, $P = 0{\cdot}01$). This indicates that not all individuals present in the population at a particular time have the same probability of recapture. In order to account

for this, a trap dependency parameter (m) was included in the model selection procedure as an individual covariate.

MODELLING RECAPTURE

Table 2 presents the best models for each of the three stages of modelling recapture. The model with the lowest AICc in stage 1 (group level covariates) had an interaction between site and time (AIC = 5022·40), but a model that included temporal variation alone had an AICc of 5022·56, and 70 compared with 111 parameters and was therefore selected. Temporal variation was simplified in stage 2 by an additive model including the parameters month, year, average rainfall over the primary session and a parameter accounting for months with reduced trapping effort (AICc = 5004·15, ΔAIC = 2·34 for the next-best model that also included density). Finally, the addition of the individual covariates coding for edge and trap dependency significantly improved the model further (AICc = 4987·44).

Recapture rates were generally highest in 2001 and lowest in 2003 when density was highest. Individuals caught in one month were more likely to be caught in the next month. Individuals also had a lower probability of being re-caught in months with reduced numbers of secondary trapping sessions. Animals on the edge of the grid had lower recapture rates. Voles were also less likely to be caught during periods of heavy rainfall.

MODELLING SURVIVAL

With the best recapture model (above) held constant, the best stage 1 model for survival included a site–time interaction and a sex–time interaction (AICc = 4944·32), but a model in which the sex effect did not vary over time had an AICc of 4945·42 and 102 compared with 122 parameters and was therefore selected. Females had 8·24% (95% CI 4·68–11·80) higher apparent survival probabilities than males. No attempts to simplify the temporal component of this model in stage 2 resulted in a model with similar fit (ΔAICc > 15 for the next best model with only 40 compared with 102 parameters). Table 3 presents the best models for each of the stages of modelling survival.

Because the best survival model included a separate coefficient for every site–month combination, it was uninformative ecologically and did not permit detailed evaluation of the factors influencing population level survival. It is of interest to note therefore that survival rates for the next best model (see Table 1) varied monthly, and were generally lower between March and September. Survival was also higher in 2003, compared with 2001 or 2002, and survival was lower following months of heavy rainfall, and following months of high temperatures. Investigation of whether cowpox prevalence could explain additional variation in survival rates at the population level was therefore undertaken using the second best model. The effect of cowpox at the individual level was examined using the best model and the ‘next-best’ model in order to check the robustness of any conclusions.

EFFECT OF COWPOX ON SURVIVAL AT THE INDIVIDUAL LEVEL

The effect of cowpox on survival at the individual level was examined using the selected model (above). Including an additive effect of the probability of infection at the time of seroconversion (indcp, Table 1) substantially improved this base model (Table 4; AICc = 4518·22, compared with AICc = 4524·19 for the base model, AICc values being lower than those above due to this analysis being carried out on a reduced data set, not including animals caught seronegative at last capture). The untransformed estimate on the logit scale for the cowpox P(I) effect was −0·9806 (95% CI −1·6075 to −0·3537). The magnitude of this effect, however, varied according to the base survival rate. Therefore, all predicted estimates of the effect of cowpox on survival are reported for a site and month with median base survival rates. Here, an uninfected individual had a median estimated survival rate of 0·74 (95% CI 0·59–0·85), compared with a rate of 0·63

(0·44– 0·79) for an individual with P(I) = 0·5, and 0·51 (0·30–0·71) for an individual with a P (I) = 1·0. The presence of recovered animals in the 'infected' group (see Materials and methods) would have reduced the magnitude of any estimated effect of cowpox on survival. We therefore used a P(I) of 1·0 to predict the survival rate of truly infected animals. Using males at the site BHP for purposes of illustration, Fig. 1 shows the proportion of individuals infected with cowpox each month, and the estimated survival rates of individuals with P(I) = 0 compared with those with P(I) = 1.

The effect of cowpox virus infection on field vole survival at the individual level was also investigated using the 'next-best' survival model as a base model (see Table 3). Including an additive effect of the probability of infection at the time of seroconversion was again substantially better than the base model, with a ΔAICc of 6, and yielded an estimated drop in survival of 22·4% for infected individuals (probability of cowpox, 1·0).

EFFECT OF COWPOX ON SURVIVAL AT THE POPULATION LEVEL

The most parsimonious model included a simple additive effect of cowpox prevalence on field vole survival but excluded average temperature in the previous month compared with the base model (AICcs: base model 4530·05, base model + cp 4527·24, base model + cp − prevt 4526·77). Again, cowpox had a negative impact on vole survival, with lower survival rates in months of higher cowpox prevalence (Table 5; parameter estimate for the slope of cowpox prevalence −3·244 (95% CI − 5·752 to − 0·735)). Using BHP in November 2001 as an example and using median levels of density and rainfall, following transformation, the model predicts a survival rate of 0·931 (0·930–0·932) for a cowpox prevalence of 0·005 (the lowest observed), and 0·838 (0·709–0·916) for a cowpox prevalence of 0·30 (the highest observed), representing a 9·3% lower rate of survival for populations with the highest cowpox prevalence. At the median level of cowpox prevalence (0·139), predicted survival is 0·897 (0·860–0·925).

DOES THE EFFECT OF COWPOX ON FIELD VOLE SURVIVAL VARY TEMPORALLY ?

Two models in which the effect of cowpox varied over time performed almost as well as the optimal model (AICc = 4526·77). For one with an interaction between cowpox prevalence and season (splitting the year into two) AICc was 4526·60. This model suggests that the negative effect on survival may be greater in summer (April–September), −5·30 (95% CI −9 to −1·60) than in winter (October–March), −0·55 (−4·88 to 3·78). A model including an interaction between year and cowpox also had an AICc value (4526·30) similar to the optimal model, and suggested that the negative effect on survival was greatest in 2003, −6·59 (−10·69 to −2·50), and lowest in 2001, −1·25 (−4·54–2·03), compared with 2002, −3·58 (−6·59 to −0·57).

MATRIX PROJECTION MODELS

The 28-day population growth rate (λ) in June declined from 1·62 to 1·50, as the fraction of the population with their survival affected by cowpox increased from 0 to 0·89: the maximum observed proportion of voles having been exposed to cowpox virus infection (Table 6).

Discussion

This study clearly shows a negative correlation between cowpox virus infection and field vole survival, with this effect apparent at both the individual level and population level of analysis. This is the first time that such an association between an endemic microparasite and processes of demographic importance has been shown in a cyclic population of wild rodents, and one of the first such demonstrations for any wildlife population. This effect was superimposed upon a pattern in which survival generally tended to be lower at higher densities (2003), during the breeding season (March–September) and over periods of either heavy rain or high temperatures, and was lower in males than in females.

The analysis at the individual level estimated that voles with a probability of infection of 1·0 in months of median base survival rates had a large and biologically significant survival rate 22·4% lower than uninfected voles during the same primary session. As noted above, a P(I) of 0·5 represented the highest possible individual P(I) included in the data, and approximately 50% of such individuals would, actually, have recovered from infection. Assuming these experience survival rates similar to uninfected animals, this would have reduced the magnitude of any estimated effect of cowpox on survival. Consequently, despite the fact that this extrapolates beyond observed P(I) values, we have used a P(I) of 1·0 to predict the survival rate of truly infected animals.

At the population level, months with lowest recorded cowpox prevalence had a 10% higher predicted survival rate compared with months with the highest cowpox prevalence. It is important to emphasize that without experimental verification, the impact of cowpox infection on field vole survival cannot be fully elucidated. Infection with cowpox may predispose individuals to infection from other pathogens, or reflect lower survival in individuals of poorer health and increased susceptibility to infection. Several studies have utilized experimental ecto- or macro-parasite removal to elucidate the negative effect of such parasites on host fitness (Oppliger, Richner & Christe 1994; Brown, Brown & Rannala 1995; Hudson, Dobson & Newborn 1998; but see Redpath *et al*. 2006; Neuhaus 2003; Smith *et al*. 2006a). However, due to a lack of specific medication, and due to logistical difficulties and economic costs of treating individuals, experimental manipulation of endemic microparasites in wild populations is problematic (but see Merino *et al*. 2000; Potti *et al*. 2002; Tomas *et al*. 2007).

The reduction in survival due to cowpox can be compared with the reduction in survival of field voles at Kielder caused by weasel predation. Graham & Lambin (2002) estimated that the average monthly increase in field vole survival due to weasel removal was 2·24% and 2·10% for adult males and females, and 0·57% and 0·78% for juvenile males and females. On that basis, and given that empirical evidence shows that cowpox virus infection prevalence varies much more widely between years than weasel abundance in Kielder (Cavanagh *et al*. 2004; Burthe *et al*. 2006), the impact of cowpox on field vole survival at Kielder appears to be much higher than that of this specialist predator.

On the other hand, individual voles only pay the survival cost of cowpox infection during one month in their life, unless this infection predisposes them to secondary infections. None the less, the projection models demonstrate that even the short-term impact of cowpox alone may reduce the 28-day population multiplication rate substantially (from 1·62 to 1·50). Moreover, because we have used the proportion of voles seroconverting as a proxy for the proportion of all voles affected by cowpox, this underestimates the prevalence of infection (and hence the impact of cowpox), as it neglects individuals that become infected but then die without subsequently being captured. In the absence of density dependence in the model, all parameter combinations predict rapid population expansion, such that the impact of cowpox alone cannot account for the observed variation in population growth during the breeding season. The simple parameterized projection model nevertheless indicates endemic infections may substantially depress population growth; future work with models including disease dynamics and density dependence will hopefully further clarify their impact.

There was some support at the population level for temporal variation in the effect of cowpox on field vole survival, with some evidence of seasonal and annual variation. The negative effect of cowpox was greater in general for any given level of cowpox prevalence in summer than in winter. A similar analysis by Telfer *et al*. (2002) found that in bank voles and wood mice, in contrast, survival rates increased with cowpox prevalence in late summer but decreased in the winter. Although the present results must be interpreted with caution, this suggests that the nature of the effect of cowpox on survival may be different for field voles than for bank voles

and wood mice. This is not unexpected, given, for example, the apparent differences in susceptibility to cowpox virus between the species (Burthe *et al*. 2006). Telfer *et al*. (2002, 2005) found that wild female bank voles and wood mice infected with cowpox virus were more likely to delay maturation, and in most cases reproduction was therefore delayed until the following year. This suggests that the increase in survival rates with high cowpox prevalence in the summer in bank voles and wood mice was due to individuals delaying costly reproduction. Thus, the contrasting results here may suggest that infected field voles at Kielder do not delay reproduction, and hence that survival rates are lowered by the simultaneous energetic demands of reproducing and mounting an immune response to cowpox virus infection.

The effect of cowpox virus infection on survival rates was lowest in 2001, increased in 2002 and was highest in 2003. This parallels changes in cycle phase and cowpox prevalence over the study period, with density increasing in general between 2001 and 2003. Survival rates generally were lower during months with high density, as might be expected. However, interaction between cowpox prevalence and host density was not significantly better than year in the statistical model, suggesting that factors other than host density *per se* are responsible for the increased effect of cowpox on survival in higher-density years. It is well established that in fluctuating small rodent populations there is significant variation in body size, timing of maturation and reproductive performance between phases of the cycle. In years of high eventual density, animals generally breed earlier in the spring, and more animals mature in their year of birth (Krebs & Myers 1974; Hansson & Henttonen 1985; Bernshtein, Zhigalsky & Panina 1989; Gilbert & Krebs 1991; Boonstra 1994; Smith *et al*. 2006b). Such phase-associated life-history changes may explain why cowpox prevalence is associated with lower survival rates in some years.

Overall, the observed reduction in survival rates associated with cowpox virus (which is likely, as explained, to be an underestimate) supports the hypothesis that endemic pathogens have the potential to shape host population dynamics. It has also been clearly demonstrated that the risk of becoming infected with cowpox virus fluctuates with a time-delay relative to field vole density 3 months in the past (Burthe *et al*. 2006). Moreover, as the majority of field voles at Kielder Forest become infected with cowpox virus at a young age (Burthe *et al*. 2006), the impact of infection on the demography of this cohort could be potentially significant in affecting population growth rates, and this is supported by the matrix projection models. In voles, several studies have suggested that phaserelated changes in juvenile survival rates and changes in age at maturity are important in driving population fluctuations (Gaines & Rose 1976; Getz *et al.* 1997; Oli & Dobson 2001; Ozgul, Getz & Oli 2004). Although, further work is necessary to investigate whether cowpox virus infection is associated with low survival rates in field vole populations during the crash and low phase of the cycle, this study clearly shows that endemic pathogens should not be dismissed as having negligible effects on the dynamics of their reservoir hosts.

Acknowledgments

The Forestry Commission provided access to study sites and weather data. Work was funded by the Natural Environmental Research Council (Studentship number NER/S/A/2000/03445 awarded to S. Burthe) and the Wellcome Trust (075202/Z/04/Z), licensed under home office project license PPL 40/1813. Gordon Brown, Jonathan Fairbairn, Matt Oliver, Laura Taylor, Gill Telford, David Tidhar and Rachel Yeates provided valuable fieldwork assistance. Alex Millon commented on the paper.

References

Anderson M, Erlinge S. Influence of predation on rodent populations. Oikos 1977;29:591–597.

Anderson RM, May RM. Regulation and stability of host–parasite population interactions. I: Regulatory processes. Journal of Animal Ecology 1978;47:219–247.

Anderson RM, May RM. Population biology of infectious diseases: Part 1. Nature 1979;280:361–367. [PubMed: 460412]

Baxby, D.; Bennett, M. Cowpox virus (Poxviridae). In: Webster, RG.; Granoff, A., editors. Encyclopedia of Virology. 2nd edn. Academic Press; London: 1999. p. 298-304.

Bennett M, Crouch AJ, Begon M, Duffy B, Feore S, Gaskell RM, Kelly DF, McCracken CM, Vicary L, Baxby D. Cowpox in British voles and mice. Journal of Comparative Pathology 1997;116:35–44. [PubMed: 9076598]

Bernshtein AD, Zhigalsky OA, Panina TV. Multi-annual fluctuations in the size of a population of the bank vole in European part of the USSR. Acta Theriologica 1989;34:409–438.

Bierman SM, Fairbairn JP, Petty SJ, Elston DA, Tidhar D, Lambin X. Changes over time in the spatiotemporal dynamics of cyclic populations of field voles (*Microtus agrestis* L.). American Naturalist 2006;167:583–590.

Boonstra R. Population cycles in microtines: the senescence hypothesis. Evolution and Ecology 1994;8:196–219.

Brown CR, Brown MB, Rannala B. Ectoparasites reduce long-term survival of their avian host. Proceedings of the Royal Society of London Series B 1995;262:313–319.

Burnham, KP.; Anderson, DR.; White, GC.; Brownie, C.; Pollock, KH. Design and Analysis of Fish Survival Experiments Based on Release–Recapture Data. American Fisheries Society; Bethesda, MD: 1987.

Burthe, SJ. The role of cowpox virus infection and vole tuberculosis in cyclic wild field vole populations. University of Liverpool; Liverpool: 2005. PhD Thesis

Burthe SJ, Telfer S, Lambin X, Bennett M, Carslake D, Smith A, Begon M. Cowpox virus infection in natural field vole, Microtus agrestis, populations: delayed density dependence and individual risk. Journal of Animal Ecology 2006;75:1416–1425. [PubMed: 17032374]

Cavanagh R, Lambin X, Ergon T, Bennett M, Graham IM, van Soolingen D, Begon M. Disease dynamics in cyclic populations of field voles (*Microtus agrestis*): cowpox virus and vole tuberculosis (*Mycobacterium microti*). Proceedings of the Royal Society of London Series B 2004;271:859–867. [PubMed: 15255106]

Chantrey, J. The epidemiology of cowpox in its reservoir hosts. University of Liverpool; Liverpool: 1999. PhD Thesis

Chantrey J, Meyer H, Baxby D, Begon M, Bown KJ, Hazel SM, Jones T, Montgomery WI, Bennett M. Cowpox: reservoir hosts and geographic range. Epidemiology and Infection 1999;122:455–460. [PubMed: 10459650]

Chao, A.; Lee, S. Estimating Population Size for Continuous Time Capture–Recapture Models Via Sample Coverage. Institute of Statistics, National Tsing Hua University; Hsin-chu, Taiwan, Republic of China: 1991. Technical Report 91-C01

Crouch AC, Baxby D, McCracken CM, Gaskell RM, Bennett M. Serological evidence for the reservoir hosts of cowpox virus in British wildlife. Epidemiology and Infection 1995;115:185–191. [PubMed: 7641833]

Feore SM, Bennett M, Chantrey J, Jones T, Baxby D, Begon M. The effect of cowpox virus infection on fecundity in bank voles and wood-mice. Proceedings of the Royal Society of London Series B 1997;264:1457–1461. [PubMed: 9364786]

Gaines M, Rose R. Population dynamics of Microtus ochrogaster in eastern Kansas. Ecology 1976;57:1145–1161.

Getz LL, Simms LE, McGuire B, Snarski ME. Factors affecting life expectancy of the prairie vole, *Microtus ochrogaster*. Oikos 1997;80:362–370.

Gilbert BS, Krebs CJ. Population dynamics of Clethrionomys and Peromyscus in southwestern Yukon 1973–89. Holarctic Ecology 1991;14:250–259.

Graham, IM. Weasels and vole cycles: an experimental test of the specialist predator hypothesis. University of Aberdeen; Aberdeen: 2001. PhD Thesis

Graham IM, Lambin X. The impact of weasel predation on cyclic field-vole survival: the specialist predator hypothesis contradicted. Journal of Animal Ecology 2002;71:946–956.

Grenfell, BT.; Dobson, AP. Ecology of Infectious Diseases in Natural Populations. Cambridge University Press; 1995.

Hanski I, Henttonen H, Korpimaki E, Oksanen L, Turchin P. Small-rodent dynamics and predation. Ecology 2001;82:1505–1520.

Hansson L, Henttonen H. Regional differences in cyclicity and reproduction in *Clethrionomys* species: are they related? Annales Zoologici Fennici 1985;22:277–288.

Hudson PJ, Dobson AP, Newborn D. Prevention of population cycles by parasite removal. Science 1998;282:2256–2258. [PubMed: 9856948]

Hurvich CM, Tsai CL. Regression and time series model selection in small samples. Biometrika 1989;76:297–307.

Innes DGL, Millar JS. Life-histories of *Clethrionomys and Microtus* (Microtinae). Mammal Review 1994;24:179–207.

Krebs CJ, Myers JH. Population cycles in small mammals. Advances in Ecological Research 1974;8:267–399.

Lambin X, Yoccoz NG. Adaptive precocial reproduction in voles: reproductive costs and multivoltine life-history strategies in seasonal environments. Journal of Animal Ecology 2001;70:191–200.

Lambin X, Elston DA, Petty SJ, MacKinnon JL. Spatial asynchrony and periodic travelling waves in cyclic populations of field voles. Proceedings of the Royal Society of London Series B 1998;265:1491–1496. [PubMed: 9744104]

Lambin X, Petty SJ, MacKinnon JL. Cyclic dynamics in field vole populations and generalist predation. Journal of Animal Ecology 2000;69:106–118.

Lebreton JD, Burnham KP, Clobert J, Anderson DR. Modelling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs 1992;62:67–118.

Merino S, Moreno J, Sanz JJ, Arriero E. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proceedings of the Royal Society of London Series B 2000;267:2507–2510. [PubMed: 11197126]

Mihok S, Turner BN, Iverson SL. The characterization of vole population dynamics. Ecological Monographs 1985;55:399–420.

Neuhaus P. Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). Proceedings of the Royal Society of London Series B 2003;270:S213–S215. [PubMed: 14667386]

Oli MK, Dobson FS. Population cycles in small mammals: the alpha-hypothesis. Journal of Mammalogy 2001;82:573–581.

Oppliger A, Richner H, Christe P. Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit (*Parus major*). Behavioural Ecology 1994;5:130–134.

Otis D, Burnham K, White G, Anderson D. Statistical inference from capture data on closed animal populations. Wildlife Monographs 1978;62:1–133.

Ozgul A, Getz LL, Oli MK. Demography of fluctuating populations: temporal and phase-related changes in vital rates of *Microtus ochrogaster*. Journal of Animal Ecology 2004;73:201–215.

Potti J, Moreno J, Yorio P, Briones V, Garcia-Borboroglu P, Villar S, Ballesteros C. Bacteria divert resources from growth for magellanic penguin chicks. Ecology Letters 2002;5:709–714.

Redpath SM, Mougeot F, Leckie FM, Elston DA, Hudson PJ. Testing the role of parasites in driving the population dynamics of a gamebird. Ecology Letters 2006;9:410–418. [PubMed: 16623726]

Sakamoto, Y.; Ishiguro, M.; Kitagawa, G. Akaike Information Criterion Statistics. KTK Scientific Publishers; Tokyo: 1986.

Scott ME. The impact of infection and disease on animal populations: implications for conservation biology. Conservation Biology 1988;2:40–96.

Smith A, Telfer S, Burthe S, Bennett M, Begon M. A role for vector-independent transmission in rodent trypanosome infection. International Journal of Parasitology 2006a;36:1359–1366. [PubMed: 16876803]

Smith M, White A, Lambin X, Sherratt JA, Begon M. Delayed density dependent season length alone can lead to multiannual rodent population cycles. American Naturalists 2006b;167:695–704.

Soveri T, Henttonen H, Rudback E, Schildt R, Tanskanen R, Husu-Kallio J, Haukisalmi V, Sukura A, Laakkonen J. Disease patterns in field and bank vole populations during a cyclic decline in central Finland. Comparative Immunology, Microbiology and Infectious Diseases 2000;23:73–89.

Telfer S, Bennett M, Bown K, Cavanagh R, Crespin L, Hazel S, Jones T, Begon M. The effects of cowpox virus on survival in natural rodent populations: increases and decreases. Journal of Animal Ecology 2002;71:558–568.

Telfer S, Bennett M, Bown K, Carslake D, Cavanagh R, Hazel S, Jones T, Begon M. Infection with cowpox virus decreases female maturation rates in wild populations of woodland rodents. Oikos 2005;109:317–322.

Tomas G, Merino S, Moreno J, Morales J, Martinez-de la Puente J. Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment in a wild passerine. Functional Ecology 2007;21:125–133.

White GC, Burnham KP. Program MARK: survival estimation from populations of marked animals. Bird Study 1999;46:120–138.

UKPMC Funders Group Author Manuscript

UKPMC Funders Group Author Manuscript

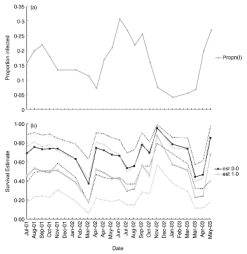

Fig. 1.

(a) Proportion of the population infected with cowpox virus per month for males at BHP throughout the 2-year study period. (b) Predicted estimates of survival for individuals with a 0 probability of being infected with cowpox, and for an individual with a 1·0 probability of being infected with cowpox. The dashed lines indicate the 95% confidence limits for the estimates. A P(I) of 0·5 represented the highest possible individual P(I) included in the data, and approximately 50% of such individuals would, actually, have recovered from infection. Assuming these experience survival rates similar to uninfected animals, this would have reduced the magnitude of any estimated effect of cowpox on survival. Consequently, despite the fact that this extrapolates beyond observed P(I) values, we have used a P(I) of 1·0 to predict the survival rate of truly infected animals.

UKPMC Funders Group Author Manuscript

Table 1

The meanings of subscripts used in the capture–recapture models. Details of which parameters were included in recapture models (p) and survival models (Φ) are given in the right-hand column

Analysis stage	Subscript	Description	Parameter type
Group level	st	Site-effect (BHP, KCS, PLJ or ROB)	Φ, p
	sx	Sex-effect	Φ, p
	t	Full time-dependence	Φ, p
Temporal	mo	Month effect (13 months per year)	Φ, p
	seas1	Season-effect (Nov–Feb, Mar–May, May–Jul, Aug–Oct)	Φ, p
	seas2	Binary season-effect (winter Oct–Mar; summer Apr–Sept)	Φ, p
	year	Year effect (2001, 2002 or 2003)	Φ, p
Covariates	rf	Average rainfall during trapping session	p
	temp	Average temperature during trapping session	p
	prevrf	Average rainfall during month previous to trapping	Φ
	prevt	Average temperature during month previous to trapping	Φ
	dens	Vole density during trapping session	Φ, p
	n	Binary score denoting months with reduced number of secondary sessions	p
	cp	Prevalence of cowpox (population level)	Φ, p
Individual covariates	e	Binary covariate denoting individuals from grid edge	p
Time-specific individual covariates	m	Trap-dependency effect	p
	indcp	Probability of cowpox virus infection at first seropositive trap session (individual level)	Φ

UKPMC Funders Group Author Manuscript

Table 2

Best model structures in each of the three stages of modelling recapture in field voles at Kielder. In each stage, the model with the lowest AICc is reported first and all models within eight of the lowest AICc are shown. The most parsimonious models, used as starting models for the next stage, are highlighted in bold. These either had the lowest AICc or were within two of the lowest AICc but had fewer parameters. While recapture was being investigated, the model structure for survival was held constant as ((sx * st) + (sx * t))

Model stage	Model	AICc	No. of parameters	Deviance
Stage 1: group level	st * t	5022·40	111	1825·55
	t	5022·56	70	1912·97
	(st * t) + sx	5024·15	112	1825·14
	(st * t) + (sx * st)	5024·82	114	1821·48
	t + st	5027·71	74	1909·72
	sx * t	5028·44	85	1887·23
	t + sx + st	5029·44	75	1909·35
Stage 2: temporal variation and covariates	**year + mo + rf + n**	**5004·15**	**65**	**1905·05**
	year + mo + rf + n + dens	5006·49	66	1905·05
	t + rf + n	5008·25	72	1894·50
	year + mo + st + rf + n	5008·29	68	1902·90
	seas + year + rf + n	5013·14	60	1924·47
Stage 3: individual covariates	**year + mo + m + n + rf**	**4987·44**	**67**	**4850·30**
	year + mo + m + n + rf	4993·74	65	4860·79

UKPMC Funders Group Author Manuscript

Table 3

Best model structures in each of the stages of modelling survival in field voles at Kielder. The model structure for recapture was held constant as the best model from Table 2 (mo + year + edge + m + n + rf). The most parsimonious models are highlighted in bold. The model with the lowest AICc is reported first and all models within eight of the lowest AICc are included. In stage 1, the starting model for the next stage had an AICc within 2 of the best model but fewer parameters. In stage 2 further models are reported to show attempts to explain temporal variation

Model stage	Model	AICc	No. of parameters	Deviance
Stage 1: group level	(st * t) + (sx * t)	4944·32	122	4689·78
	(st * t) + sx	**4945·42**	**102**	**4734·10**
	(st * t) + (sx * st)	4947·73	104	4732·11
Stage 2: temporal variation and covariates	**(st * t) + sx**	**4945·42**	**102**	**4734·10**
	(st * dens) + prevrf + prevt + mo + year + sx	4961·14	40	4880·02
	(st * dens) + prevrf + mo + year + sx	4963·06	39	4883·99
	(st * dens) + prevrf + prevt + mo + sx	4969·87	38	4892·86
	(st * year) + prevrf + mo + dens + sx	4974·87	42	4889·64
	st + dens + prevrf + prevt + mo + year + sx	4981·01	37	4906·06

UKPMC Funders Group Author Manuscript

Table 4

The effect of cowpox on survival of field voles. The base model was $\phi_{(st*t)+sx}\, p_{mo+year+edge+m+n+rf}$, and the AICc result for this models is presented first (AICc lower than in Table 3 due to this analysis being carried out on a reduced data set, not including animals caught seronegative at last capture). The most parsimonious model is highlighted in bold

Modelling stage	Survival	Recapture	AICc	No. of parameters	Deviance
Base model	(st * t) + sx	mo + year + rf + n + m + edge	4524·19	101	4314·33
Investigating the effect of cowpox on survival	**(st * t) + sx + incp**	**mo + year + rf + n + m + edge**	**4518·22**	**102**	**4306·21**

UKPMC Funders Group Author Manuscript

Table 5

The effect of cowpox on survival and recapture of field voles at the population level. The base model used was $\phi_{(st*dens)+prevrf+prevt+mo+year+sx}$ $p_{mo+year+edge+m+n+rf}$. In each stage, the model with the lowest AICc is reported first and all models within eight of the lowest AICc are included. The most parsimonious models are highlighted in bold

Modelling stage	Survival model	Recapture model	AICc	No. of par	Deviance
Base model	(st * dens) + prevrf + prevt + mo + year + sx	mo + year + rf + n + m + edge	4530·05	40	4448·83
Investigating the effect of cowpox on recapture	(st * dens) + prevrf + prevt + mo + year + sx	mo + year + rf + n + m + edge + cp	4533·14	41	4451·91
Investigating the effect of cowpox on survival	**(st * dens) + prevrf + mo + sx + year + cp**	**mo + year + rf + n + m + edge**	**4526·77**	**40**	**4445·55**
	(st * dens) + prevrf + prevt + mo + sx + year + cp	mo + year + rf + n + m + edge	4527·24	41	4443·96
	(st * dens) + mo + year + prevrf (sx * cp)	mo + year + rf + n + m + edge	4528·45	41	4445·16
	(st * dens) + mo + year + sx + cp	mo + year + rf + n + m + edge	4534·17	39	4455·01
	(st * dens) + prevrf + sx + mo + (year * cp)	mo + year + rf + n + m + edge	4526·30	42	4440·95
	(st * dens) + prevrf + sx + year + (mo * cp)	mo + year + rf + n + m + edge	4529·79	50	4427·89

UKPMC Funders Group Author Manuscript

Table 6

The impact of changes in adult field vole survival rates in June due to cowpox infection on monthly population growth rates (λ). Population growth rates were estimated using a matrix projection model, assuming juvenile survival of 0·61. Predicted population growth rates were calculated for uninfected populations (P(I) = 0), for the minimum observed P(I) (0·46), for the average P(I) (0·71), and for the maximum observed P(I) (0·89). The difference in predicted population growth rates is reported for each proportion infected compared with an uninfected population (P(I) = 0). Growth rates were calculated for females only

	P(I) = 0	**P(I) = 0·46**	**P(I) = 0·71**	**P(I) = 0·89**
Survival rates (Φ)	0·77	0·69	0·65	0·61
Monthly population growth rate (λ)	1·62	1·56	1·53	1·50
Δ λ		0·06	0·09	0·12