Epidemiol. Infect. (2000), **124**, 551–562. Printed in the United Kingdom

A longitudinal study of an endemic disease in its wildlife reservoir: cowpox and wild rodents

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(*Accepted 10 November 1999*)

SUMMARY

Cowpox is an orthopoxvirus infection endemic in European wild rodents, but with a wide host range including human beings. In this longitudinal study we examined cowpox in two wild rodent species, bank voles *Clethrionomys glareolus* and wood mice *Apodemus sylvaticus*, to investigate the dynamics of a virus in its wild reservoir host. Trapping was carried out at 4-weekly intervals over 3 years and each animal caught was uniquely identified, blood sampled and tested for antibodies to cowpox. Antibody prevalence was higher in bank voles than in wood mice and seroconversion varied seasonally, with peaks in autumn. Infection was most common in males of both species but no clear association with age was demonstrated. This study provides a model for studying other zoonotic infections that derive from wild mammals since other approaches, such as one-off samples, will fail to detect the variation in infection and thus, risk to human health, demonstrated here.

INTRODUCTION

Empirical support for theoretical studies of the role of parasites and pathogens in the population dynamics of their hosts is rare [1]. Indeed, most studies on infectious disease in natural populations have been opportunistic investigations of epidemics caused either by pathogens not previously encountered by the host, such as myxomatosis in European rabbits [2], or against a background of immunosuppression, as suggested for seal morbillivirus [3, 4]. In other cases, endemic infections have been suggested to explain observed time series of host abundance which appear unaffected by other demographic variables [5, 6]. Few studies, however, have addressed the role of endemic diseases which cause little obvious mortality or clinical signs. This study sets out to provide the empirical basis required to investigate fully the nature of host–pathogen interactions between an endemic viral disease, cowpox, and its wild rodent reservoir hosts.

Cowpox virus, despite its name, rarely infects cattle and is most often diagnosed in domestic cats [7]. It is also a zoonosis although human cases are rare [8]. Wild rodents are the 'reservoir' hosts in which cowpox circulates naturally. This study can therefore also provide a good model for diseases which circulate in wild animal reservoirs and present a zoonotic risk to humans generally, such as hantavirus, monkeypox and ebola [9–12]. These diseases are often described as new and 'emerging' infections in man and have become an increasing cause for concern. Understanding their dynamics in their wildlife reservoirs is crucial to understanding the epidemiology of these diseases in humans and deciding on measures to prevent them. Most studies of zoonotic infections in

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wildlife reservoirs have involved cross-sectional studies that present pathogen prevalence at only one point in time. Longitudinal monitoring of a pathogen in the field, however, allows the number of individuals in the population required to maintain the infection through successive generations to be estimated, and the periods when disease incidence in the reservoir species is highest to be pinpointed, which may represent the time of highest potential zoonotic risk to humans.

Cowpox virus is a member of the genus Orthopoxvirus in the family Poxviridae [13]. Serological surveys have shown that it is a widespread endemic infection in European wild rodents [14]. Antibody and, at a very much lower prevalence, virus have been detected in wild ground squirrels (yellow susliks *Citellus fulvus*) and gerbils (*Rhombomys opimus*, *Meriones libicus* and *Meriones meridianus*) in Turkmenistan and Georgia [15, 16] in root voles (*Microtus oeconomus*) on the Kolskiy Peninsula in northern Russia [17] and, by PCR (polymerase chain reaction), from various rodents in Norway [18]. In Great Britain, antibody has been found in the occasional house mouse (*Mus musculus*) but the highest seroprevalence is in bank voles (*Clethrionomys glareolus*), wood mice (*Apodemus sylvaticus*) and field voles (*Microtus agrestis*) which are believed to be the reservoir hosts [19–21]. This has been further supported by the detection of cowpox-specific DNA by PCR and sequencing of viral thymidine kinase and fusion genes in bank voles and wood mice [21].

Bank voles and wood mice are susceptible to infection with between 2 and 20 plaque-forming units (p.f.u.s) of cowpox virus, both by the skin and oronasal routes [22]. However, the virus does not cause obvious signs of disease in wild or laboratory animal populations, nor does it obviously affect survival. We previously reported experimental studies, however, which demonstrated that cowpox can affect fecundity in both bank voles and wood mice by delaying significantly the onset of reproduction [23]. This demonstrated that an endemic microparasite such as cowpox virus could have an effect on a process of clear demographic importance. The present study of the dynamics of cowpox virus infection in its rodent hosts is part of a larger investigation of the effect of endemic infections on the dynamics of their hosts in the field.

In this longitudinal study we report on the incidence of cowpox virus in two mixed populations of two wild rodent species, bank voles and wood mice, over a 3-year period. We examine the host–pathogen dynamics in the two populations and look at disease incidence in relation to species, sex and age.

METHODS

Study sites

Study sites were established at two square 1 hectare sites in mixed woodland habitat on the Wirral Peninsula in north-west England. The first was at Rake Hey (grid ref.: 303 838) where the wooded area covered approximately 4 hectares. The 1 hectare grid was on the edge of the woodland, bounded by fields on two sides and woodland on the two others. The second site was 3 km away at Manor Wood (grid ref.: 294 816) where the wooded area covered approximately 8 hectares. The 1 hectare grid was bounded on one side by a pondage area and on the other three sides by woodland. A 10×10 grid was marked out at both sites with 100 trap stations, permanently situated at 10-m intervals. Two Longworth traps (Penlon Ltd, Oxfordshire, UK) were placed at each station. The traps were baited with whole wheat grain and filled with autoclaved hay for bedding. Trapping sessions were at 4-weekly intervals, and in each session, traps were set over 3 days and nights. Traps were checked twice daily except on the third day when the traps were lifted after being checked once. All bedding material and obvious waste was removed from traps containing animals and the traps were cleaned with 70% ethanol prior to being reset. Traps were sterilized in an autoclave between trapping sessions. The data presented here are for the period April 1995–April 1998 for Rake Hey and March 1995–March 1998 for Manor Wood.

Trapping procedure

Captured animals were identified using subcutaneous microchip transponders with a unique nine-digit number which could be detected using a handheld Power Tracker Reader II (Labtrac by AVID plc, East Sussex, UK). Transponders were injected into the scruff of the neck of each animal on first capture and provided a permanent means of identification. The species, sex and reproductive condition of each animal was recorded and each was weighed to the nearest gram using a handheld Pesola balance (Pesola Ltd, Switzerland). A 20–40 μl blood sample was taken from each animal from the tip of the tail. Each animal

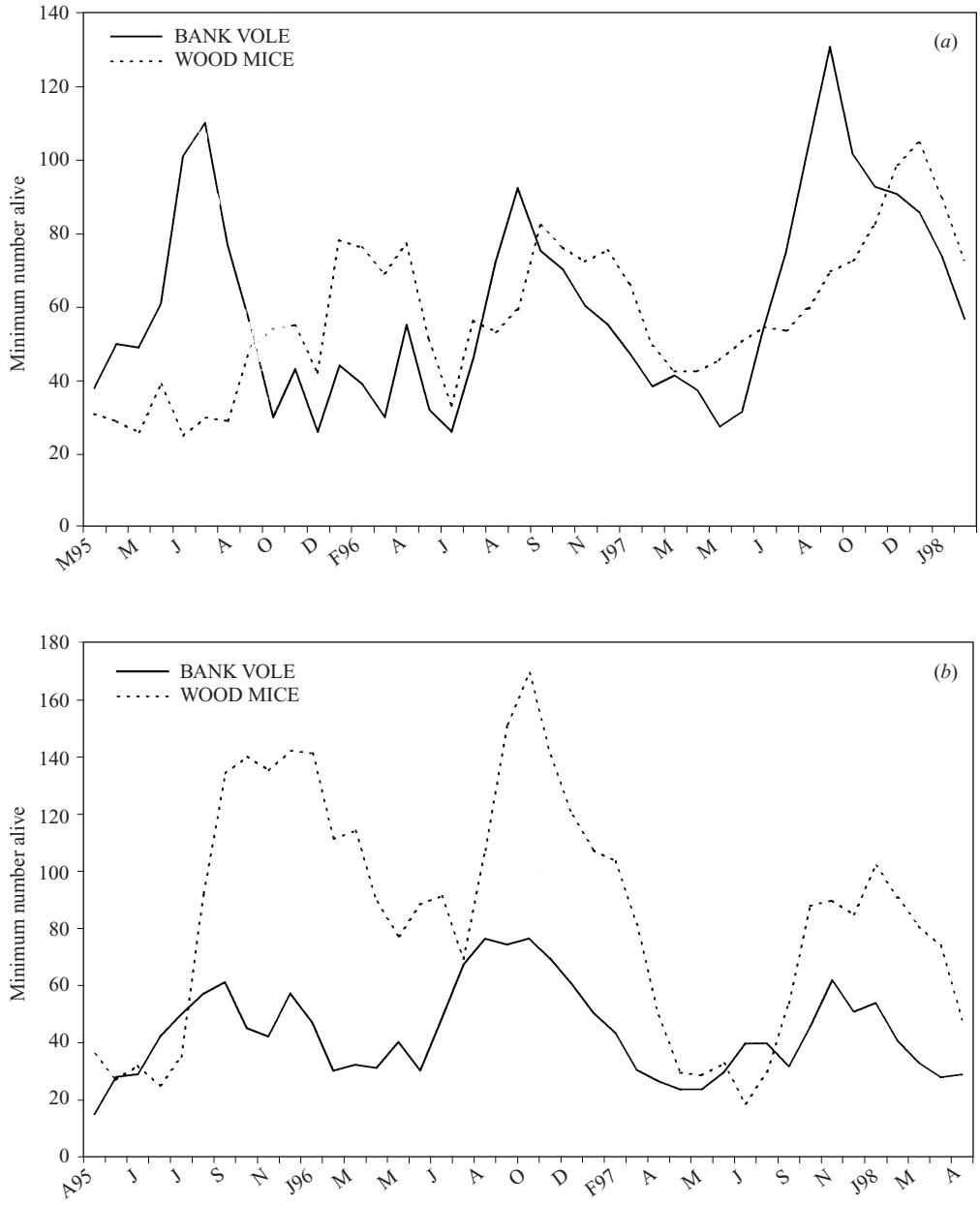

Fig. 1. Minimum number of bank voles and wood mice alive in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

was then released at the exact site of capture. Animals recaptured during the same trapping session were not blood-sampled again.

Serology

Serum samples were stored at −20 °C. Cowpox virus antibody was determined by an immunofluorescence (IF) assay described by Crouch and colleagues [20] and Bennett and colleagues [22]. Antibody titres of 20 or greater were taken as positive.

Data analysis

Analyses of population and virus dynamics and the interaction between the two were carried out. Rodent dynamics were determined using minimum number alive estimates of bank vole and wood mouse numbers for each site by adding the total number of individuals caught in a given trapping session to the number that were not caught in that session but which were caught subsequently and on a previous trapping session [24]. The number of cowpox antibody-positive bank voles

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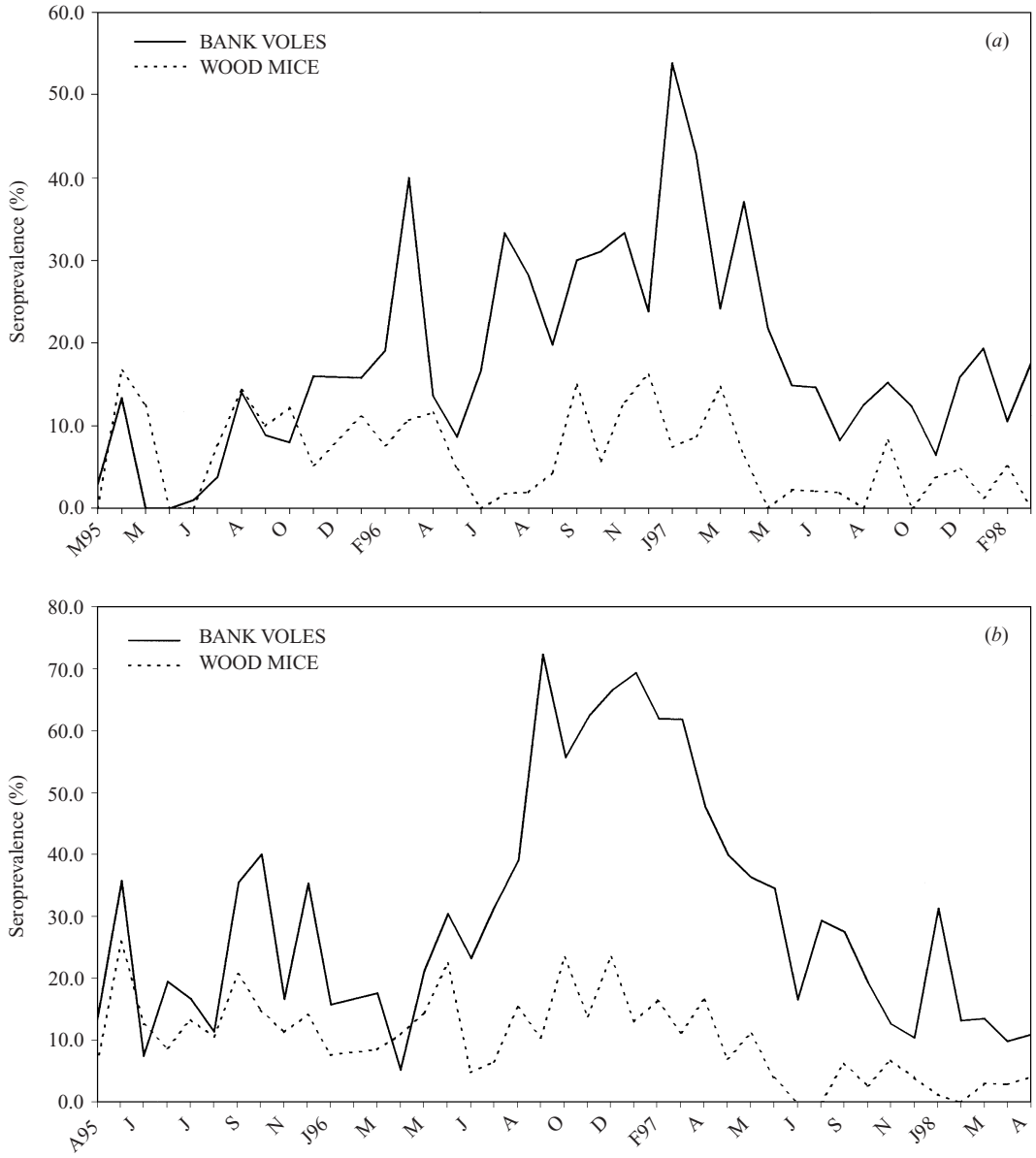

Fig. 2. Cowpox seroprevalence in bank voles and wood mice in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

and wood mice was determined by IF results, and the seroprevalence was expressed as the proportion of the total number of each class of host (species, sex) that were positive. The cowpox antibody-positive hosts may either have been currently infected with cowpox virus or have recovered from an infection.

The nature of the data set, with animals being caught repeatedly, meant that it was possible, for many animals, to determine when they first became cowpox antibody-positive, that is when they seroconverted. Animals which were positive on first capture were also assumed to have seroconverted in the previous month if they were less than adult weight (see below) and therefore likely to be less than 3 months old. This number of seroconverters provided an estimate of the incidence of cowpox virus infection in each population in the period immediately prior to each trapping session rather than past infection, since seroconversion typically occurs about 2 weeks after initial infection and infection itself typically lasts for around 4 weeks [25]. The number of animals that seroconverted between trapping sessions was also used to calculate the proportion seroconverting out of the total number of individuals 'available' to seroconvert; that is, those that could have seroconverted whether they did so or not. This proportion is referred

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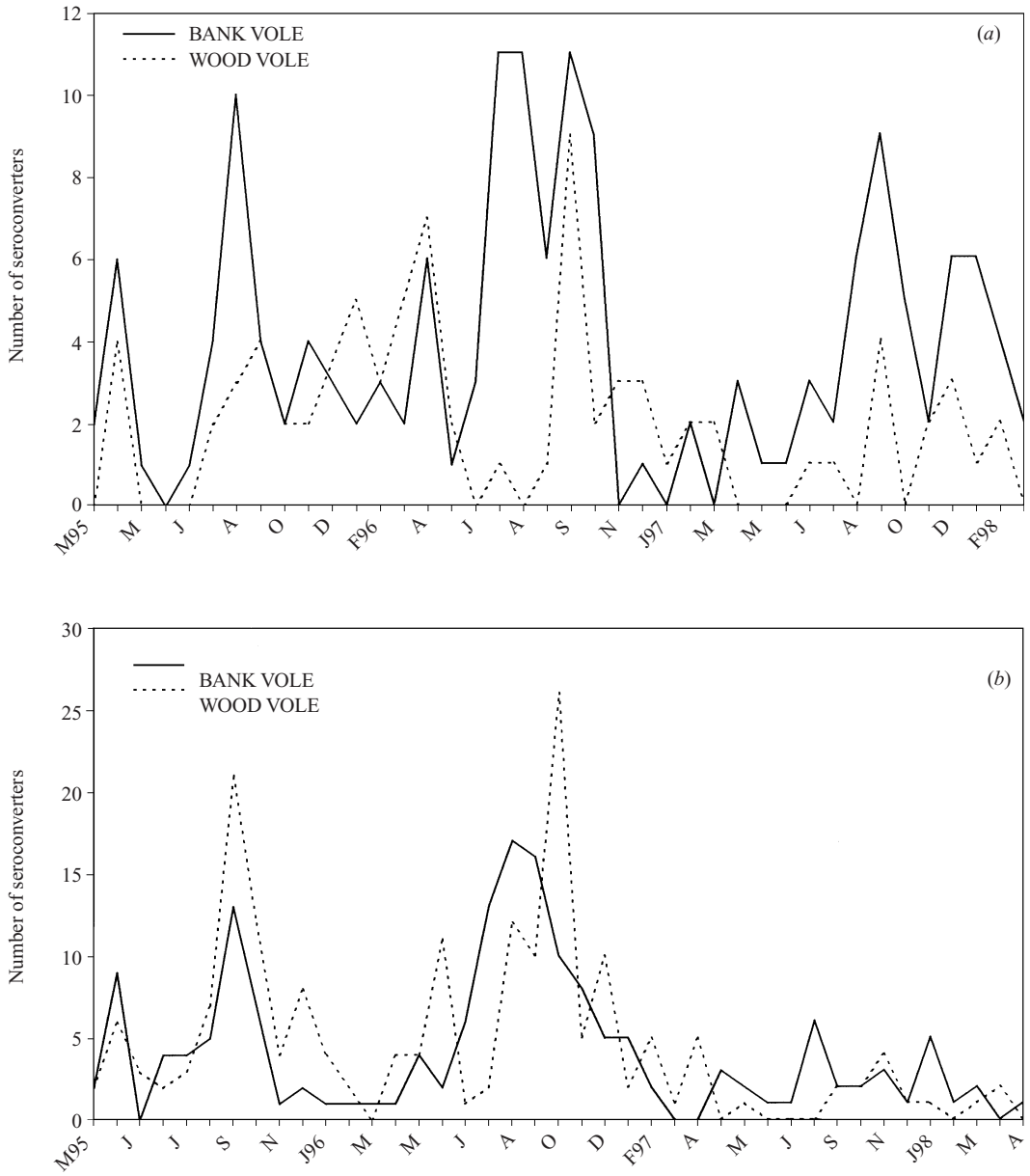

Fig. 3. Number of bank voles and wood mice seroconverting to cowpox in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

to as the seroconversion quotient. These analyses were carried out for both species at both sites. Note that the number seroconverting in a particular class of host indicates the importance of that host-class for the dynamics of the pathogen; whereas the seroconversion quotient is a measure of the potential importance of the pathogen for the dynamics of the host.

Data were also analysed according to sex and age. Age classes at seroconversion were determined using body weight to differentiate juveniles and subadults or 'young' animals (bank voles < 19 g, wood mice < 20 g) and adults (bank voles $\geqslant 19$ g, wood mice $\geqslant 20$ g) [26–28]. However, in a small number of cases animals were assigned to the adult category despite being less than the suggested adult weight, either because their capture-history indicated that they had previously achieved adult weight, or because the animal had been captured on four or more trapping sessions previously.

Seroconversion quotient data were presented as three-point moving averages so that trends in the data could be seen more clearly when comparing different species, sexes and age groups.

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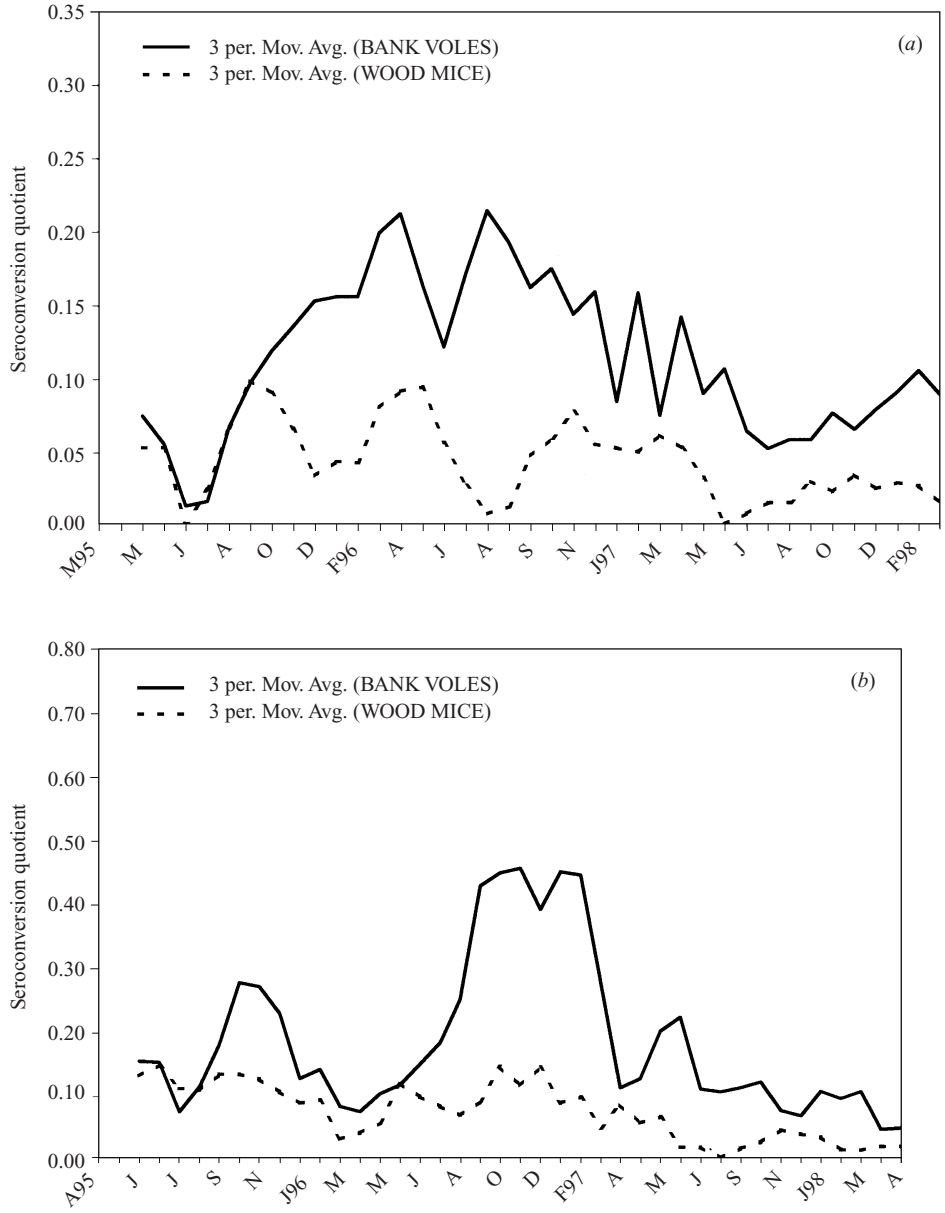

Fig. 4. Cowpox seroconversion quotient in bank voles and wood mice in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

RESULTS

Rodent population size

During the 3-year period from April 1995 until April 1998, 476 bank voles and 788 wood mice were caught at Rake Hey, and between March 1995 and March 1998, 750 bank voles and 664 wood mice were caught at Manor Wood. Estimates of the population size at each time interval using the minimum number alive showed that numbers of both species fluctuated seasonally, with bank vole peaks occurring in late summer and early autumn and peaks for wood mice occurring 2–3 months later (Fig. 1*a*, *b*). Numbers of bank voles fluctuated between 15 and 76 at Rake Hey and between 26 and 130 at Manor Wood per trapping session. Numbers of wood mice fluctuated between 25 and 169 at Rake Hey and between 25 and 104 at Manor Wood per trapping session.

Serology

Blood samples were obtained in over 90% bank voles captures and over 87% for wood mice caught in each

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Table 1. *Sex- and species-related differences for number of animals, number of seroconverters and proportion of seroconverters at each study site*

Study sites	Species	Sex	Number	Number of seroconverters	Proportion of seroconverters
Rake Hey	Bank voles	Male	249	109	0·44
		Female	178	53	0·30
	Wood mice	Male	420	111	0·26
		Female	349	72	0·21
Manor Wood	Bank voles	Male	352	87	0·25
		Female	325	64	0·20
	Wood mice	Male	380	49	0·13
		Female	278	27	0·10

trapping session at Manor Wood and over 89% of bank voles captures and 91% for wood mice in each trapping session at Rake Hey over the 3-year period. Animals were not sampled on some occasions because they were in poor physical condition or poor weather conditions made sampling difficult.

The seroprevalence; i.e. percentage of cowpox antibody-positives in the total sample, varied between 0 and 54% for bank voles and between 0 and 17% for wood mice at Manor Wood, and between 0 and 72% for bank voles and 0 and 26% for wood mice at Rake Hey (Fig. 2*a*, *b*). Hence, the seroprevalence for bank voles was much higher than for wood mice at both sites.

A seasonality was apparent, for seroconversion, which is an indication of actual infection, with numbers seroconverting tending to peak in late summer and early autumn for both species (Fig. 3*a*, *b*). The numbers of animals seroconverting overall were similar in the two species. The seroconversion quotient, however, varied between 0 and 0·30 for bank voles but only between 0 and 0·16 for wood mice at Manor Wood, and between 0 and 0·70 for bank voles but only between 0 and 0·23 for wood mice at Rake Hey (Fig. 4*a*, *b*). The quotient was thus higher for bank voles than wood mice at both sites. Also, at Rake Hey at least, the bank vole quotient showed a seasonality, with peaks in early autumn, which was not observed in wood mice.

Sex-related differences

There were more males than females of both species captured at both sites and more of the captured males than females of both species seroconverted at both sites (Table 1). Moreover, the proportion of captured animals that ever seroconverted was also greater for males than for females in both species at both sites (Table 1). This was significant for bank voles at Rake Hey ($\chi^2 = 8{\cdot}64$, $P < 0{\cdot}005$), but also significant overall when χ^2 values and degrees of freedom were added ($\chi^2 = 16{\cdot}22$, $P < 0{\cdot}005$). When the data were examined longitudinally, three point moving averages revealed that the seroconversion quotient for males was also higher than that observed in females for both species at each site over time, although the differences were more marked in bank voles than wood mice (Fig. 5*a*, *b*).

Age-related differences

The numbers of young and adult animals seroconverting are shown in Table 2. These numbers, however, are affected on the one hand by the fact that individuals, if they survive, are young for a shorter period than they are adult, but on the other hand by the fact that an animal seroconverting when young is then not available to seroconvert as an adult. It is therefore not possible to express the number of seroconverters in each category as an overall proportion of the total number of young and adult animals because over time animals convert from one age category to another. The seroconversion quotients for each sample therefore offer the best indication of the effect of age on the dynamics of the virus, and these (Fig. 6*a*, *b*) suggest no consistent difference overall between the quotients of young and adult animals of either species at either site.

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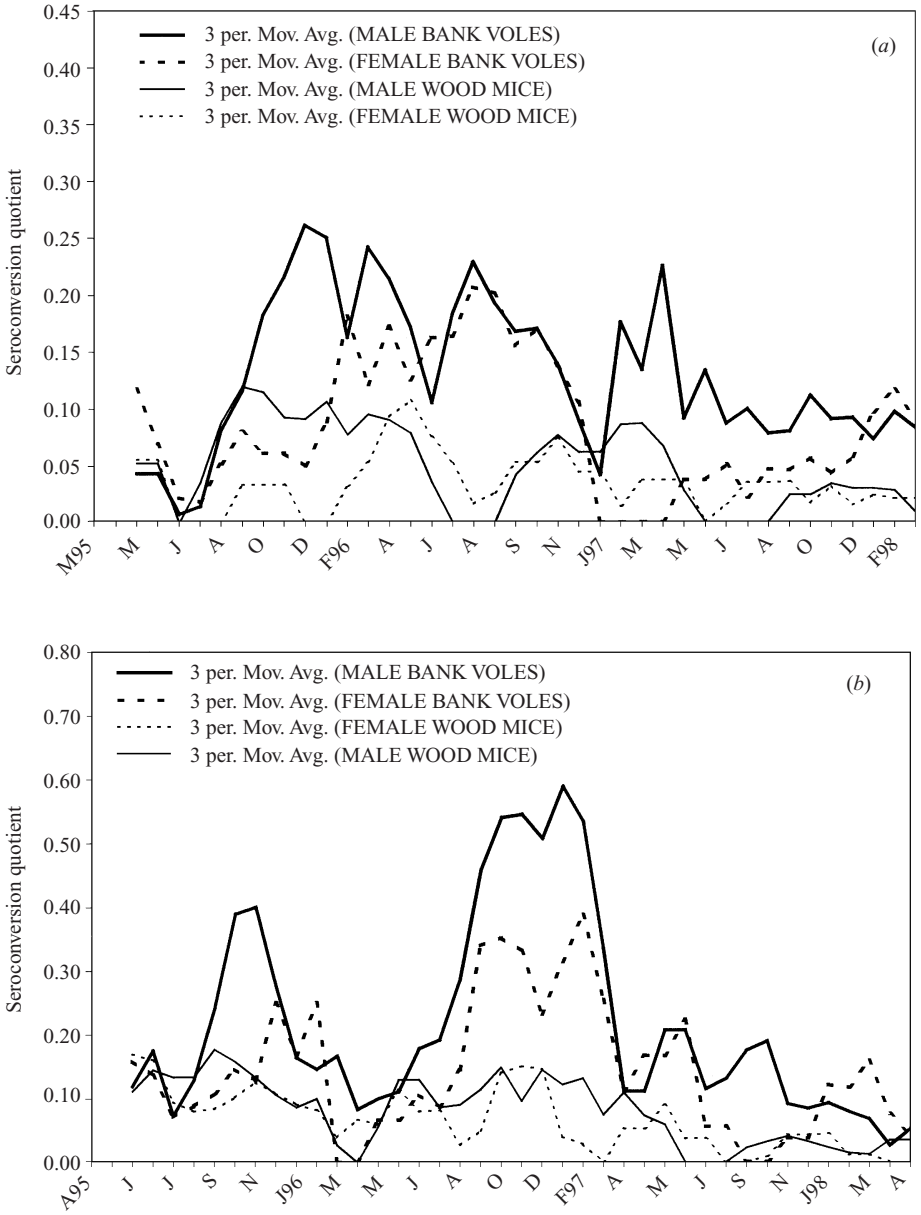

Fig. 5. Cowpox seroconversion quotient in bank voles and wood mice in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

Table 2. *Age- and species-related differences for number of seroconverters at each study site*

Study site	Species	Age grouping	Number of seroconverters
Rake Hey	Bank voles	Young	92
		Adult	70
	Wood mice	Young	88
		Adult	95
Manor Wood	Bank voles	Young	70
		Adult	81
	Wood mice	Young	28
		Adult	48

DISCUSSION

Studies of disease in natural populations have tended to come either as a result of outbreaks of 'new' diseases on an epidemic scale in previously unaffected populations (e.g. myxomatosis [2], seal morbillivirus [3, 4] or as a consequence of their potential as reservoirs for zoonotic infections of threat to human populations (e.g. hantavirus [28]). In both cases, the outcomes have been opportunistic studies, whereas the present investigation highlights the value of longitudinal studies in addressing underlying mechanistic questions and understanding the dynamics of

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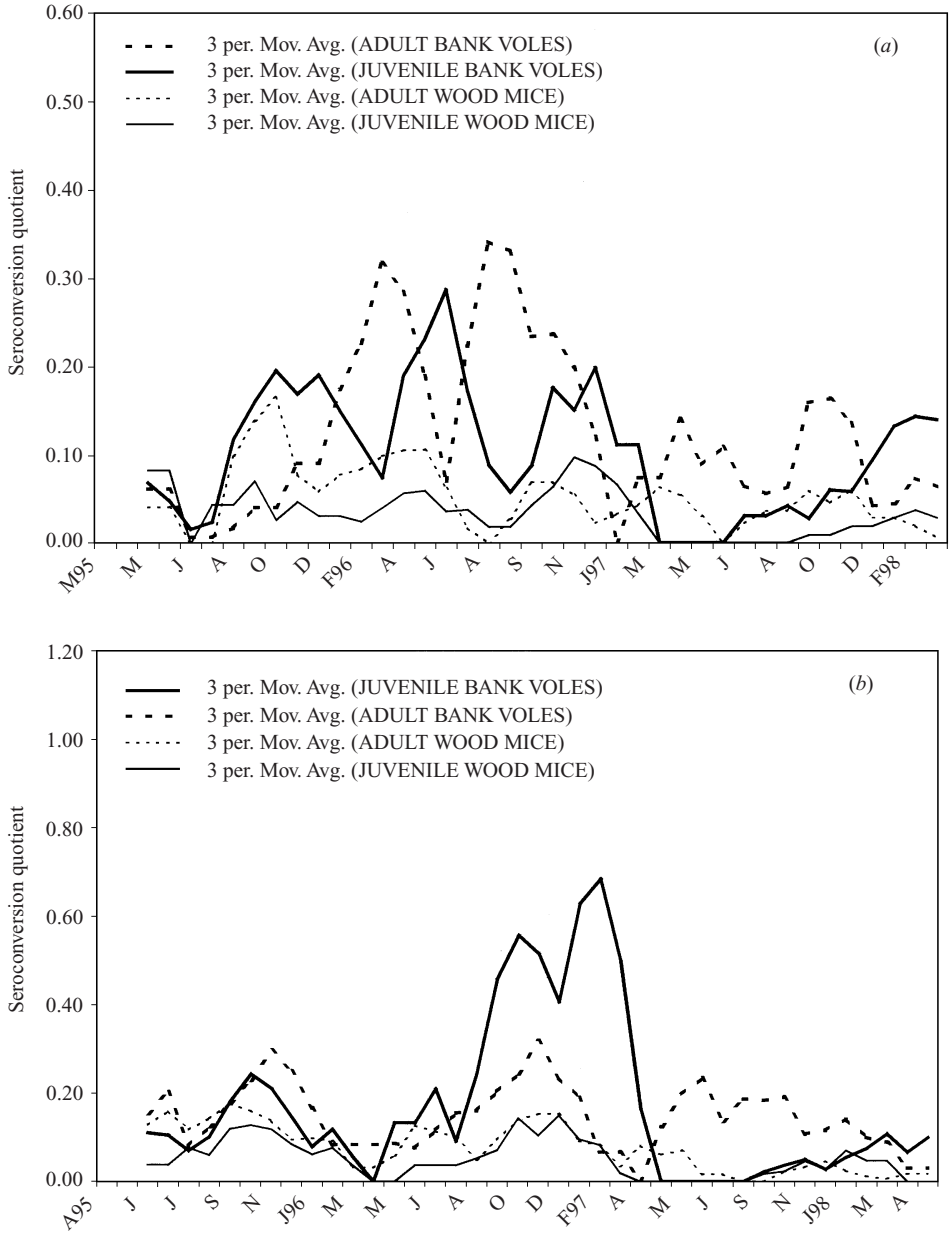

Fig. 6. Cowpox seroconversion quotient in bank voles and wood mice in (*a*) Manor Wood (March 1995–March 1998); (*b*) Rake Hey (April 1995–April 1998).

endemic disease in natural wildlife populations. Analysis of longitudinal data allow long term patterns of infection to be identified at the individual and population level, including the effect of species, sex and age differences. This information can then be used in models to predict disease incidence in host populations. For diseases with a zoonotic potential these data can be used further to predict potential risk and minimize incidence of human disease [12, 28, 29].

The two woodland populations of wild rodents in this study are typical of those found throughout Britain [30]. The population estimates for bank voles and wood mice based on minimum number alive were consistent with previous findings from other studies [31–34] with bank vole numbers peaking in late summer and early autumn and wood mice numbers peaking in late autumn and early winter. The numbers of bank voles and wood mice at Manor Wood were similar, whereas in Rake Hey the number of wood mice was consistently higher than the number of bank voles. This may have been connected to habitat differences between the two sites. Bank voles tended to be confined to areas with good ground cover from low vegetation at Rake Hey, whereas in more open

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areas only wood mice were caught. The level of cover for Manor Wood tended to be more consistent throughout the wood and both species were caught throughout the 1 hectare grid (S. M. Hazel, unpublished observations). Any possible effects of trapping and sampling on the animals were likely to have been negligible, since studies carried out elsewhere have shown that even when animals are anaesthetized and blood sampled by more invasive methods, such as from the orbital sinus, the effect on survival is negligible [35, 36]. Differences in the minimum number alive estimates for males and females, however, may have been attributable to trapping bias caused by males being trapped more easily, because they tend to encounter more traps in their range than females [33].

The numbers of cowpox antibody-positive bank voles and wood mice did not differ greatly at either site, but there was a much higher seroprevalence in bank voles than wood mice. Seroprevalence was observed to vary considerably for both species at each site over time. Many studies of infectious disease in wild mammals rely on intermittent, 'snapshot' samples of seroprevalence or disease prevalence [37] or may cull animals from the population at intervals for testing which can itself have profound effects on disease dynamics [38]. The data presented here, however, illustrate that such intermittent samples, even if taken in the same limited number of months each year, would not reflect the changing prevalences observed within and between years. Moreover, antibody prevalence, although widely used to study infection in natural populations, may also be misleading, because antibody cannot be taken as an indication of current infection, as stressed recently for hantavirus infection by Alexeyev and colleagues [39]. By contrast, in the current study the individual identification of each animal meant that it was possible to detect, using the longitudinal data sets, when an individual animal seroconverted. Furthermore, using PCR, viral DNA has been detected in blood cell samples in the month prior to seroconversion and in the month of seroconversion, and it has been shown in controlled experiments that viral DNA can be detected up to 4 weeks post infection [25]. Hence, by detecting the time of seroconversion we also identify the period over which the animal is actually infected with cowpox virus. In this way it is possible to determine the current infectious status of every individual and the population as a whole [40].

The number of seroconverting animals therefore offers the best indication of the dynamics of the virus within the wild rodent population. For a zoonotic disease this information is particularly pertinent since it indicates when most animals are likely to be infectious and so helps identify periods of greater risk to human health. Cowpox incidence in cats and humans is reported to be greatest in late summer and early autumn [13]. From Figure 3, it can be seen that this period coincides with peak numbers seroconverting in both species.

The similarity in the number of animals in both species which seroconverted suggests that the two species were of similar importance as reservoirs of cowpox virus. The seroconversion quotients, however, indicate that cowpox has a greater potential role to play in bank voles than wood mice, as bank voles had higher quotients. Moreover, in controlled experiments, bank voles are more susceptible to infection than wood mice [22].

Two important steps in developing an understanding of the role of a pathogen in a natural population are, first, understanding the dynamics of the transmission of the pathogen within the host population, and second, determining the effect of the pathogen, if any, on host population dynamics. With regard to the first, analysis of the transmission dynamics in the bank vole populations suggests that the numbers of susceptible and infectious animals can be used to predict numbers of newly-infected bank voles using standard epidemiological models [40, 41] though with frequency- not density-dependent transmission as conventionally assumed. A similar result has been obtained for cowpox in wood mice, though with little evidence of transmission between species [40]. The first step can therefore be made for this system. Investigating the possible effects of the pathogen on host dynamics will be the subject of future analyses.

Differences were also observed between males and females. The greater number of males seroconverting may in itself have been a trapping artifact reflecting the greater number of males caught overall. The higher proportions of males seroconverting, however, suggest, first, that males are likely to be more important as a reservoir for cowpox virus, and second, that cowpox virus has a greater potential role to play in males than females. These differences suggest either that females were less susceptible to infection or that their behaviour made them less likely to encounter infectious individuals. Since there is no evidence in experiments that females are less susceptible to infection [23], differences in behaviour are more strongly implicated. During the breeding season, when

bank vole and wood mouse populations are increasing, the territorial behaviour of individuals also increases as densities become greater. Females tend to defend discrete territories while males tend to overlap female territories. The males therefore encounter more conspecifics and are also likely to be involved in territorial disputes [42, 43]. Differences in pathogen incidence between sexes of the same species has also been reported for hantavirus infection in *Peromyscus* sp., where differences were also attributed to territorial aggression, longer survival of males and greater breadth of travel [44].

The data on the effect of age suggest that overall young and adult animals are equally likely to contract cowpox in both species. This is in spite of the fact that in experiments, young bank voles are much more susceptible to cowpox infection than older animals (unpublished observations). This emphasizes, again, that the probabilities of animals contracting infections in natural population reflect both their intrinsic susceptibilities and their patterns of behaviour.

Cowpox prevalence was consistently higher at Rake Hey than at Manor Wood. Between-site variation has been observed in studies of other infections, where sites separated geographically have shown variation in the incidence of an endemic disease in wild rodents. In a study in central Arizona, hantavirus incidence in *Peromyscus* sp., varied between sites 0·6 km apart, and appeared to be associated with the pattern of vegetation [44]. In the present system, too, the relationship between habitat, rodent distribution, disease incidence and transmission dynamics of the disease is worthy of further investigation.

Overall, it is clear that by monitoring natural populations longitudinally, it is possible to establish the dynamics of pathogens circulating in them more clearly. The information derived has applications for conservation, understanding the nature of host–pathogen relationships and establishing zoonotic disease risk.

ACKNOWLEDGEMENTS

We are grateful for grant support from NERC (GR3/9304). J. C. was supported by a BBSRC Veterinary Research Fellowship. K. B. and R. C. were supported by NERC studentships. This work was carried out under Home Office Project Licence 40/01038.

REFERENCES

1. Grenfell BT, Dobson AP. Ecology of infectious diseases in natural populations. Cambridge: Cambridge University Press, 1995.
2. Flowerdew JR, Trout RC, Ross J. Myxomatosis and ecological effects in the United Kingdom. Rev Sci Tech Off Int Epiz 1992; **11**: 1109–13.
3. Osterhaus ADME, De Swart HW Vos, Ross PS, Kenter MJH, Barrett T. Morbillivirus infections of aquatic mammals: newly identified members of the genus. Vet Microbiol 1995; **44**: 219–29.
4. Swinton J, Harwood J, Grenfell BT, Gilligan CA. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. J Anim Ecol 1998; **67**: 54–68.
5. Mihok S, Turner BN, Iversen SL. The characterization of vole population dynamics. Ecol Monog 1985; **55**: 399–420.
6. Laine K, Henttonen H. The role of plant production in microtine cycles in northern Fennoscandia. Oikos 1983; **40**: 407–18.
7. Baxby D. Is cowpox misnamed? – a review of 10 human cases. BMJ 1977; **1**: 1379–81.
8. Baxby D, Bennett M, Getty B. Human cowpox; a review based on 54 cases, 1969–93. Br J Dermatol 1994; **131**: 598–607.
9. Mills JN, Ellis BA, Kelly KT, et al. A longitudinal study of Junin virus activity in the rodent reservoir of Argentine hemorrhagic fever. Am J Trop Med Hyg 1992; **47**: 749–63.
10. Fenner F. Human monkeypox. In: Morse SS, ed. Emerging viruses. New York: Oxford University Press, 1993: 176–83.
11. Brown DWG. Threat to humans from virus infections of non-human primates. Rev Med Virol 1997; **7**: 239–46.
12. Mills JN, Childs JE. Ecological studies of rodent reservoirs: their relevance for human health. Emerg Infect Dis 1998; **4**: 529–37.
13. Baxby D, Bennett M. Cowpox virus. In: Granoff A, Webster RG, eds. Encyclopedia of virology. London: Academic Press, 1994: 261–67.
14. Boulanger D, Crouch A, Brochier B, et al. Serological survey of orthopoxvirus infection of wild mammals in areas where a recombinant rabies virus is used to vaccinate foxes. Vet Rec 1996; **138**: 247–9.
15. Marennikova SS, Maltseva NN, Korneeva VI, Garanina NM. Outbreak of pox disease among carnivora (Felidae) and Edentata. J Infect Dis 1977; **135**: 358–66.
16. Tsanava SA, Sakvarelidze LA, Shelukhina EM. Serologic survey of wild rodents in Georgia for antibodies to orthopoxviruses. Acta Virol 1989; **33**: 91.
17. Lvov SD, Gromashevskyi VL, Marennikova SS, et al. Poxvirus isolation from *Microtus oeconomus* Pal. 1776 in Colsky peninsula. Vop Virus 1988; **1**: 92–4.
18. Tryland M, Sandvik T, Mehl R, Bennett M, Traavik T, Olsvik O. Serosurvey for orthopoxviruses in rodents and shrews from Norway. J Wildl Dis 1998; **34**: 240–50.

19. Kaplan C, Healing TD, Evans N, Healing L, Prior A. Evidence of infection by viruses in British field rodents. J Hyg 1980; **84**: 285–94.
20. Crouch AC, Baxby D, McCracken CM, Gaskell RM, Bennett M. Serological evidence for the reservoir hosts of cowpox virus in British wildlife. Epidemiol Infect 1995; **115**: 185–91.
21. Chantrey J, Meyer H, Baxby D, et al. Cowpox: reservoir hosts and geographic range. Epidemiol Infect 1999; **122**: 455–60.
22. Bennett M, Crouch AJ, Begon ME, et al. Cowpox in British voles and mice. J Comp Pathol 1997; **116**: 35–44.
23. 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. Proc Roy Soc Lond B, 1997; **264**: 1457–61.
24. Krebs CJ. Demographic changes in fluctuating populations of *Microtus californicus*. Ecol Monog 1966; **36**: 239–73.
25. Chantrey J. Studies on the epidemiology of cowpox virus in its rodent reservoir hosts. Liverpool, England: University of Liverpool, 1999.
26. Innes DGL, Millar JS. Correlates of life-history variation in *Clethrionomys* and *Microtus* (Microtinae). Ecoscience 1994; **2**: 329–34.
27. Fernandez FAS, Evans PR, Dunstone N. Population-dynamics of the wood mouse *Apodemus sylvaticus* (Rodentia, *Muridae*) in a sitka spruce successional mosaic. J Zool Lond 1996; **239**: 717–30.
28. Mills JN, Yates TL, Ksiazek TG, Peters CJ, Childs JE. Long-term studies of hantavirus reservoir populations in the southwestern United States: Rationale, potential, and methods. Emerg Infect Dis 1999; **5**: 95–101.
29. Perez-Eid C, Hannoun C, Rodhain F. The alsatian tick-borne encephalitis focus: presence of the virus among ticks and small mammals. Eur J Epidemiol 1992; **8**: 178–86.
30. Macdonald DW, Barrett P. Mammals of Britain and Europe. Harper Collins Publishers, 1993.
31. Flowerdew JR. Field and laboratory experiments on the social behaviour and population dynamics of the wood mouse (*Apodemus sylvaticus*). J Anim Ecol 1974; **43**: 499–511.
32. Gurnell J. Seasonal changes in numbers and male behavioural interaction in a population of wood mice, *Apodemus sylvaticus*. J Anim Ecol 1978; **47**: 741–55.
33. Flowerdew JR. The population dynamics of wood mice and yellow-necked mice. Symp Zool Soc Lond 1985; **55**: 315–38.
34. Alibhai SK, Gipps JHW. The population dynamics of bank voles. Symp Zool Soc Lond 1985; **55**: 277–313.
35. Swann DE, Kuenzi AJ, Morrison ML, DeStefano S. Effects of sampling blood on survival of small mammals. J Mammal 1997; **78**: 908–13.
36. Parmenter CA, Yates TL, Parmenter RR, et al. Small mammal survival and trapability in mark-recapture monitoring programs for hantavirus. J Wildl Dis 1998; **34**: 1–12.
37. Jay M, Asher M, Chomel B, et al. Seroepidemiological studies of hantavirus infection among wild rodents in California. Emerg Infect Dis 1997; **3**: 183–90.
38. Graham TB, Chomel BB. Population dynamics of the deer mouse (*Peromyscus maniculatus*) and Sin Nombre Virus, California Channel Islands. Emerg Infect Dis 1997; **3**: 367–70.
39. Alexeyev OA, Ahlm C, Elgh F, et al. A minority of seropositive wild bank voles (*Clethrionomys glareolus*) show evidence of current Puumala virus infection. Epidemiol Infect 1998; **121**: 419–25.
40. Begon M, Hazel SM, Baxby D, et al. Transmission dynamics of a zoonotic pathogen within and between wildlife host species. Proc Roy Soc Lond B. In press.
41. Begon M, Feore SM, Bown K, Chantrey J, Jones T, Bennett M. Population and transmission dynamics of cowpox in bank voles: testing fundamental assumptions. Ecol Letters 1998; **1**: 82–6.
42. Montgomery WI, Gurnell J. The behaviour of *Apodemus*. Symp Zool Soc Lond 1985; **55**: 89–115.
43. Gipps JHW. The behaviour of bank voles. Symp Zool Soc Lond 1985; **55**: 61–87.
44. Abbott KD, Ksiazek TG, Mills JN. Long-term hantavirus persistence in rodent populations in Central Arizona. Emerg Infect Dis 1999; **5**: 102–12.