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Cowpox: reservoir hosts and geographic range

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SUMMARY

It is generally accepted that the reservoir hosts of cowpox virus are wild rodents, although direct evidence for this is lacking for much of the virus's geographic range. Here, through a combination of serology and PCR, we demonstrate conclusively that the main hosts in Great Britain are bank voles, wood mice and short-tailed field voles. However, we also suggest that wood mice may not be able to maintain infection alone, explaining the absence of cowpox from Ireland where voles are generally not found. Infection in wild rodents varies seasonally, and this variation probably underlies the marked seasonal incidence of infection in accidental hosts such as humans and domestic cats.

INTRODUCTION

In his *Inquiry* [1], Edward Jenner gave not only the first account of what became known as smallpox vaccination, but also a description of the clinical signs and, in his view, likely epidemiology of cowpox in humans and cattle. He also raised and discussed two questions about the epidemiology of cowpox which until recently have remained unanswered. What is the reservoir host of cowpox? And why is there no cowpox in Ireland? In this report, we review the evidence for rodents being the reservoir hosts of cowpox virus, and, for the first time, produce direct evidence that cowpox is indeed endemic in bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) in Great Britain and, by inference, much of Europe. We then use these and other data to suggest why cowpox does not exist naturally in Ireland, and discuss the implications of this for our understanding of the ecology of cowpox virus overall.

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Jenner himself doubted that cattle were the main host of cowpox [1], a view confirmed by modern serosurveys [2]. He suggested the horse instead [1]. There is little doubt that an orthopoxvirus did once circulate amongst European horses [3, 4] and that the horse virus may have contributed to the development of the modern smallpox vaccine, vaccinia virus [3–5]. However, there is no evidence that horses are, or have ever been, commonly infected with cowpox virus. The most commonly recognized host of cowpox virus is the domestic cat [6, 7], which is also a frequent liaison host for human infection [8]. In addition, cowpox virus has been isolated from a variety of zoo animals [9], and occasionally from domestic dogs [7, 8].

To our knowledge, fully characterized cowpox virus has only been isolated from western Eurasia, in an area approximately bounded by Norway and Northern Russia, Moscow, Turkmenia, Northern Italy, France and Great Britain. The epidemiology of cowpox in 'accidental' hosts, particularly humans and domestic cats, combined with the known experimental host range and limited geographic range, suggest that wild mammals, possibly rodents, are the

reservoirs of infection [2, 6, 8]. Some direct evidence for this comes from the eastern extent of the virus's range. In Turkmenia, antibody to an orthopoxvirus was detected in 15% of suslicks (*Rhombomys opimus*) and 18% of gerbils (*Citellus fulvus*), and cowpox virus was isolated from the tissues of 3/1275 rodents tested [11]. Antibody has also been detected in 9% of gerbils (*Meriones libicus*) in Georgia [12]. While this combination of serological and isolation data suggests that the virus is at least in part maintained in these species, their limited geographic distribution means that suslicks and gerbils cannot be the reservoir hosts elsewhere in Europe.

Cowpox virus has also been isolated from laboratory rats in Russia [13], from which it spread to zoo animals and humans [11, 14]. However, wild rats are unlikely to be true reservoir hosts for two reasons. First, if cowpox were endemic in rats, then one might expect cowpox to be found world-wide, rather than limited to Western Eurasia; a similar argument, of course, applies to domestic animal hosts, such as cattle. Second, there is little evidence for their infection in the field [12, 15]. Cowpox virus has also been isolated from a root vole (*Microtus oeconomus*) in Northern Russia [16], but, like rats, this species has a wider geographic range than cowpox virus. Furthermore, no serological survey has been done in this species.

Serological surveys in Great Britain, the Low Countries, France, Austria and Norway have produced evidence of orthopoxvirus infection in both voles (*Microtus* and *Clethrionomys* species), and wood mice [17–20]. In the Norwegian study, antibody was also detected in lemmings (*Lemmus lemmus*), and active infection detected by PCR of various tissues from seropositive species [20]. However, virus has not been isolated from Western European rodents taken from the wild. In fact, although susceptible to infection with low doses (< 1 p.f.u.) of cowpox virus, it has so far proved difficult to re-isolate from voles and wood mice, even after experimental infection [21]. Furthermore, the antibody and PCR assays used have only allowed identification of the causative virus(es) to the genus level. Identification of the virus species is important since there is increasing evidence of orthopoxviruses other than cowpox infecting Eurasian wildlife, particularly in the East and South [22–24].

Orthopoxvirus antibody has also been detected in wild red foxes (*Vulpes vulpes*) in Eastern Europe [25, 26]. This may be due to cowpox virus infection, but farmed foxes are also known to be susceptible to infection with ectromelia virus [23], and red foxes were found to be relatively resistant to infection with a British strain of cowpox virus [19].

In this report we describe briefly the results of a longitudinal serological study of orthopoxvirus infection in two British wild rodent populations, and the use of PCR and sequencing to confirm that the antibody was caused by infection with cowpox virus. This combination of serology with direct and specific demonstration of virus infection demonstrates for the first time that cowpox virus infection is endemic in these species. Furthermore, we demonstrate that, unlike in Great Britain and continental Europe, there is no evidence of infection in wood mice in Ireland.

MATERIALS AND METHODS

Rodent samples

For the longitudinal study, small wild mammals (mainly bank voles and wood mice, but also occasional field voles, *Microtus agrestis*) were captured, live, using Longworth small mammal traps at two woodland sites on the Wirral Peninsula, North West England. Two hundred traps were set at each site for 3 nights during 1 week in every 4. Two traps were placed at each node of a 100×100 m area in each wood permanently marked out as a 10×10 m grid. Individual animals were identified using subcutaneous transponders (Avid). Blood samples were collected from the tip of the tail of each animal the first time it was caught in each trapping week.

Of the species that serosurveys and/or PCR indicate might be cowpox reservoirs, only the wood mouse occurs naturally in Ireland [27]. Further sera were therefore collected from 149 wood mice from three sites in North Down and three in South Down, Northern Ireland. These areas were chosen as being remote from the area in south west Ireland where the bank vole has relatively recently been introduced. Blood samples from these animals were collected by terminal exsanguination.

Serology

Serum antibody to cowpox virus was detected using an immunofluorescence assay as described previously [18, 21].

PCR and sequence analysis

After removal of serum by centrifugation, blood cell pellets were stored at -20 or -80 °C, and DNA

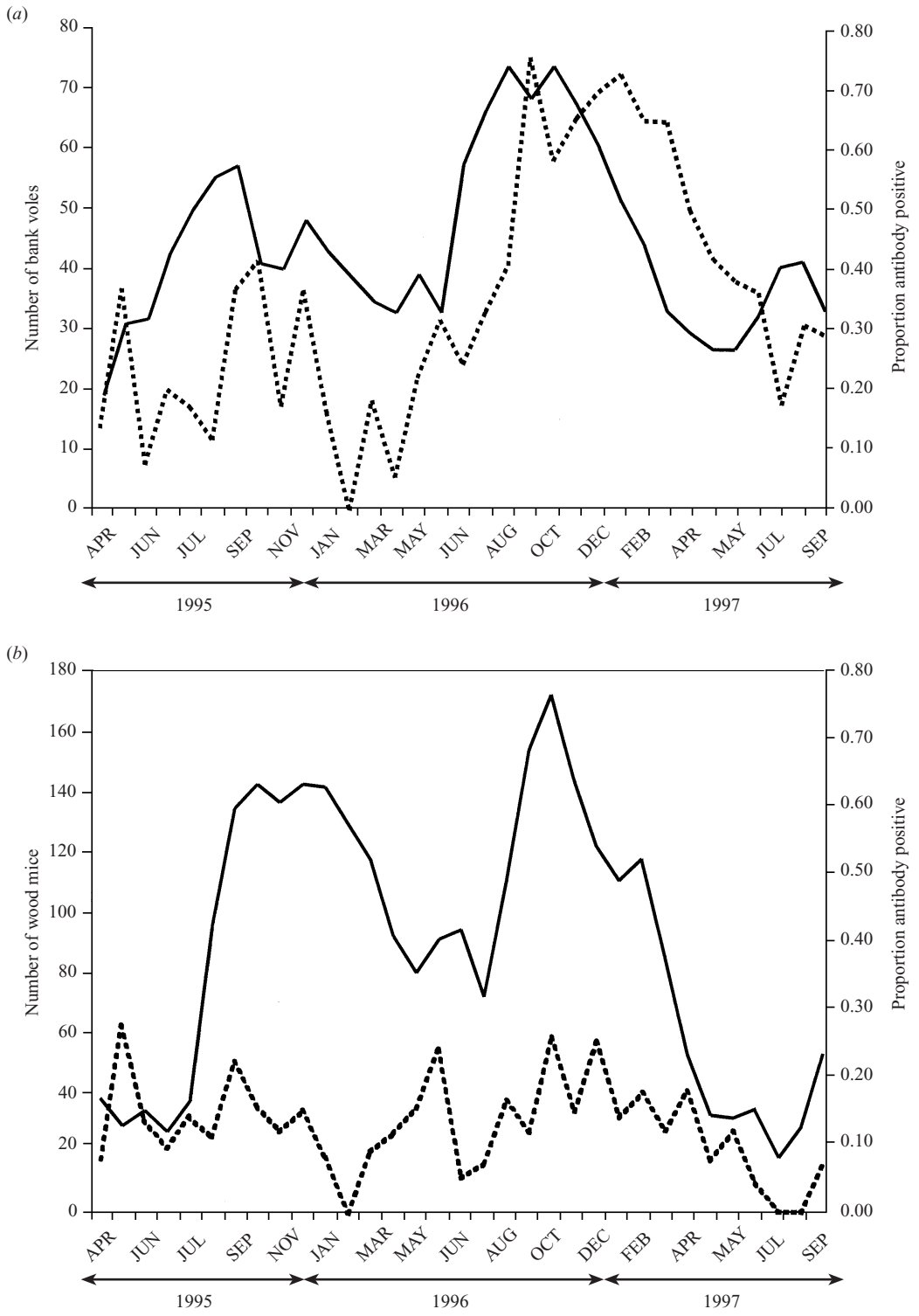

Fig. 1. (*a*) Seasonal variation in cowpox virus infection in wild bank voles at a wood in North West England. Antibody prevalence (number seropositive/number caught that month) · · · · ·, and total population size (minimum number alive) ——. (*b*) Variation in cowpox virus infection in wild wood mice in the same wood as (*a*). Antibody prevalence · · · · · and population size —— calculated as in (*a*).

extracted as described elsewhere [28]. DNA was screened using a nested PCR targeted at the thymidine kinase gene. The first round of the TK–PCR was based on that of Thomas and others [29], using the primers VTK1 (ATGAACGGCGGACATATTCA-GTTG) and VTK-2 (TTATGAGTCGATGTAACA-

CTTTCT), but was followed by a nested PCR on product from the first reaction using the primers NTK1 and NTK2 (ATAGCTCAATATAAATGCG-TGAC and GCATTTCATACACACAGCAGTTA respectively). As the TK gene sequence varies little between orthopoxviruses, the PCR-positive samples were further subjected to a PCR directed at the orthopoxvirus fusion protein gene [30], sequence analysis of which permits recognition of orthopoxvirus species, and can sometimes identify clusters within cowpox virus (to be published elsewhere). Samples were therefore subjected to a nested PCR using the outer primers FP1 and FP2 (ATGGACGGAACT-CTTTTCCC and TAGCCAGAGATATCATAGCC-GC respectively), and then a pair of internal primers, FP3 and FP4 (CTGAATTTTTCTCTACAAAGGC-TGCTAA and TCAGCGTGATTTTCCAACCTAA-ATAG respectively). The nucleotide sequences of amplicons from the fusion gene-PCR were determined (ABI, automated sequencing) and aligned using the Wisconsin GCG [31] software package. Phylogenetic relationships were determined using the PHYLIP [32] software packages.

RESULTS

The results of the longitudinal serological survey for one wood are shown in Figure 1: similar patterns were seen in both woods. A clear seasonality in the prevalence of antibody, and, by implication seroconversion, was observed in bank voles. The prevalence was fairly stable at around 10% for much of the year, but increased to almost 80% in late summer and early autumn when the size of the host population also peaked. Variation in both infection rates and host dynamics also occurred between years. Similar, but less marked trends, were seen in wood mice, although in this species the prevalence only once reached 27%. Only 12 field voles were caught in the woods during the study period, 11 of which were antibody-positive.

A TK–PCR has previously been developed to study the experimental pathogenesis of cowpox (to be published elsewhere), and in preliminary experiments was found to detect a cell-associated viraemia which persisted for approximately 1 week in captive wood mice and up to 4 weeks in bank voles. Here, the TK–PCR was therefore applied only to selected blood samples collected from wild rodents. From the field study, 88 blood cell pellets from 61 bank voles, and 86 samples from 63 wood mice, were identified which had been collected between 1 month before and 1 month after the time of seroconversion and stored at −80 °C. Eight of these samples, five from bank voles and three from wood mice, were positive by TK–PCR. The TK–PCR-positive samples were further subjected to a PCR directed at the orthopoxvirus fusion gene, and those from four bank voles and two wood mice were positive. The sequences of all the fusion gene amplicons were compared with those of amplicons of (i) five British cowpox virus isolates from domestic cats, (ii) cowpox virus strain Brighton (the international type strain), (iii) ectromelia virus, and (iv) vaccinia virus Lister. As can be seen from the tree in Figure 2, the sequence analysis clearly demonstrates that the virus infecting the voles and wood mice was, indeed, cowpox virus.

All the sera from wood mice collected in Ireland were tested for cowpox antibody by immunofluorescence assay, and all were negative.

DISCUSSION

By a combination of serology and PCR, we have demonstrated clearly for the first time that the reservoir hosts of cowpox virus in Great Britain include the bank vole and, possibly, the wood mouse. Given that this and previous [17–19] surveys, combined with experimental work [21], have shown that field voles are both susceptible to cowpox and frequently seropositive in the wild, but that antibody is rare in other species, we suggest that the British reservoir species should also include field voles.

The clear autumn peak of infection in voles and, to a lesser extent, wood mice, probably underlies the marked autumnal incidence of cowpox in both domestic cats and man [6–8]. Preliminary analysis of the bank vole data suggests that infection is strongly influenced by population size, and that the incidence of infection (as determined by seroconversion) can be predicted from knowledge of prior infection rates and the number of susceptibles available [33]. Further field data are being collected for more thorough analysis.

We, like Jenner, are unaware of any reports of cowpox in any species in Ireland. Jenner attributed its absence there to different social and husbandry practices in Ireland than in England [1]. However, of the putative reservoir hosts, only the wood mouse is native to Ireland, and all those tested in this study had no detectable cowpox antibody. Wood mice are less susceptible to experimental infection than bank voles

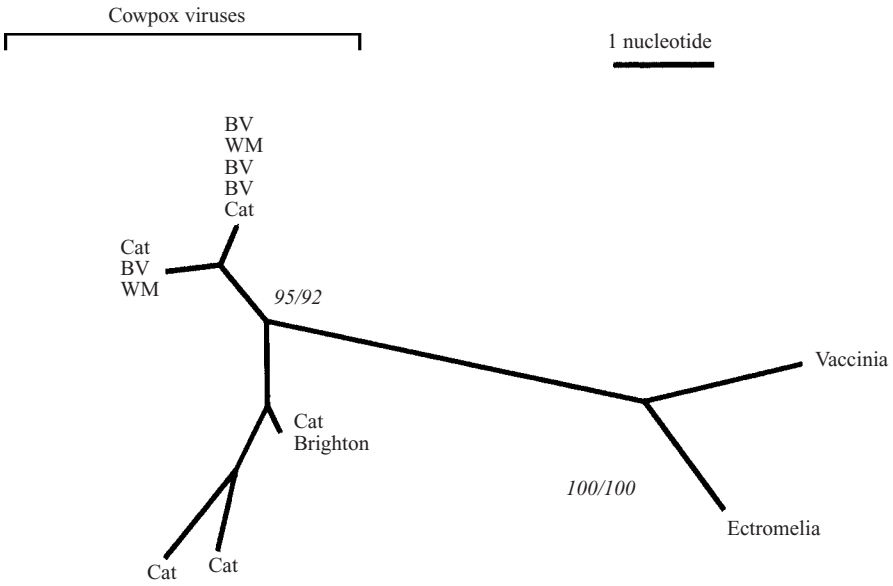

Fig. 2. Neighbour-joining tree of nucleotide sequences of 120 bp orthopoxvirus fusion gene amplicons from 4 bank voles (BV), 2 wood mice (WM), 5 domestic cat cowpox isolates, cowpox virus Brighton (the international strain), ectromelia virus and vaccinia virus. In the tree shown, distances were determined using the Jukes Cantor method, but similar relationships between isolates were seen using other distance methods and by parsimony analysis. Numbers beside internal branches indicate bootstrap probabilities (100 replicates) for Jukes Cantor distance/parsimony trees where both values were $> 50\%$.

[21], and they appear to have a shorter viraemia after experimental infection. Furthermore, this study shows the seroprevalence in wood mice to be generally less than in bank voles. These factors might together make wood mice less likely to maintain endemic infection in the absence of other more competent species. Thus, wood mice may not, in fact, be true 'reservoir' hosts even on the British mainland, but merely common 'accidental' hosts. We hope to collect and test sera from the small population of bank voles recently introduced into South West Ireland, and from sympatric wood mice, to see if cowpox virus has been introduced with the voles. Investigation of near and distant wood mouse populations would also provide the opportunity for determining the spread of the virus through a naïve population, and any effects on host population dynamics.

Thus, we have directly demonstrated cowpox virus in British wild rodents. This, together with the high seroprevalence, confirms that these are true reservoir hosts. Furthermore, the absence of antibody in the one potential reservoir species found in Ireland, confirms and explains the absence of cowpox there. Indeed, the geographic range of bank and field voles fits well the limited host range of cowpox virus generally, with no reports of cowpox from areas such as the Iberian Peninsula or Southern Italy, where these species are not found (although wood mice are). The geographic range of the virus may be extended by its ability to be maintained in some other species, for example suslicks and gerbils in Turkmenia, or it may be that these species, perhaps like the wood mouse, require the occasional re-introduction of virus from local voles.

Cowpox virus provides a readily studiable model for investigating the relationship between endemic infectious agents and their natural hosts. In particular, it may provide a model relevant to other zoonotic orthopoxviruses with wildlife reservoirs, such as monkeypox. We are continuing to collect data from the two main sites described in this report, with the aim of investigating further the relationship between cowpox and host population dynamics, and between cowpox and other infectious agents in the same populations.

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REFERENCES

1. Jenner E. An inquiry into the causes and effects of the variolae vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow pox. London: Sampson Low, 1798.
2. Baxby D. Is cowpox misnamed? A review of 10 human cases. BMJ 1977; **i**: 1379–81.
3. Baxby D. Jenner's smallpox vaccine. The riddle of vaccinia virus and its origin. London: Heinemann Educational Books, 1981.
4. Maceachran D. Horse-pox. Vet J 1880; **10**: 381–4.
5. Depaul M. Expériences faites à l'Académie Impériale de Médecine avec le cow-pox ou vaccin animal. Paris: Ballières, 1868.
6. Bennett M, Gaskell CJ, Baxby D, Gaskell RM, Kelly DF, Naidoo J. Feline cowpox virus infection. J Small Anim Pract 1990; **31**: 167–73.
7. Bomhard D, von Pfleghaar S, Mahnel H. Zur Epidemiologie, Klinik, Pathologie und Virologie der Katzen-Pocken-Infektion. Kleintierpraxis 1992; **37**: 219–30.
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. Pilaski J, Rosen-Wölff XX. Poxvirus infection in zoo-kept mammals. In: Darai G, ed. Virus diseases in laboratory and captive animals. Boston: Martinus Nijhoff Publishing, 1987: 83–100.
10. Smith KC, Bennett M, Garrett DC. Skin lesions caused by poxvirus infection in a dog. Abstract in Proceedings of the European Society of Veterinary Pathology Conference, 1998.
11. Marennikova SS, Shelukhina EA, Efremova, EV. New outlook on the biology of cowpox. Acta Virol 1984; **57**: 461–4.
12. Tsanava SA, Sakvarelidze LA, Shelukhina EM. Serologic survey of wild rodents in Georgia for antibodies to orthopoxviruses. Acta Virol 1989; **33**: 91.
13. Marennikova SS, Shelukhina EM, Fimina V. Pox infection in white rats. Lab Anim 1978; **12**: 33–6.
14. Marennikova SS, Maltseva NN, Korneeva VI, Garanina NM. Outbreak of pox disease among carnivora (Felidae) and Edentata. J Infect Dis 1977; **135**: 358–66.
15. Webster JP, Macdonald DW. Parasites of brown rats (*Rattus norvegicus*) on UK farms. *Parasitol* 1995; **111**: 247–55.
16. Lvov SD, Gromashevskyi VL, Marennikova SS, et al. Poxvirus isolation from *Microtus oeconomus* Pal. 1776 in Colsky peninsula. Vop Virus 1988; **1**: 92–4.
17. 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.
18. 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.
19. Boulanger D, Crouch A, Brochier B, et al. Serological survey of orthopoxvirus infection of mammals in areas where a recombinant rabies virus is used to vaccinate foxes. Vet Rec 1996; **138**: 247–9.
20. 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.
21. Bennett M, Crouch AJ, Begon ME, et al. Cowpox in British voles and mice. J Comp Path 1997; **116**: 35–44.
22. Tantawi HH, Zaghloul TM, Zakaria M. Poxvirus infection in a rat (*Rattus norvegicus*) in Kuwait. Int J Zoonoses 1983; **10**: 28–32.
23. Mahnel H, Holejsovsky J, Bartak P, Czerny C-P. Kongenitale Ektromelie bei pelzttieren durch orthopoxvirus muris. Teirarztliche Praxis 1993; **21**: 469–72.
24. Baxby D, Hill BJ. Characteristics of a new poxvirus isolated from Indian buffaloes. Arch Gesamte Virusforsch 1971; **35**: 70–9.
25. Henning K, Czerny CP, Meyer H, Muller T, Kramer M. A seroepidemiological survey for orthopoxvirus in the red fox (*Vulpes vulpes*). Vet Microbiol 1995; **43**: 251–9.
26. Muller T, Henning K, Kramer M, Czerny CP, Meyer H, Ziedler K. Seroprevalence of orthopoxvirus-specific antibodies in red foxes (*Vulpes vulpes*) in the federal state Brandenburg, Germany. J Wildl Dis 1996; **32**: 348–53.
27. Corbet GB, Harris S. The handbook of British mammals, 3rd edn. Oxford: Blackwell Science, 1991.
28. Livesley MA, Carey D, Geru L, Nuttall P. Problems of isolating *Borrelia burgdorferi* from ticks collected in United Kingdom foci of Lyme-disease. Med Vet Entomol 1994; **8**: 172–8.
29. Thomas I, Brochier B, Languet B, et al. Primary multiplication site of the vaccinia-rabies glycoprotein recombinant virus administered to foxes by the oral route. J Gen Virol 1990; **71**: 37–42.
30. Meyer H, Osterrieder N, Czerny C-P. Identification of binding-sites for neutralizing monoclonal-antibodies on the 14-kda fusion protein of orthopoxviruses. Virol 1994; **200**: 778–83.
31. Program Manual, Wisconsin Package, version 8 Madison Wisconsin: Genetics Computer Group, 1994.
32. Felsenstein, J. PHYLIP: phylogenetic inference package (version 3.2) *Cladistics* 1989; **5**: 164–6.
33. Begon M, Feore S, Bown K, Chantrey J, Jones T, Bennett M. The population dynamics of cowpox virus infection in bank voles: testing fundamental assumptions. Ecology Lett 1998; **1**: 82–6.