The effect of cowpox virus infection on fecundity in bank voles and wood mice

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SUMMARY

Although epidemic infectious diseases are a recognized cause of changes in host population dynamics, there is little direct evidence for the effect of endemic infections on populations. Cowpox virus is an orthopoxvirus which is endemic in bank voles (*Clethrionomys glareolus*), wood mice (*Apodemus sylvaticus*) and field voles (*Microtus agrestis*) in Great Britain. It does not cause obvious signs of disease nor does it affect survival, but in this study we demonstrate experimentally that it can reduce the fecundity of bank voles and wood mice by increasing the time to first litter by 20–30 days. The pathogenic mechanisms causing this effect are at present not known, but this finding suggests that natural subclinical infection could have a considerable effect on the dynamics of wild populations.

1. INTRODUCTION

Following a long period of neglect, the potential role of parasites and pathogens in the population dynamics of their hosts is now well recognized (Grenfell & Dobson 1995). Theoretical studies (e.g. Anderson & May 1978, 1981) have been highly influential in bringing about this change of perception. However, empirical confirmation of theoretical possibilities has remained rare (Dobson & Hudson 1995; Gulland 1995). For directly transmitted microparasites (bacteria, viruses and protozoa) of wild vertebrates especially, studies have tended to be either opportunistic investigations of epidemics, with measurable increases in mortality, e.g. myxomatosis in rabbits (Flowerdew *et al.* 1992) and seal morbillivirus (Harwood *et al.* 1989), or evocations of endemic infections where other effects on demographic variables appear unable to explain observed population time series, e.g. Laine & Henttonen (1983) and (Mihok *et al.* 1985). Few researchers seriously doubt that most natural populations support infections of microparasites which are endemic but have no obvious or widespread effects on mortality. The importance of such infections for the dynamics of their hosts, however, remains profoundly uncertain.

At least two types of data would be of particular value: (i) documentation of a demographic effect of a pathogen from the analysis of field data, and (ii) direct demonstration of an effect of the pathogen on a process of clear demographic importance. Here, we report the effects on fecundity and the lack of an effect on survival, under experimental conditions, of a viral disease known to be endemic in two species of wild rodents. This is part of a larger study which is also examining host–pathogen population dynamics in the field.

The hosts concerned are bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*), and the pathogen is cowpox virus. Cowpox virus is a member of the genus *Orthopoxvirus*, and is endemic in Europe and some western states of the former USSR (Baxby & Bennett 1994). Although natural infection and disease occurs in cattle, man, domestic cats and various captive mammals in zoological collections (Zwart *et al.* 1971; Gibbs *et al.* 1973; Marennikova *et al.* 1977; Baxby *et al.* 1982; Pilaski 1988; Bennett *et al.* 1990; Baxby *et al.* 1994), such cases are relatively uncommon, and the reservoir hosts are generally accepted to be wild rodents. Antibody and, at a much lower prevalence, virus have been detected in wild ground squirrels (yellow suslicks) (*Citellus fulvus*) and gerbils (*Rhombomys opimus*, *Meriones libicus* and *Meriones meridianus*) in Turkmenistan and Georgia (Marennikova *et al.* 1984; Tsanava *et al.* 1989), from root voles (*Microtus oeconomus*) on the Kolskiy Peninsula in northern Russia (Lvov *et al.* 1988), and evidence of infection has been obtained by PCR (polymerase chain reaction) from various rodents in Norway (Sandvik & Tryland 1996). In Great Britain and parts of western continental Europe, a high prevalence of cowpox virus antibody has been detected in wild bank voles, field voles and wood mice (Kaplan *et al.* 1980;

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Crouch *et al*. 1995; Boulanger *et al*. 1996; authors' unpublished data).

We have previously demonstrated that bank voles and wood mice are susceptible to infection with between 2 and 20 plaque-forming units (PFUs) of cowpox virus, both by skin and oronasal infection (Bennett *et al*. 1997), and that such infection causes little obvious disease. Indeed, no specific clinical signs were observed after oronasal infection in either species. In this paper we describe further experiments which show that cowpox may, however, significantly affect the reproductive potential of bank voles and wood mice, and discuss the potential significance of this for the dynamics of wild populations.

2. MATERIALS AND METHODS

(*a*) *Virology and serology*

Stocks of low-passage cowpox virus, strain L97 (Gaskell *et al*. 1983; Naidoo *et al*. 1992), were prepared by single passage on fowl chorioallantoic membrane and stored at −80 °C until used. Virus infectivity was titrated on Vero cells. Serum samples were kept at −20 °C, and cowpox virus antibody was determined in an immunofluorescence (IF) assay essentially as described previously (Crouch *et al*. 1995), but using a 1 : 1 mixture of FITC-conjugated polyclonal anti-mouse and anti-rat IgG (Sigma). Sera were tested at dilutions of 1 : 20, 1 : 40 and 1 : 80, and titres of ⩾ 1 : 20 were taken as positive (Crouch *et al*. 1995; Bennett *et al*. 1997).

(*b*) *Animals*

The housing and maintenance of breeding colonies of bank voles and woodmice, originally obtained from Dr J. Clarke (Department of Zoology, University of Oxford, UK) have been described previously (Baker & Clarke 1987; Bennett *et al*. 1997). The colonies had no clinically apparent disease and were housed under semi-barrier or barrier conditions. No cowpox virus antibody has been detected in any animal other than those deliberately infected.

(*c*) *Experimental design*

Bank voles and wood mice were weaned at approximately 18 days old, and distantly-related, mixed-sex pairs were established in separate cages. Each pair of animals were both inoculated oronasally (Bennett *et al*. 1997) with 40 µl of either cell culture medium alone or medium containing 800 PFUs of cowpox: 20 µl were applied to the nostrils and 20 µl orally. Overall, 36 pairs of bank voles and 31 pairs of wood mice were inoculated with virus, and 33 pairs of bank voles and 29 pairs of wood mice with control medium.

General health was monitored daily. Occasional blood samples for serology were obtained from live animals from the tip of the tail; otherwise blood was collected by cardiac puncture under terminal anaesthesia. It is difficult to re-isolate virus from these species, and no clinical signs are seen after oronasal infection (Bennett *et al*. 1997), so antibody status at the end of the experiment was used to determine whether or not any animal had been successfully infected. The time to first litter was measured from the day each pair was inoculated to the day when the first litter was produced. Animals were kept until their first litter was weaned. A maximum of 120 days was allowed for the experiment as experience with the stock colonies indicates that pairs of animals that have not reproduced by then are unlikely to do so. Pairs of animals that failed to reproduce within a maximum period of 120 days were killed.

Table 1. *Effect of cowpox on the fecundity of bank voles and wood mice*

(*As determined by serology at end of experiment: m, male; f, female; +, antibody positive; −, antibody negative.)

species	treatment	infection status*	no. pairs	no. first litters produced	time to first litter (days): mean, range, (s.e.)	litter size: mean, range, (s.e.)
bank vole	virus inoculated	m + , f +	14	9	61.3, 31–104, (7.9)	3.22, 1–5 (0.4)
		m + , f−	4	2	84.5, 83–86 (1.5)	4, 4–4 (0)
		m−, f +	5	3	69.7, 34–114, (23.5)	4, 4–4 (0)
		m−, f−	13	5	38.6, 26–69, (7.9)	3.46, 1–5, (0.29)
	control	—	33	19	43.9, 28–101, (4.5)	3.39, 2–5, (0.22)
wood mouse	virus inoculated	m + , f +	19	13	74.7, 40–108, (7.2)	3.60, 1–5, (0.37)
		m + , f−	6	3	67, 38–90, (15.3)	2.3, 1–4, (0.88)
		m−, f +	4	3	82.7, 49–102, (16.9)	2.3, 1–4, (1.20)
		m−, f−	2	0	—	—
	control	—	29	14	55.9, 28–98, (5.1)	4.14, 2–6, (0.31)

3. RESULTS

The results are shown in table 1. Of those animals inoculated with virus, 37 out of 72 bank voles and 48 out of 62 wood mice developed an antibody response. No mock-infected animals produced antibody. No overt clinical signs were seen in any animals at any time. Thus, as in previous work (Bennett *et al*. 1997), infection had no demonstrable effect on either the mortality or morbidity of the hosts. There were no significant differences between any experimental groups in the proportion of pairs producing a litter, nor in the size of the litters produced. Differences were observed, however, in time to production of first litter.

In virus-infected bank vole pairs where both the male and the female developed antibody, 9 out of 14 produced litters at a mean time of 61 days after pairing. Among 33 mock-infected pairs, however, 19 produced litters, at a mean time of 43 days after pairing. This difference is significant ($t_{26} = 2.85$, $p < 0.05$). Out of 23 pairs in which any one or both parents were successfully infected, 14 produced litters, and the mean time to birth was 66 days. When compared with mock-infected voles, the difference in time to first litter is again significant ($t_{31} = 2.06$, $p < 0.01$).

From the 19 pairs of virus-infected wood mice, 13 produced litters at a mean time of 75 days after pairing, whereas 14 out of 29 pairs of mock-infected mice produced litters at a mean time of 56 days. This difference is significant ($t_{25} = 2.15$, $p < 0.05$). When all pairs in which one or both parents were infected are included in the analysis, the mean is also 75 days and the difference is more significant ($t_{31} = 2.33$, $p < 0.03$). Thus, under these conditions, cowpox virus infection delayed the production of the first litter by around 20–30 days in both host species.

In addition to the above groups of animals, 13 pairs of bank voles and two pairs of wood mice were inoculated with virus but did not become infected as determined serologically. The two pairs of wood mice did not produce litters, while the mean time to first litter among five pairs of voles was 39 days, which is very similar to that for mock-infected pairs.

4. DISCUSSION

The studies described here clearly demonstrate that oronasal infection with cowpox virus can reduce the reproductive potential of bank voles and wood mice without causing any overt disease or mortality. Although the natural routes and rates of transmission of cowpox virus amongst wild rodents remain unknown, this at least demonstrates the possibility that an endemic virus infection might significantly influence the dynamics of a wild mammal population without causing any other obvious signs of disease.

That no obvious morbidity, apart from the effect on fecundity, was seen in any animals in this study confirms the findings of earlier work (Bennett *et al*. 1997). No obvious signs of disease have been seen in wild bank voles or wood mice either, despite seroprevalence rates of up to 75% in bank voles and 24% in wood mice in some populations at certain times of year (authors' unpublished observations). Although the routes of infection for cowpox are uncertain in small rodents, oronasal infection is an important route of transmission for several poxviruses including smallpox virus in man, vaccinia virus in rabbits, ectromelia virus in laboratory mice, capripoxviruses in sheep and goats and avipoxviruses in birds (Fenner *et al*. 1988; Gledhill 1962; Westwood *et al*. 1966; Tripathy 1991; Kitching 1994) and cowpox virus is known to replicate to high titres, and sometimes to cause disease, in the respiratory tracts of some species (Baxby *et al*. 1982; Marrennikova *et al*. 1984; Bennett *et al*. 1989, 1990). The dose of virus used in this study was probably higher than that likely to be met naturally, but oronasal inoculation is a relatively inefficient means of dosing rodents of this size because much of the inoculum is sneezed out or swallowed: consequently, the effective infecting dose of virus was likely to be much smaller than 800 PFUs. The practical difficulties inherent in this dosing regime are demonstrated in this study by the number of virus-inoculated animals that did not develop antibody. It could also be argued that the animals used here were likely to be less susceptible to the effects of infection than their wild counterparts, as they were housed under optimum husbandry conditions, with balanced nutrition, and were apparently free of other infectious diseases.

It has long been recognized that infectious agents which cause obvious morbidity and mortality, and which are often characterized by epidemic spread, affect wild mammal populations: for example, myxomatosis in European rabbits (Flowerdew *et al*. 1992), phocine morbillivirus in seals (Osterhaus *et al*. 1995) and canine distemper in lions (Roelke-Parker *et al*. 1996) and black-footed ferrets (Appel & Summers 1995). However, endemic infections, probably because they often cause minimal or no obvious disease and mortality, have generally been regarded as relatively unimportant as determinants of host population dynamics. This has been especially true of microparasites, although a small number of studies of nematode infection in lagomorphs and game birds, for example, have demonstrated parasite infection causing increased vulnerability to predation (Hudson *et al*. 1992; Ives & Murray, 1997; Murray *et al*. 1997) or decreases in natality (Yuill 1964; Dunsmore 1981; Hudson 1986; Dobson & Hudson 1992).

Although much work remains to be done on the relationship between cowpox virus and its endemic hosts, the present study does exemplify the type of mechanism whereby a superficially harmless infection could significantly affect its host at the population level. Reduction in fecundity in the manner described here is at once a subtle and profound outcome of infection: subtle because it would be difficult to recognize in a field population, but profound since the absence of a litter has essentially the same effect at the population level as loss of that litter through mortality after birth. The delay observed here was around 20–30 days. During a 6–8 month long breeding season, wild bank voles, if they survive, can produce litters of 3–5 young up to five times a year, while wood mice can have litters

of 2–9 young up to four times a year, and the gestation time for both species is around 20 days (Macdonald & Barratt 1993). Even for those that survive the whole breeding season, therefore, a delay of up to 30 days in just one litter might represent a reduction in fecundity of approximately 25%. For others, the reduction may be far greater—for those that succumb to some other source of mortality in the first months of life, for example, such a delay may reduce fecundity to zero. On the other hand, our unpublished field data show that some individuals develop antibody much later in life: here the reduction in overall fecundity would be much less, even if a comparable delay in litter production was induced, which is itself unknown.

The times to first litter in pairs where only one parent became infected did not differ greatly according to the sex of the infected parent and were similiar to those where both parents were infected. In the wild, however, infected males, for example, may obtain a disproportionately low (or high) number of matings. The implications for the dynamics of the population may then depend on the detailed pattern of matings and may differ according to the sex of the animals infected. The effect of infection may also differ between species since the seroprevalence among bank voles in the field is much greater than that observed in wood mice (unpublished data) and bank voles are susceptible to a dose ten times lower than that required to infect wood mice (Bennett *et al*. 1997).

Further work is needed to investigate and compare the interrelationship of cowpox virus with voles and mice, both in field populations and under experimental conditions. In particular, the effects of dose, route, and age at infection are being studied, along with the influence of other environmental parameters on the outcome of infection under laboratory conditions, while long-term studies of the epidemiology of the virus in wild populations are also underway.

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