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Serological evidence for the reservoir hosts of cowpox virus in British wildlife

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SUMMARY

The reservoir host of cowpox virus in Western Europe is not known, but epidemiological evidence from human and feline infections indicates that the virus is probably endemic in small wild rodents. Therefore, serum and tissue samples were collected from a variety of wild British mammals and some birds, and tested for evidence of *Orthopoxvirus* infection. Antibody reacting with cowpox virus was detected in 9/44 (20%) bank voles (*Clethrionomys glareolus*), 8/24 (33%) field voles (*Microtus agrestis*), 17/86 (20%) wood mice (*Apodemus sylvaticus*) and 1/44 house mice (*Mus musculus*), but in no other animal species tested. Although virus was not isolated from any animal, this serological survey, together with other evidence, suggests that bank and field voles and wood mice are the main reservoir hosts of cowpox virus in Great Britain.

INTRODUCTION

Cowpox virus is an *Orthopoxvirus* endemic in Europe and some Western states of the former USSR [1]. Infection and disease have been reported in cattle and man [2–4], a variety of mammals in European zoological collections [5] and domestic cats [6, 7]. Despite its name bovine cowpox is rare and a survey of 1076 British cattle found an antibody prevalence of only 0·7% [8]. The domestic cat is the most frequently recognized host of cowpox virus in Western Europe [1, 6, 7, 9] but surveys have again found no evidence that this species is the reservoir host of the virus [6, 10]. The suggestion that the virus circulates in small wild mammals [8] is now generally accepted and evidence is accumulating to support the role of rodents as reservoirs of the virus. A survey of wild rodent populations in the UK [11] detected antibody reactive with ectromelia virus, an *Orthopoxvirus* antigenically closely-related to cowpox virus, in wood mice (*Apodemus sylvaticus*), field voles (*Microtus agrestis*) and Skomer bank voles (*Clethrionomys glareolus skomerensis*), but not mainland bank voles (*C. glareolus*). Virus has not yet been isolated from any wild Western European rodents, although cowpox virus has been isolated from wild susliks (*Rhombys opimus*) and gerbils (*Citellus fuleris*) in

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Turkmenia [12], and from the red-tailed Libyan jird (*Meriones libyans*) in Georgia (former USSR) [13].

A better understanding of the epidemiology of cowpox in Western Europe would be useful both in order to determine the sources of infection in man and domestic animals [1, 14] and to permit more informed assessment of the risk of genetic hybridization between endemic cowpox virus and released recombinant vaccinia vaccines [15–17]. This paper reports the results of surveys of British wildlife for antibody to cowpox virus carried out during 1975–93, and indicates that voles and wood mice are the most likely reservoir hosts.

MATERIALS AND METHODS

Serum and tissues were collected from several animal species at various sites in England and Wales between 1975–93. Some were collected at Whipsnade Zoological Park in 1977, others from four survey sites during 1991–3 (Table 1), and the remainder from a variety of sites throughout the survey period.

Antibody detection

Sera were inactivated at 56 °C for 20 min and stored at −20 °C until tested. Cowpox virus strains Brighton or L97 [18–20] grown on chorioallantoic membranes (CAM) of chick embryos or in Vero cells were used in all assays.

Virus neutralization

Sera from deer, foxes, badgers, hares, rabbits, squirrels, insectivores and some rodents were screened by a virus neutralization (VN) assay described previously [4, 8] using serum diluted 1/20 and incubated with virus for 2 h at 35 °C. The VN titres of positive sera were determined by further test, and taken as the reciprocal of the dilution of serum which reduced virus infectivity by 50% [4, 8].

Immunofluorescence

Sera from most voles, mice and rats were tested in immunofluorescence (IF) assays. Sera were serially diluted twofold in phosphate-buffered saline (PBS) from an initial dilution of 1/20 and added to 96-well, flat-bottomed microtitre plates containing ethanol-fixed Vero monolayers showing cowpox virus cytopathic effect. Prior to use the plates were washed with PBS. Absorption of test and FITC-conjugated sera was for 1 h each at 20 °C. Vole and mouse sera were detected by a rabbit anti-mouse IgG-FITC conjugate, and rat sera by an anti-rat IgG-FITC conjugate (Sigma), both used at a dilution of 1/64. The IF titre was taken as the reciprocal of the highest dilution of test serum at which specific intracytoplasmic fluorescence was detected in infected cells. Each batch of sera included positive controls from experimentally-infected bank voles and wood mice.

Haemagglutination-inhibition

Where sufficient material was available IF-positive vole and wood mouse sera were also tested in haemagglutination-inhibition (HAI) assays. Our modification of the traditional method [21] used 96-well U-bottomed microtitre plates and a unit volume of 50 μl [22]. Sera were titrated from an initial dilution of 1/50 and

Table 1. *Prevalence of* Orthopoxvirus *antibody in seropositive species caught at different sites*

Site/species	Antibody detected* (positive/tested) IF and/or VN	% + ve†	HAI‡
Wirral 1 (Cheshire)			
Bank vole (*C. glareolus*)	4/10	40	1/4
Wood mouse (*A. sylvaticus*)	7/16	44	6/7
House mouse (*M. musculus*)	1/1	100	nt
Wirral 2 (Cheshire)			
Bank vole	0/2	0	nt
Wood mouse	0/9	0	nt
House mouse	0/4	0	nt
Woodchester (Gloucestershire)			
Bank vole	1/2	50	nt
Field vole (*M. agrestis*)	7/12	58	3/5
Wood mouse	2/17	12	nt
Preston Mountford (Shropshire)			
Bank vole	1/4	25	nt
Field vole	1/3	33	nt
Wood mouse	0/2	0	nt
House mouse	0/1	0	nt
Whipsnade (Bedfordshire)			
Field vole	0/8	0	nt
Wood mouse	0/28	0	nt
Miscellaneous§			
Bank vole	3/26	12	1/1
Field vole	0/1	0	nt
Wood mouse	8/14	57	4/6
House mouse	0/38	0	nt

* Any IF or VN titre $\geqslant 20$ or HAI titre $\geqslant 50$ (the lowest dilution used) was recorded as positive.

† Positive by IF and/or VN.

‡ Only IF-positive vole and wood mouse sera were tested by HAI.

§ Includes several further sites on the Wirral Peninsula, and sites at Ormskirk (Lancashire) and Newbury (Berkshire).

the HAI titre taken as the reciprocal of the greatest dilution of serum which inhibited haemagglutination. Immune sera from cats and experimentally-inoculated bank voles were used as positive controls.

Virus isolation

Turbinate, lung, skin, small and large intestine, kidneys, spleen, and reproductive organs from each animal were pooled and stored at −70 °C until tested. They were then thawed, finely chopped, and freeze-thawed three times before attempted virus isolation on Vero cells and/or CAM as described previously [22, 23].

RESULTS

The samples were collected over a long period and from a variety of locations; in some cases a particular location provided only one specimen. VN was used more

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extensively during 1975–90 and IF after 1991, but results obtained on experimental and field sera with these tests were in very close agreement thus allowing results obtained early in the survey to be compared with those obtained later.

Detection of antibody by VN and IF

Overall, antibody to *Orthopoxvirus* was detected in 9/44 (20%) bank voles (*C. glareolus*), 8/24 (33%) field voles (*M. agrestis*), 17/86 (20%) wood mice (*A. sylvaticus*) and 1/44 house mice (*M. musculus*) (Table 1). IF antibody titres were in the range from 20 to $\geqslant 320$ and VN titres in the range from 40 to $\geqslant 100$.

Prevalence rates varied between populations. For example no seropositive animals were detected in the Whipsnade and Wirral 2 populations, but at Wirral 1, only 400 m from Wirral 2, antibody was detected in 7/16 wood mice and 4/10 bank voles (Table 1). Although this may reflect the small numbers sampled, it also suggests differences in the prevalence rates at different sites. The seropositive house mouse was trapped in an area where seropositive bank voles and wood mice were prevalent, whereas most of the other house mice tested came from buildings where voles and wood mice were infrequently found. Overall, no obvious difference in prevalence of antibody to *Orthopoxvirus* was detected between sexes of any species.

No *Orthopoxvirus* antibody was detected in samples from the numbers indicated of the following species: yellow-necked mouse, *Apodemus flavicolis* (7); rat, *Rattus norvegicus* (87); grey squirrel, *Scuirus carolensis* (39); hedgehog, *Erinaceus europeaeus* (12); mole, *Talpa europea* (5); shrews, *Sorex* spp (9); hare, *Lepus capensis* (15); rabbit *Oryctolagus cuniculus* (12); roe deer, *Capriolus capriolus* (27); badger, *Meles meles* (31); fox, *Vulpes vulpes* (4); birds, miscellaneous species (12).

Detection of antibody by HAI

HAI antibody was detected in only a proportion of those animals found positive by other methods (Table 1). Of 23 wood mouse and vole sera found positive by IF, and tested by HAI, only 15 (65%) had HAI antibody. HAI titres were in the range from 50 to $\geqslant 200$.

Virus isolation

No *Orthopoxvirus* was isolated from any tissues of any species. In all 236 animals were sampled, including 56 bank voles, 21 field voles, 82 wood mice and 23 house mice. A poxvirus isolated from skin lesions on a sparrow (*Passer domesticus*), was shown by appropriate cross-neutralization tests to be an avipoxvirus.

DISCUSSION

The results of the survey using IF and VN assays demonstrated that *Orthopoxvirus* infection is most common in bank voles (20%), field voles (33%), and wood mice (20%). However, the numbers tested of some species were sometimes very low and infection in different or larger populations of other species cannot be discounted.

HAI antibody persists for shorter periods than other antibodies after human [24] and bovine [4] infection and its presence may be taken as an indicator of

recent infection [4, 8, 24, 25]. This may account for the relative insensitivity of HAI compared to VN and IF in this study, although the high starting dilution (1/50) used of the small amounts of serum remaining after other tests had been completed would also reduce the sensitivity of HAI assays. It is of interest that the previous survey [11], which used HAI to detect *Orthopoxvirus* antibody, found lower prevalences overall than we did by IF.

The total numbers tested in antibody positive species were small, and too-detailed analysis is inappropriate. However, from serological surveys of other poxvirus infections in various species it is reasonable to propose that a particular species may be regarded as a reservoir host if antibody prevalences of greater than 8–12% are detected, particularly if there is evidence of recent (HAI positive) and distant (IF or VN positive, HAI negative) infection [25]. Here such evidence was obtained for bank and field voles and wood mice. However, analysis of results from different sites showed high prevalence at some (e.g. Wirral 1) but no seropositive animals at adjacent (Wirral 2) or distant (Whipsnade) sites. This result may be due to the low numbers sampled. However, similar results were obtained with both bank voles and wood mice (Table 1) which suggests that it may also reflect qualitative differences in the distribution of virus in rodent colonies at different sites. If so, this may be important in planning future surveys.

In the absence of virus isolation, the virus which elicited the antibody response cannot be fully identified. The high sensitivity of our cowpox virus isolation procedures has been established by our studies on human and feline cowpox [3, 22], but it may be that the main sites of virus replication in infected animals were not sampled or that only small amounts of virus are ever found in naturally-infected animals. Wild-caught, captive-bred bank voles are highly susceptible to experimental cowpox virus infection, but only small amounts of virus were isolated [26]. More detailed studies of the pathogenesis of cowpox in voles and wood mice are underway.

Cowpox virus is the only known *Orthopoxvirus* indigenous to Great Britain and its epidemiology in non-endemic hosts, particularly domestic cats, is strongly suggestive of a rodent reservoir [3, 6, 7, 14]. In a previous survey which detected *Orthopoxvirus* antibody in some wild British rodents, it was assumed that the antibody detected was elicited by ectromelia virus [11]. However, our preliminary studies have shown that bank voles are much less susceptible to ectromelia infection than they are to cowpox [26]. Furthermore, ectromelia virus, although infectious for house mice [27, 28] has not been found to occur naturally in wild mice except those in contact with infected laboratory animals [28, 29]. However, only 1 of 44 house mice tested in this study had *Orthopoxvirus* antibody and if this were due to infectious ectromelia then we would have expected a much higher prevalence of antibody in this species.

The high prevalence of antibody in some populations could indicate recently-introduced epidemic infection, but if so we would have expected to isolate virus. Alternatively, the results could indicate a high incidence of an endemic infection of low pathogenicity, a conclusion supported by our studies on experimentally-infected bank voles and field mice (26, in preparation). Our results could also be explained by the presence of a hitherto unrecognized *Orthopoxvirus* instead of or as well as cowpox virus. However, we think this is most unlikely. Cats acquire

cowpox, presumably from a rodent reservoir and we know of no animal species which acts as a reservoir host for more than one *Orthopoxvirus* species.

Thus, we believe that the antibody detected in this survey was due to cowpox virus infection and that bank voles, field voles and wood mice are important reservoir hosts of cowpox virus in Great Britain. However, the small number of samples tested does not exclude the existence of endemic infection in other species. Further work is now underway to investigate the epidemiology of *Orthopoxvirus* infection in known seropositive colonies of voles and wood mice and to identify conclusively the virus(es) involved.

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