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SEROLOGICAL SURVEY FOR ORTHOPOXVIRUS INFECTION OF WILD MAMMALS IN AREAS WHERE A RECOMBINANT RABIES VIRUS IS USED TO VACCINATE FOXES

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ABSTRACT

Several fox vaccination campaigns against rabies have been undertaken in Belgium by using a vaccinia-rabies recombinant virus distributed in baits in the field. However, foxes and other wild animals that may ingest the baits could be infected at the same time by another orthopoxvirus, such as cowpox virus, which circulates in wildlife. Recombination between the two viruses could therefore occur. A serological survey for antibodies to orthopoxvirus, and particularly to cowpox virus, was undertaken in foxes and in several other wild species. Antibodies were detected only in two rodent species, in 16 of 25 bank voles (64 per cent) and in two of 29 woodmice (7 per cent). The risk of virus recombination in wildlife can therefore be considered to be extremely low.

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Sylvatic rabies can be efficiently controlled by the vaccination of foxes with a vaccinia-rabies recombinant virus which expresses the immunogenic glycoprotein of rabies virus (Kieny and others 1984). The insertion of the rabies glycoprotein coding sequence in the vaccinia thymidine kinase gene markedly attenuates the virulence of the recombinant virus (Buller and others 1985). The efficacy and innocuity of this vaccine has been demonstrated in target and non-target wild species that could compete with the red fox for the bait (Blancou and others 1986, Brochier and others 1988, 1989, Pastoret and others 1992). Several large-scale fox vaccination campaigns with this vaccine have led to the almost complete elimination of rabies in Belgium (Brochier and others 1991, 1993). The safety of the vaccine has been tested in several European wild species: Daubenton 's bat (*Myotis daubentoni*), wild boar (*Sus scrofa*), Eurasian badger (*Meles meles*), woodmouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), red bank vole (*Clethrionomys glareolus*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), water vole (*Arvicola terrestris*), common buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), carrion crow (*Corvus corone*), magpie (*Pica pica*) and jay (*Garrulus glandarius*). Neither clinical signs nor pox lesions were ever observed in the vaccinated animals during a minimum observation period of 28 days (Brochier and others 1989). This vaccine is therefore preferable to conventional attenuated strains of rabies virus which are pathogenic to some non-target species including rodents (Winkler and others 1976, Wandeler and others 1982, Leblois and Flamand 1988, Artois and others 1992, Bingham and others 1992). However, it has been suggested that genetic recombination could occur between the engineered vaccine virus distributed in baits and other closely related viruses, such as cowpox virus, which circulate in wild animals that may take the bait (Baxby and others 1986).

Cowpox virus is found only in Europe and parts of western Asia. Despite its name, cowpox virus occurs only rarely in cattle, and surveys of cattle in the United Kingdom have found an antibody prevalence of only O,7 per cent (Baxby 1977). The domestic cat is the most frequently recognised host of cowpox virus in the UK, but surveys have again found no evidence that this species is the reservoir host for the virus (Bennett and others 1990). It is now generally accepted that the reservoir host for cowpox virus is a small wild rodent, and indeed serosurveys in the UK (Kaplan and others 1980, Crouch and others 1995) have detected a high prevalence of antibody to orthopoxvirus in bank and field voles (*C glareolus* and *M agrestis*) and in woodmice (*A sylvaticus*). Similar results have been obtained in Germany (Pilaski and Jacoby 1993). Although cowpox virus has not been isolated so far from any western European rodents, it has been isolated from wild rodents in eastern Europe (Marennikova and others 1978, Marennikova 1979). Cowpox virus is thus believed to circulate in populations of wild rodents (Baxby 1977, Marennikova and others 1978, Baxby and others 1986) from which it may be transmitted to other species, particularly domestic cats and man (Marennikova and others 1977, Egberink and others 1986, Bennett and others 1990, Maenhout and others I 991, Baxby and others 1994). Foxes, and possibly other species, might also be infected in the same way, although there is no evidence for this; moreover the fox seems to be poorly susceptible to this infection (Boulanger and others 1995).

A serological survey of foxes and other wild animals that had previously been shown to compete in consuming baits, for example, mustelids, wild boars and rodents (Kalpers and others 1987,

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Brochier and others 1988), was therefore undertaken to help assess the risk of recombination of the engineered vaccine virus in the field.

Materials and methods

ANIMALS

Blood samples were obtained from 70 adult foxes (*Vulpes vulpes*) and 55 juveniles captured in Belgium or i n France (Table I), from 24 roe deer (*Capreolus capreolus*), three mouflon (*Ovis aries*), two polecats (*Mustela putorius*) and two stone martens (*Martes foina*) captured in the provinces of Brabant or Luxembourg in Belgium (Table I) and from 22 wild boars captured in the province of Luxembourg . Blood samples were also collected from 25 bank voles, 29 woodmice, one dormouse (*Eliomys quercinus*) and one house mouse (*Mus musculus*) captured in different areas in Belgium (Table I), and from I0 Eurasian badgers captured in a rabies-free area of France (Moulin, Departement of Allier).

SEROLOGICAL ANALYSES

All the sera were tested for antibody to orthopoxvirus in indirect immunotluorescence (IF) assays, and all except the rodent sera in haemaggluti nation inhibition (HAI) assays.

IMMUNOFLUORESCENCE ASSAY

The IF assays were done as described by Crouch and others (1995). Fox serum was tested with anti-dog IgG conjugate, wild boar serum with anti-pig IgG conjugate, mouflon with anti-sheep IgG conjugate, roe deer serum with antibovine IgG conjugate, badger, polecat and stone marten serum was tested with anti-dog and anti-cat IgG conjugates, and mouse, woodmouse and bank vole serum with anti-mouse conjugate. Roe deer sera were also tested with an anti-deer IgG monoclonal antibody and anti-mouse IgG-fluorescein isothiocyanate (FITC) conjugate, and badger and stone marten sera with an anti-badger IgG monoclonal antibody followed by an anti-mouse IgG-FITc conjugate. Anti-deer (M75) and anti-badger (CF2) monoclonal antibodies were kindly supplied by Malcolm Stock, Central Veterinary Laboratory (now Veterinary Laboratories Agency), Weybridge. All the FITC conjugates were polyclonal anti-IgG whole molecule and were obtained from Sigma Pharmaceuticals and used at a dilution of 1:64. Any IF titre ≥10 was considered as positive.

Immune cat sera (Bennett and others 1989) and anti-cat IgGFITC conjugate were used as a positive control on every plate, as were positive sera from bank voles, woodmice or foxes experimentally infected with cowpox virus (Crouch 1994, Boulanger and others 1995, Crouch and others 1995) whenever appropriate.

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HAEMAGGLUTINATION INHIBITION ASSAY

The HAI assays were based on the method of McCarthy and Helbert (1960), modified for 96-well U-bottomed microtitre plates (Bennett and others 1985). A unit volume of 50 µl was used throughout. The HAI titre was taken as the reciprocal of the greatest dilution of serum which fully inhibited haemagglutination. Any HAI titre ≥16 was recorded as positive. Immune cat serum was used as a positive control.

TABLE 1: *Place of capture of wild mammals and results of tests for orthopoxvirus antibody*

Species	Province or area	Number positive/number tested
Fox (*Vulpes vulpes*)		
Adults (70)	Brabant	0/47
	Luxembourg	0/5
	France	0/18
Juveniles (55)	Brabant	0/19
	Luxembourg	0/36
Bank vole (*Clethrionomys glareolus*)	Anvers	0/2
	Hainaut	2/3
	Liège	6/9
	Luxembourg	1/2
	Namur	7/9
Dormouse (*Eliomys quercinus*)	Namur	0/1
House mouse (*Mus musculus*)	Liège	0/1
Woodmouse (*Apodemus sylvaticus*)	Anvers	0/3
	Hainaut	0/7
	Liège	2/9
	Luxembourg	0/1
	Namur	0/9
Polecat (*Mustela putorius*)	Luxembourg (vaccinated area)	0/2
Stone martin (*Martes foina*)	Luxembourg (vaccinated area)	0/2
Badger (*Meles meles*)	Allier Departement in France (unvaccinated area)	0/10
Wild boar (*Sus scrofa*)	Luxembourg	0/22
Roe deer (*Capreolus capreolus*)	Brabant	0/10
	Luxembourg	0/14
Mouflon (*Ovis aries*)	Luxembourg	0/3

Results

Antibody to orthopoxvirus was detected by IF in sera from 16 of 25 bank voles (64 per cent positive) and two of 29 woodmice (7 per cent) (Table I). No antibody was detected in any of the 125 foxes, two polecats, I0 badgers, two martens, 22 wild boars, 24 deer or three mouflon examined by both IF and HAI, or in the mice examined by IF.

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Discussion

Antibody reactive with orthopoxvirus was detected in bank voles and woodmice, but not in any of the other species tested. Antibody to orthopoxvirus has been previously detected in bank voles and woodmice in the UK by Kaplan and others (1980) and Crouch and others (1995). The serological techniques used in this study do not make it possible to identify precisely the virus responsible for the antibody detected. However, cowpox virus is the only orthopoxvirus known to circulate in continental Europe and the authors' unpublished observations have demonstrated that bank voles are susceptible to cowpox virus infection but not to infection with vaccinia or ectromelia viruses. This strongly suggests that the antibody detected in this study and in the previous surveys by Kaplan and others (1980) and Crouch and others (1995) was produced in response to cowpox virus infection.

None of the other species tested was positive. Specific conjugates were not available for many of the species tested, but the authors have previously used anti-dog FITC conjugate to detect orthopoxvirus antibody in experimentally infected foxes (Boulanger and others 1995). Anti-bovine conjugates have been used to detect antibody i n deer (Losson and LeFevre 1989), and anti-pig and anti-sheep conjugates would be expected to bind, respectively, to wild boar and mouflon immunoglobulins . The badger and roe deer sera were also tested by using badger and deer specific monoclonal antibodies. Anti-dog conjugates have also been used to detect antibody in ferrets and badgers. The light chains of mustelid immunoglobulins are known to be antigenically similar (Taranin and others 1991) but to ensure sensitivity both anti-cat and anti-dog conjugates were used with the polecat and stone marten sera. Antibody to the poxvirus haemagglutinin antigen is a sensitive indicator of recent infection (McCarthy and others 1958, Baxby 1977), and all except the rodent sera were also tested for this antibody and found to be negative.

Although only a small number of samples was obtained from each species, the results suggest that most of the species likely to take the vaccine-containing bait (foxes, badgers and wild boars) (Brochier and others 1990) are not frequently infected with any orthopoxvirus. However, the results do not totally exclude the possibility of any of these species being an occasional host for cowpox virus. The domestic cat is the most frequently identified host of cowpox virus in Great Britain, but surveys have found only a low prevalence of antibody in randomly sampled populations (Bennett and others 1986, Nowotny 1994, authors' unpublished observations). Two of 29 (7 per cent) woodmice did have detectable orthopoxvirus antibody, as did 17 of 86 (20 per cent) woodmice in a British survey by Crouch and others (1995). Although woodmice do often consume recombinant vaccine in baits, the rabies-vaccinia recombinant virus has been shown to be non-pathogenic in this species (Brochier and others 1989) and should therefore multiply at a very low level; as a result recombination between the vaccine and a wild orthopoxvirus is very unlikely to occur.

None of the foxes tested were seropositive. However, a recent serological survey in Germany (Henning and others 1995) detected low titres (mostly 1:16 in a competitive ELISA) of orthopoxvirus antibody in 6·5 per cent of the foxes tested. The difference may be due to the different

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geographical origin of the samples or the different serological assays used and the different interpretations of those assays. It would be necessary to confirm these results by testing identical samples by both techniques before drawing any definitive conclusion. In any case, recombination between the vaccine and cowpox viruses is unlikely in foxes because foxes are very resistant to cowpox virus infection (Boulanger and others 1995).

Recombination also seems to be unlikely in other species tested, such as the badger and wild boar in which no orthopoxvirus antibody was detected. Surveys in Great Britain have also failed to detect antibody in badgers (Crouch and others 1995, unpublished observations). More samples need to be tested from other mustelids to assess the risk of recombination in them.

Taken together, the results of this and other surveys and of the experimental studies by Brochier and others (1990), Boulanger and others (1995) and Crouch and others (1995) strongly suggest that the opportunity for recombination between the rabies-vaccinia recombinant vaccine virus and wild orthopoxviruses, particularly cowpox virus, is extremely small in European wildlife.

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