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EPIDEMIOLOGY AND CONTROL OF FOX RABIES IN EUROPE

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ABSTRACT

During recent years, most of the research on the control of sylvatic rabies has concentrated on developing methods of oral vaccination of wild rabies vectors. In order to improve both the safety and the stability of the vaccine used, a recombinant vaccinia virus which expresses the immunizing glycoprotein of rabies virus (VRG) has been developed and extensively tested in the laboratory as well as in the field. From 1989 until 1995, several million VRG vaccine doses have been dispersed in western Europe for vaccination of red foxes. In Europe, the use of VRG has lead to the elimination of sylvatic rabies from large areas, which have consequently been freed from vaccination.

This may have consequences on the regulation of pets movements within the whole European Union.

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Introduction

The present European terrestrial epizootic of rabies has spread some 1400 km westward from Poland since 1939. For several years, the front of the epizootic advanced 20-60 km per year [1, 2]. This terrestrial epizootic is sylvatic: the reservoir of infection is in wild-ife. While all susceptible species, both wild and domestic, are involved, the red fox (*Vulpes vulpes*) is involved in more than 75% of cases. The red fox is both the vector of the disease and its reservoir. It plays a key role in the maintenance of the disease but it does not usually transmit it directly to humans. Humans are mainly at risk from affected domestic animals such as cattle and cats [3].

The percentage of rabid foxes is underestimated for several reasons. In fact, the proportion of rabies cases reported is likely to be as low as 2-10%; this can cause serious problems in detecting outbreaks [4] and, conversely, the impact of the epizootic on wildlife is therefore enormous [5]. For example, the occurrence of the disease in cubs in the den is always neglected, but could be frequent [6]. The fox seems to be the only species maintaining the present terrestrial epizootic; if rabies were to be eliminated from the fox population it would cease to be a problem in other wildlife or domestic species.

In western Europe, rabies is considered a source of economic loss and, above all, hampers the movement of animals between the different member states of the European Union. This has serious implications for the 'open market' since some member states are currently rabies free and wish to maintain their disease-free status. Therefore, the control of rabies required a common strategy to be established at an European Union level.

The history of urban rabies in Great Britain

Rabies may be maintained in two not necessarily interrelated cycles, urban and sylvatic. Urban rabies, affecting stray and feral dogs and cats, is by far the more dangerous to humans, accounting for an estimated 99% of all recorded human cases and for 92% of all human postexposure treatments [3]. Sylvatic rabies is characterized by the involvement of one or two main species in particular locations and this pattern remains stable over many years [7]. In continental Europe, sylvatic rabies has been reported since the Middle Ages but has been intensively studied only since its current upsurge began at the beginning of World War II [5] before which urban rabies had virtually disappeared.

Rabies was a common disease in Great Britain for many centuries [5, 8]. The first allusion to the disease seems to have been in 1026 in the laws of Howell the Good of Wales (cited by Fleming [9]). The prevalence of the disease increased from 1735 and by 1776 rabies was widespread in dogs throughout Britain. Mad dogs were reported in London from 1752 to 1862. Despite a significant fox population, the disease was not observed in wildlife except for two outbreaks in deer (at Barnsley

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in 1856 when 100 animals were affected and in Richmond Park, London, in 1886, when 257 fallow deer (*Cervus dama*) died but did not pass on the disease to red deer (*Cervus elaphus*) in the same park).

Between 1889 and 1898 over 160 human cases of rabies were recorded in Britain. From 1897 onwards, the implementation of orders which included powers for the muzzling of dogs, the elimination of stray dogs, the tracing of movement of rabid dogs and their contacts and the regulation and control of the importation of dogs, led to the eradication of the disease for the first time in 1902.

Rabies was reintroduced in 1918 and 328 further animal cases were found in the south of England before it was finally eradicated in 1922. However, a further 21 recorded human deaths have resulted from infection acquired abroad. Except for two dogs which became rabid after release from quarantine, import regulations, including quarantine of imported dogs and cats, have kept Britain free of animal rabies since 1922.

During the 49 year period 1922-1970, 29 animals died of rabies in quarantine. However, in the 21 year period (1971-1991) since the immunization of cats and dogs with inactivated vaccines of cell culture origin within quarantine was introduced, only two dogs have died in quarantine with evidence of rabies in the brain. Since 1971, of nearly 200,000 imported cats and dogs immunized within quarantine, none have died of rabies after release. It should also be noted that among all the cases of rabies in animals imported into Britain, none was due to a previous contamination with fox rabies in another country belonging to the European Union.

In fact, urban rabies did disappear from other western European countries during the same period as in Britain.

The biology of rabies virus infection in foxes

The present epizootic of fox rabies in western Europe seems to have started at the Russian-Polish border and the prevailing hypothesis is that the virus originated in dogs and became adapted, through successive passages, for foxes [10].

The virus is highly pathogenic for the fox: a dose of 0.3 mouse intracerebral LD_{50} is sufficient to kill one fox in two, whereas domestic carnivores require a dose 100,000 times greater to produce the same effect. When the disease front penetrates a new area, the foxes within the area suffer an epizootic of rabies. When the fox population has been severely reduced, the incidence of the disease decreases and remains low for a 'silent' period of some 2-3 years. After the initial epizootic, secondary enzootic outbreaks recur, often at intervals of 3-5 years [11].

Superimposed upon the annual variance in the incidence of reported cases of vulpine rabies is a seasonal pattern. Cases tend to peak in late winter and reach a through in midsummer. The late winter/early spring peak of rabies seems to be linked to the mating season of foxes.

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Besides vaccination in recent years the advance of the rabies epizootic, which had previously progressed 20-60 km per year in Europe, seems to have stopped. This is exemplified by the situation in France. It may be due to strain variations or to the ecology of transmission. In Europe, the fox is certainly the animal species most susceptible to rabies infection. The proportion of rabid foxes that excrete the virus is very high: 93-100% of rabid foxes harbour the virus in the salivary glands and excrete it in large amounts in saliva. Furthermore, the virus can be excreted for up to 29 days before the onset of the disease [12].

The incubation of the disease in foxes depends mainly on the dose of virus with which the animal has been infected and varies from 10 to 41 days. The clinical disease lasts 3-5 days. The signs are variable [13]. The furious form is relatively rare; instead, most animals become apathetic and develop paralysis. Rabies is transmitted by biting. Since the apathetic form of the disease predominates in the fox, rabid foxes do not usually wander far from their original territory [14]. This may explain why the front of a fox rabies epizootic progresses slowly.

Control of fox rabies

Prophylactic measures taken in the past, like the destruction of foxes to reduce fox population, did not prevent the spread of the epizootic. During recent years, most of the research on the control of fox rabies has concentrated on the development of methods of oral vaccination of the fox [15] and this method has already been extensively used in all the contaminated countries belonging to the European Union. Research has focused on oral vaccination because it is the only means of immunizing a sufficient proportion (75%) of wild foxes, through the distribution of vaccine baits. Therefore the only vaccines that could be used were either attenuated strains of rabies virus or live vectored vaccines. Inactivated rabies vaccines are useless when given orally [16].

In 1986, in order to develop a common strategy for the European Union, a co-ordinated trial of oral vaccination of foxes was undertaken in several European countries using the SAD B19 attenuated strain of rabies virus in order to assess both the efficacy and the feasibility of the method [17]. The results of these campaigns confirmed the efficacy of fox vaccination for the control of sylvatic rabies.

However, the use of attenuated rabies virus remains controversial as far as safety and stability are concerned, as these virus strains are still pathogenic for laboratory and wild rodents or wildlife species such as the chacma baboon (*Papio ursinus*) and target species such as the striped skunk (*Mephitis mephitis*) [18]; moreover, these strains may still be pathogenic to man. Human beings exposed to SAD-derived attenuated strains of rabies must be treated with a conventional inactivated rabies vaccine since it elicits good cross-protective immunity. The SAD-derived attenuated strain may also be inefficient for some rabies vectors such as the raccoon (*Procyon lotor*) in North America.

The pathogenicity of attenuated rabies virus strains can be abolished by mutating arginine residues at position 333 of the rabies virus glycoprotein [19]. This has led to the development of a

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new attenuated vaccine strain already used in the field [20]. Nevertheless another inconvenience of attenuated strains of rabies virus is their heat sensitivity [21] which reduces their potential efficacy in field conditions.

Thus, in order to improve both the safety and stability of the vaccines used for fox vaccination in the field, a recombinant vaccinia virus which expresses the immunizing glycoprotein of rabies virus (VRG) has been developed and tested in the field for oral vaccination of foxes against rabies [22-26].

Development and deliberate release of a vaccinia-rabies glycoprotein recombinant virus (VRG) for oral vaccination of foxes against rabies

The recombinant vaccinia-rabies glycoprotein virus (VRG) has been tested for efficacy and safety in the fox [23, 27]. The duration of protection conferred by VRG, a minimum of 12 months in cubs and 18 months in adult animals corresponds to the length of protection required for fox vaccination in the field, due to the high turnover of fox population. The efficacy of VRG contained in a machine-made baiting system has been tested [25, 26] and shown to be effective.

VRG was shown to be non-pathogenic in the fox [24, 27] whatever the inoculation dose or route of administration. No transmission of immunizing amounts of VRG was found to occur in adult or young foxes, with the exception of one adult fox bitten by another freshly vaccinated. VRG only multiplies locally [28].

The influence of vaccination with VRG on the onset of the disease and on the delay before death, in foxes previously infected with wild rabies virus, has been investigated. The results show that 'early' and 'late' death phenomena occur as a consequence of interactions between oral vaccination with VRG and rabies infection, but preclude the risk of the emergence of asymptomatic carriers of wild-rabies virus after vaccination [29, 30].

Field trials with baits have shown that several nontarget wildlife species compete with foxes for bait consumption. It must also be taken into account that, within the orthopoxvirus group, vaccinia virus has a wide range of host species. In fact, bait uptake monitoring and tetracycline (biomarker included within the bait) detection controls, performed after vaccination campaigns, proved that mustelids, wild boars (*Sus scrofa*) and domestic carnivores may ingest the vaccine baits. Moreover, a significant proportion of the baits are partly eaten by small mammals. It was therefore important to verify the safety of VRG for non-target species (both domestic and wild).

Several non-target wild species have been chosen for testing in Europe because of their opportunistic feeding behaviours and their presence in the areas where the vaccine must be distributed [29-31]; among them wild boar, Eurasian badger (*Meles meles*) and several

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micromammals. No clinical signs of rabies and/or poxinflicted lesions were observed in the vaccinated animals during the observation period (28 days minimum after vaccination).

Taking into account all the available experimental data concerning the safety of the VRG for target and non-target species and its efficacy in foxes, limited field trials of fox vaccination with the recombinant virus were authorized first by the Belgian [32, 33] and then by the French public health authorities.

Towards elimination of rabies within the European Union

The last trial of deliberate release of the VRG on a 2200 km^2 area of southern Belgium was intended to test the feasibility of rabies elimination on a large area [33]. The 25,000 baits containing VRG and tetracycline as a biomarker were dropped by helicopter on three occasions (November 1989, April 1990 and October 1990). After the third phase of vaccination, 81% (64/79) of inspected foxes were tetracycline positive. Only one rabid fox was recorded, at the periphery of the baited area, and this was tetracycline negative.

Despite the dramatic decrease in the number of rabid foxes recorded after vaccine-bait distribution, the efficacy of the vaccination campaign remains difficult to evaluate because systematic collection of foxes is not logistically feasible. Nevertheless, because notification of cases of rabies in cattle and sheep is mandatory in Belgium, the incidence of rabies in livestock provides a reliable indicator of the prevalence of rabies in the wild. No case of livestock rabies has been recorded in the study zone since the second phase of vaccination.

On this occasion, we have also investigated the economics of the vaccine-baits dispersal programme. The average yearly cost of rabies in Belgium (1980-1989), including postexposure treatments of humans, animal diagnosis, compensation to farmers for the culling of infected livestock and the culling of wild foxes, was estimated to be 400,000 ECUs per 10,000 km^2, or 88,000 ECUs per year for the area under study. These figures did not include the cost of vaccination of domestic animals nor the salaries of civil servants. In comparison we estimate the overall expenditure during the three campaigns of vaccine-bait distribution to be 118,000 ECUs. Because vaccination following elimination can in principle, be interrupted or subsequently limited to the borders of the vaccinated zone, longterm maintenance of a rabies-free area by peripheral vaccination with VRG is economically justifiable.

The use of VRG has now been extended to all the contaminated areas in Belgium and the Grand Duchy of Luxembourg as well as to large areas in France. Rabies is approaching the stage of elimination in these three countries [31, 34].

The quasi elimination of rabies in Belgium has already had other beneficial effects, besides the improvement of animal health. Firstly the number of human postexposure treatments has decreased in proportion to the decrease of rabies incidence in animals (mainly cattle). Secondly the diminution of rabies incidence in wildlife has had a beneficial effect on the survival of

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threatened wild species, such as the Eurasian badger in the contaminated area. Estimation of the badger population in the treated area shows a gradual increase in numbers.

Towards new European regulations?

As mentioned in Section 1, rabies in western Europe is considered a source of public health concern, economic loss and hampers the movement of animals between the different member states of the European Union. Some member states, such as the UK, Spain and Portugal, are currently rabies free and wish to maintain their disease-free status. Therefore, the control of rabies requires a common strategy established at the level of the European Union. This has already begun since the European Union pays for half of the fox rabies elimination programme costs. The elimination programmes are discussed between the different countries concerned, before implementation.

Within the European Union, a country will be declared rabies free after 2 years of epidemio-surveillance without any reported case of rabies in either wildlife or domestic animals. If previously infected countries become rabies free, one may consider the possibility of changing the rules governing animal traffic within the European Union. For example, quarantine containment in the UK for pets coming from other rabies-free areas of the Union could be abolished, keeping in mind that fox rabies was never introduced in Spain by pets coming from contaminated countries in the past and that among the rabies cases in UK quarantine kennels, none originated from another member state of the Union. The risk of introducing fox rabies into the UK through pets from other member states is practically nil. But this is another controversial issue. The vaccinia-rabies glycoprotein recombinant virus vaccine represents a real kink between the work of Jenner and Pasteur [35].

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