SPECIAL ARTICLE

The development and use of a vaccinia-rabies recombinant oral vaccine for the control of wildlife rabies; a link between Jenner and Pasteur

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SUMMARY

To improve both safety and stability of the oral vaccines used in the field to vaccinate foxes against rabies, a recombinant vaccinia virus, which expresses the immunizing G protein of rabies virus has been developed by inserting the cDNA which codes for the immunogenic glycoprotein of rabies virus into the thymidine kinase (TK) gene of the Copenhagen strain of vaccinia virus. The efficacy of this vaccine was tested by the oral route, primarily in foxes. The immunity conferred, a minimum of 12 months in cubs and 18 months in adult animals, corresponds to the duration of the protection required for vaccination of foxes in the field. Innocuity was tested in foxes, domestic animals, and in numerous European wild animal species that could compete with the red fox for the vaccine bait. No clinical signs or lesions were observed in any of the vaccinated animals during a minimum of 28 days post vaccination. Moreover, no transmission of immunizing doses of the recombinant occurred between foxes or other species tested. To study the stability of the vaccine strain, baits containing the vaccine were placed in the field. Despite considerable variations of environmental temperatures, the vaccine remained stable for at least one month. Because bait is taken within one month, it can be assumed that most animals taking the baits are effectively vaccinated. To test the field efficacy of the recombinant vaccine, large-scale campaigns of fox vaccination were set up in a 2200 km² region of southern Belgium, were rabies was prevalent. A dramatic decrease in the incidence of rabies was noted after the campaigns. The recombinant is presently used to control wildlife rabies in the field both in several European countries and in the United States.

INTRODUCTION

On Monday, 26 October 1885, Louis Pasteur announced his first results on post-exposure vaccination against rabies in a session of the Academy of Sciences in Paris. He reported that he had successfully vaccinated Joseph Meister with an attenuated vaccine and that the experiment had been repeated in another subject, Jean-Baptiste Jupille. At the end of the treatment, Joseph Meister had resisted challenge using a virulent 'fixed virus' [1]. Pasteur's vaccine was subsequently improved, step by step, resulting in the inactivated vaccines that are produced in cell culture today [2].

The original experiments, in which vaccinated human beings were exposed to virulent rabies, were preceded by rigorous experiments in dogs. Preventive vaccines developed in animal trials largely contributed to the eradication of canine rabies in Western Europe by the beginning of this century. However, after the Second World War, rabies reappeared in several European countries linked to a wildlife rabies reservoir, namely the red fox (*Vulpes vulpes*). Fox rabies is presently controlled in Western Europe by vaccination. This method has led to the elimination of sylvatic rabies from large areas in several countries consequently where vaccination is no longer needed [3, 4]. Conventional, attenuated rabies virus vaccines

are still used by some countries for this purpose, notwithstanding their lack of safety [2] and stability. However, a recombinant vaccinia rabies virus offers a better alternative because of its safety, efficacy and stability. Such a recombinant is a real link between the pioneer work of Edward Jenner, the bicentenary of whose first vaccination is celebrated in 1996, and Louis Pasteur the centenary whose death was commemorated in 1995 [5].

THE RATIONALE FOR WILDLIFE VACCINATION AGAINST RABIES

Rabies still prevails in many countries [6]. It may be maintained in two not necessarily interrelated cycles, urban and sylvatic. Urban rabies, which affects stray and feral dogs and cats, is by far the most dangerous to humans, accounting for an estimated 99% of all recorded human cases and for 92% of all human post-exposure treatments. Sylvatic rabies is characterized by the involvement of one or two main wild reservoir species in particular locations, and this pattern remains stable over many years. The species involved in maintaining the infection may vary according to geographical and ecological conditions. In North America, for instance, several wildlife species play a distinct role, such as the raccoon (*Procyon lotor*), the striped skunk (*Mephitis mephitis*), the red fox (*Vulpes vulpes*), the coyote (*Canis latrans*) and the Arctic fox (*Alopex lagopus*).

The present European epizootic of terrestrial rabies has spread 1400 km westward from Poland since 1939. Although overall the epizootic involves all susceptible species both wild and domestic, the red fox is involved in > 75% of cases. In Western Europe, the fox appears to be the only species maintaining the present terrestrial epizootic. Thus, if rabies were eliminated from the fox population it would cease to be a problem in other wildlife or domestic species and human infections would no longer occur.

This paper discusses the control of fox rabies through vaccination. However, many different epidemiological cycles either rural or sylvatic, exist in the world involving many different animal species. Thus, the overall aim must be to develop control measures, e.g. through vaccination that can be applied in as many different situations as possible. Therefore, our strategy for development of a wildlife vaccine was to use a stable orthopoxvirus vector such as vaccinia virus which has a wide host range, rather than attenuated rabies virus [7]. Recombinant viruses

expressing the rabies glycoprotein have also been constructed from an orthopoxvirus isolated from the raccoon. Although racoonpoxvirus recombinants were immunogenic for raccoons [8], they have not yet been used extensively.

DEVELOPMENT AND TESTING OF THE VACCINIA-RABIES RECOMBINANT VACCINE

Construction of the vaccinia rabies recombinant virus

The surface glycoprotein of rabies virus is the only antigen capable of eliciting the formation of virus-neutralizing antibodies, and confers immunity [6]. Thus, this glycoprotein is an ideal candidate for use in the construction of subunit vaccines. The rabies virus glycoprotein gene has been inserted into the thymidine kinase (TK) gene of the Copenhagen strain of vaccinia virus generating a selectable TK⁻ virus known as VVTGg RAB [9]. Expression of the exogenous protein-coding sequences in vaccinia virus essentially involves two steps. First, the exogenous coding sequence is aligned with a vaccinia promoter and inserted *in vitro* at a site within a non-essential segment of vaccinia DNA cloned into a suitable bacterial plasmid replicon. Secondly, the flanking vaccinia sequences permit homologous recombination *in vivo* between the plasmid and the viral genome. Double reciprocal recombination results in transfer of the DNA insert from the plasmid to the viral genome, where it is propagated and expressed.

Efficacy of the VVTGg RAB in target species

VVTGg RAB has been tested for efficacy and safety in the fox, raccoon and striped skunk which are the main rabies target species in Western Europe and North America. The results of tests for efficacy in foxes can be summarized as follows [10, 11]. All but one of 26 adult captive foxes inoculated by various routes developed high titres of rabies neutralizing antibodies, and resisted wild rabies virus challenge on day 28 after vaccination. Immunity conferred by 10⁸ plaque-forming units, given orally lasted for at least 18 months. This is more than adequate because most foxes in the wild are < 24 months of age. Foxes receiving less than the recommended dose showed a clear dose-dependent response. The liquid form of VVTGg RAB was more efficient than the freeze-dried one when administered by the oral route. A second dose of VVTGg RAB induced an increase of rabies

neutralizing antibody. When given orally to fox cubs the vaccine induced significant levels of rabies virus antibody and protected 11/12, the duration of immunity exceeding 12 months.

Oral administration is the only route appropriate for vaccination of wild animals. Accordingly, the vaccine must be presented in a form suitable for ingestion. The vaccine vehicle (bait plus container) may affect the contact of the virus with the oral mucosa, and thereafter, if the baiting system is inappropriate, lower the efficacy of the vaccine. The efficacy of VVTGg RAB contained in a new machine-made baiting system has been tested [12]. Twenty-two captive young foxes with antibody to rabies virus were divided into three experimental groups of six, and a control group of four. Foxes in the first three groups were fed one, two or three vaccine baits, respectively, on successive days. The four unvaccinated foxes were housed separately. All the baited foxes ingested at least one bait, as shown by the incorporation of tetracycline into their bones. Thirty days after baiting, seroconversion to the rabies glycoprotein was observed in 15/18 foxes, and seroconversion to vaccinia in 14/18. Of these 18 foxes, 16 resisted a wild rabies virus challenge after 90 days. One cub was protected against rabies despite the absence of detectable rabies antibody. These results demonstrate that the baiting-sachet system used permits an efficient release of the virus suspension into the mouth.

Safety of the VVTGg RAB for the target species

A vaccine must not only be efficacious but also safe for the target species. VVTGg RAB was non-pathogenic for the fox whatever the dose (10^2–10^{10} $TCID_{50}$) or route of administration (oral, intramuscular, intraduodenal, subcutaneous, intradermal, conjunctival or intranasal). It is preferable that a vaccine virus used for oral vaccination of wildlife is not transmitted horizontally to unvaccinated animals. In order to test for horizontal transmission, unvaccinated control animals were held in close contact with vaccinated ones. No transmission of immunizing doses of VVTGg RAB was found to occur in young or adult foxes, in those circumstances with the expection of one adult bitten by a freshly inoculated one [10, 11].

When assessing a recombinant vaccine, it is also of great importance to detect any change in tissue tropism of the vector. Experiments were designed to determine the multiplication site of the recombinant

virus and the parental strain of vaccinia virus in foxes, by virus isolation, titration and indirect immuno-fluorescence. The polymerase chain reaction was also used to detect specific virus DNA in several fox organs. Foxes were fed with 10^8 $TCID_{50}$ of either VVTGg RAB or vaccinia virus, and were killed 12, 24, 48 or 96 h after inoculation by the oral route. Using these different techniques, VVTGg RAB or vaccinia virus could be detected only during the first 48 h following vaccination by the oral route, but only in the tonsils, buccal mucosa and soft palate [11, 13]. Results of other experiments demonstrated that tonsillectomy of foxes does not completely impede seroconversion.

Because no virus could be detected in the salivary glands of foxes, the risk of transmission from one animals to another through saliva can be discounted. Furthermore, the fact that VVTGg RAB only multiplies in restricted sites minimizes the potential risk of recombination with other naturally-occurring orthopoxviruses. In addition, serological surveys [14, 15] have shown that the prevalence of cowpox virus, the orthopoxvirus which circulates in European wildlife, is low in its reservoir species and experimental studies have shown that the fox, the main target species of vaccination, is only moderately susceptible to cowpox infection [16]. Therefore, the risk of recombination between vaccinia virus and cowpox virus occurring in the wild is minimal. In the previously described experiments, no difference was observed between the multiplication sites of either VVTGg RAB or vaccinia virus, demonstrating that recombination did not modify the tropism of the virus. In particular, virus was never detected in the brain [11, 16].

It is also of major importance to avoid potential epizootiological risks, such as the emergence of asymptomatic carriers of wild rabies virus. This situation could occur in the field if animals incubating rabies then took up the vaccine. The influence of vaccination with VVTGg RAB on the onset of the disease and on the delay before death in foxes previously infected with wild rabies virus, has been investigated [11]. All foxes, vaccinated or not, died from rabies, but animals vaccinated soon after infection with rabies died after a shorter period than unvaccinated controls. On the other hand, animals vaccinated belatedly (14) days) after infection with rabies died after the unvaccinated controls. These results show that 'early' and 'late' death phenomena occur as a consequence of interactions between oral vaccination with VVTGg RAB and rabies infection,

but preclude the risk of the emergence of asymptomatic carriers of wild rabies virus after vaccination.

Safety of the VVTGg RAB for non-target species

Field trials with attenuated strains of rabies virus have shown that several non-target wildlife species compete with foxes for bait [17, 18]. It must also be taken into account that vaccinia virus has a wide-host range [19]. Bait uptake surveillance and tetracycline (biomarker) detection controls, performed after vaccination campaigns, proved that mustelids (e.g. badgers), wild boar (*Sus scrofa*) and domestic carnivores ingest the vaccine baits. Moreover, a significant proportion of the baits is partially eaten by small mammals. Therefore, it is important to verify the safety of VVTGg RAB for both domestic and wild non-target species. Several European non-target wild species have been tested because of their opportunistic feeding behaviour and their presence in the areas where the vaccine must be distributed. Safety of the vaccine has been tested in daubenton bat (*Myotis daubentoni*), wild boar, Eurasian badger (*Meles meles*), woodmouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), 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*) [18]. Clinical signs and/or pox lesions were never seen in the vaccinated animals during observation periods of up to 28 days. Similar experiments were performed in North America [11]. A complete list of the many species tested is given elsewhere [4].

Efficacy trials in the field with the vaccinia-rabies recombinant virus

Taking into account all the available experimental data concerning the safety of the VVTGg RAB for target and non-target species and its efficacy in foxes, initial limited field trials of fox vaccination were authorized first by the Belgian [20], and then by the French Public Health authorities. In the Belgian trial a total of 250 vaccine-baits (chicken heads) were delivered manually during 17–18 October 1987 over a 6 km² area situated in the central part of a military zone.

With the safety of the VVTGg RAB confirmed by this small trial and the laboratory studies, the Belgian authorities agreed to an enlarged open field trial [17]. This was conducted in a 435 km² area in the south of the country, chosen because it has the lowest average human population density in the country (42 inhabitants/Km) but a high incidence of fox rabies. Furthermore, the region has a varied fauna, including most of the animal species likely to take the bait. Each bait contained a 2·2 ml suspension of 10–8 $TCID_{50}$ of vaccine within a plastic sachet; tetracycline (150 mg) was also incorported as a long-term biomarker of bait uptake.

After the vaccination campaign, 222 dead wild animals belonging to 19 species were collected in the vaccination area. After necropsy, the following organs were removed: brain for rabies diagnosis, jaws for tetracycline detection, and blood for the titration of vaccinia and rabies antibodies. Tetracycline was detected in foxes, stone martens (*Martes foina*), feral domestic cats, woodmice, wild boars and carrion crows; these animals were strong competitors of the foxes for the bait. Twelve months of monitoring failed to detect any ecological or public health hazard. The vaccine was very stable even after natural freezing and thawing cycles.

Three fox vaccination campaigns using VVTGg RAB were then carried out in order to check for efficacy in November 1989, April 1990 and October 1990, in a Belgian area of 2200 km² with a mean baiting density of 15 baits/km [21]. After each vaccination campaign foxes found dead, or shot by hunters, were collected for rabies diagnosis and bone tetracycline analysis. During the three campaigns 10 rabid and 178 healthy foxes were collected. The bait uptake rate determined from tetracycline status of 23 adult foxes, 9 of which were rabid, during the first period was 74%. During the second collection period bait uptake rates were 80% (25/31) in adult foxes, but only 49% (27/55) in juveniles. No rabid fox was recorded during this period out of 86 sampled. After the third phase of vaccination (October 1990) 81% (64/79) of inspected animals were tetracyline positive. Only one rabid animal was detected. This was found at the periphery of the baited area, and was tetracycline negative.

Despite this dramatic decrease in the number of rabid foxes, the success of the vaccination campaign was difficult to evaluate because systematic collection of foxes is not feasible. However, notification of rabies in cattle and sheep is mandatory in Belgium. Therefore, the incidence of rabies in domestic livestock provides a reliable indicator of the prevalence of

rabies in the wild because transmission of rabies to domestic animals occurs through the bite of a rabid wild animal. No case of livestock rabies has been recorded in the study zone since the second phase of vaccination; this confirms the success of these trials.

Large scale vaccination campaigns

Five major campaigns conducted during the 3 years 1989–91 and covering the whole of the infected area in Belgium induced a drastic decrease in the incidence of rabies and eliminated the disease from the major part of the initial infected area [22]. As shown by the geographical distribution of rabies cases in 1992, the spatial pattern of rabies has changed; all of 17 cases of fox rabies occurred within 20 km of the French border and more than 70% of these were < 5 km from the border. During this period, France reported numerous rabies cases in the contiguous area.

The successive 'defence' campaigns carried out along the border in 1992, combined with vaccination operations in neighbouring countries, resulted in the complete elimination of fox rabies in Belgium by 1993. However, during this year, two cases (one badger and one domestic cat) were detected close to international borders. According to the recommendations of the World Health Organization, epidemiological surveillance by culling a maximum number of foxes had been increased and 488 rabies-negative foxes were collected in 1993 [22]. As a second consequence of fox rabies control, only one case of rabies was reported in domestic carnivores during 2 years. This reduction could have a beneficial effect on the free movement of pets within the European Community by leading to the abolition of quarantine containment in some disease-free countries.

The number of people who were vaccinated after contact with a suspected rabid animal has also decreased markedly; 71 people were treated in 1993, of whom only six were exposed to an animal confirmed as infected. Unfortunately, in 1994 rabies was again confirmed in 41 foxes and 18 domestic animals in an area of Belgium close to the French border. These introduction justified further restricted vaccination campaigns [23]. Nucleotides sequencing for molecular epidemiological investigations showed that a common rabies virus strain was prevalent both in France and Belgium [23].

VVTGg RAB efficacy has also been attested to some North American rabies vectors such as raccoon and coyote. In 1992, the first large scale field trial of raccoon vaccination was started on the mainland in New Jersey State. Since then, several campaigns have also been conducted in Massachussets and New York States for raccoon vaccination and in Texas for coyote vaccination [4].

CONCLUSIONS

Due to its efficacy, safety and heat stability, the vaccinia rabies recombinant vaccine offers an excellent alternative to attenuated strains of rabies virus for the control of wildlife rabies. In addition to the efficiency of the vaccine-bait combination, the temporal and spatial strategy of bait delivery is of major importance for achieving immunization of the fraction of the fox population required for elimination of rabies. In this sense, the heat stability of the VVTGg RAB-bait system is an important advantage because it provides more flexibility in planning a campaign. For instance, for fox vaccination in Europe, the vaccine can be distributed during the cold season when the population density is the lowest of the year; at this time, a baiting campaign can more easily ensure the protection of the required fraction of the fox population.

In Western Europe, the use of the recombinant vaccine has led to the elimination of sylvatic rabies from large areas in several countries. Further vaccination is therefore no longer necessary. The continued absence of rabies from these areas provides proof that rabies virus has been eliminated from the fox population. The current epidemiological situation is characterized by the persistence of foci of rabies near national borders and by the reinfection of areas previously freed of rabies. Despite very good cross-border cooperation, some reinfections were due to the difficulty of coordinating vaccination plans between neighbouring countries, other were due to misplaced confidence in the results obtained early in the campaign. Therefore, vaccination campaigns should continue for at least 18 months after the last detected case and when neighbouring countries are still infected.

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