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Biological control of wild animal infections

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Until recently, there were few examples of biological control of wild animal infections. The most significant development in this field is the use of a vaccinia–rabies recombinant virus or other recombinants for the control of rabies by oral vaccination of vectors, both in Europe and in North America.

Current Opinion in Biotechnology 1991, **2**:465–469

Introduction

Until recently, there have been few examples of biological control of wild animal infections. As far as biotechnological control is concerned, new developments have mainly focused on biopesticides, such as genetically engineered baculoviruses [1•,2]. In these cases, the infection of insects is used in a similar way to conventional pesticides, so that the insect population is controlled.

Biotechnological control of wildlife infections is difficult to bring about, mainly because wild animals are free-ranging, making them difficult to handle by conventional veterinary means. Vaccination is the most appropriate way of controlling infections at a population level but, in these cases, the only practical route of administration is the oral one. Several attempts have been made in the past to control wildlife infections through parenteral administration of vaccines either directly (e.g. distemper virus infection of the Black footed ferret, *Mustela nigripes*, in the USA) [3], or via a gun (e.g. vaccination of roan antelopes, *Hippotragus equinus*, against anthrax in South Africa) [4].

In both cases, very few animals were vaccinated for conservation purposes. Larger scale vaccination campaigns against rabies by parenteral administration of inactivated vaccines to foxes have also been undertaken in Germany, but these were quickly replaced by oral vaccination campaigns because of practical difficulties.

Sylvatic rabies provides the best example of biotechnological control of a wildlife infection. In northern countries, rabies is a zoonotic disease, the epidemiology of which is solely linked to a wildlife reservoir. In western Europe, the main vector is the red fox (*Vulpes vulpes*), whereas in northern America, it is either the red fox, the raccoon (*Procyon lotor*) or the striped skunk (*Mephitis mephitis*). In this respect, 'vector' refers to the animal host that is most susceptible to rabies in a region at a given time, and that is solely responsible for maintaining the infection. The control of the infection within the vector species thus permits overall control of the infection and, most importantly, eliminates the risk of transmission to man.

In western Europe, control measures by reducing fox populations were only temporarily effective and did not prevent the disease from spreading. For this reason, other methods such as oral immunization of foxes needed to be assessed. Research has focused on oral vaccination, the only procedure allowing the immunization of a sufficient proportion (75%) of wild foxes through the distribution of vaccine baits. Since 1978, several European countries have organized large-scale field trials of oral vaccination of foxes using the standard, modified, attenuated strain of B19 rabies virus, Street Alabama Dufferin (SAD) [5,6]. The promising results obtained from these vaccination campaigns attest to the feasibility and the efficacy of the method. 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, are heat-sensitive [7] and may still be pathogenic for man. Pathogenicity of attenuated rabies virus strains can be abolished by mutating Arg residues at position 333 of the glycoprotein, although such strains can still revert to virulence [8,9••]. Thus, in order to improve both the safety and stability of the vaccines used, a recombinant vaccinia virus, which expresses the immunizing glycoprotein of rabies virus, has been developed and tested in the field for oral vaccination of wildlife vectors against rabies.

Construction of the vaccinia–rabies recombinant virus

The glycoprotein of rabies virus is the sole viral protein present on the external surface of the membrane. It is the only viral antigen capable of eliciting the formation of rabies virus-neutralizing antibodies (VNAs) and has been shown to be capable of conferring immunity to rabies.

Abbreviations

SAD—Street Alabama Dufferin; **$TCID_{50}$**—median tissue culture infective dose; **TK**—thymidine kinase; **VNA**—virus-neutralizing antibody.

 ISSN 0958-1669

Thus, the rabies virus glycoprotein seems to be an ideal candidate for use in the construction of subunit vaccines.

Nucleotide sequence analysis of the glycoprotein gene reveals an open reading frame of 524 amino acids. Recent technical advances have permitted the development of vaccinia virus as a cloning and expression vector [10•]. Expression of exogenous protein-coding sequences in vaccinia virus involves essentially 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. Second, 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, wherein it is propagated and expressed. The rabies virus glycoprotein gene has been inserted into the thymidine kinase (TK) gene of vaccinia virus in this way, generating a selectable TK^- virus [11,12] known as VVTGgRAB.

Similar recombinants have been prepared [13] using both vaccinia virus or a raccoon poxvirus [14] for raccoon oral vaccination and, more recently, adenovirus recombinants have been constructed. However, the VVTGgRAB recombinant is the only one to have been tested in the field so far.

Efficacy and safety of the recombinant vaccinia–rabies virus in the target species

VVTGgRAB has been tested for both efficacy and safety in the main rabies-target species in western Europe and North America: fox, raccoon and striped skunk [15,16,17,18••]. The results of tests for efficacy in foxes can be summarized as follows. All but one out of 26 adult captive foxes inoculated by various routes developed high titres of rabies VNAs, and resisted wild rabies virus challenge on day 28 after vaccination. Immunity conferred by VVTGgRAB (10^8 plaque-forming units; oral route) lasted for at least 18 months, which is more than adequate, as most foxes in the wild are under 24 months of age. Foxes receiving less than the recommended dose showed a clear dose-dependent response. The liquid form of VVTGgRAB seems to be more efficient than the freeze-dried version, when administered by the oral route. A second administration of VVTGgRAB induces an increase of rabies VNA titres (booster effect). When administered to fox cubs by the oral route [$10^{7.2}$ median tissue culture infective dose ($TCID_{50}$)], the vaccine induces significant levels of rabies virus antibody and protects 92% of foxes (11/12) against rabies, with the duration of immunity exceeding 12 months [19].

Oral administration is the only route appropriate for the vaccination of wild animals. Accordingly, the vaccine must be presented in a form suitable for ingestion. The vaccine vehicles (bait plus container) can 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 VVTGgRAB (10^8 $TCID_{50}$) contained in a new machine-made baiting system has been tested recently [20•]. Twenty-two captive young foxes were divided into three experimental groups of six, and a control group of four. Foxes in the first three groups were fed one, two and three vaccine baits, respectively, on successive days. The four unvaccinated foxes were housed separately. As shown by the incorporation of a tetracycline biomarker into their bones, all the baited foxes ingested at least one bait. Thirty days after baiting, seroconversion to rabies was observed in 15 out of 18 of the foxes and seroconversion to vaccinia in 14 out of 18. Of the 18 baited foxes, 16 resisted a wild rabies virus challenge 90 days after baiting. One cub was protected against rabies despite the absence of detectable anti-rabies antibody. These results demonstrate that the baiting-sachet system used permits an efficient release of the virus suspension into the mouth.

A vaccine virus must not only be efficacious but also innocuous for the target species. VVTGgRAB was observed to be non-pathogenic in the fox whatever the dose of inoculation (10^2–10^{10} $TCID_{50}$) or route of administration (oral, intramuscular, intraduodenal, subcutaneous, intradermic, conjunctival or intranasal).

It is preferable that a vaccine virus used for oral vaccination of wildlife is not horizontally transmitted to unvaccinated animals. In order to test for horizontal transmission, unvaccinated control animals have been held in close contact with vaccinated ones. Using this assessment method, no transmission of immunizing amounts of VVTGgRAB were found to occur in adult or young foxes, with the exception of one adult fox bitten by a freshly inoculated one.

It is also of major importance to preclude epizootiological risks, such as the emergence of asymptomatic carriers of wild rabies virus [21]. This situation could occur in the field by the vaccination of naturally infected animals during the incubation period. The influence of vaccination with VVTGgRAB on the onset of the disease and on the delay before death in foxes previously infected with wild rabies virus, has been investigated. All foxes, vaccinated or not, died from rabies. Animals vaccinated early after challenge died after a shorter period of incubation than unvaccinated controls. On the other hand, animals vaccinated belatedly after challenge died after the controls. These results show that 'early' and 'late' death phenomena occur as a consequence of interactions between oral vaccination with VVTGgRAB and rabies infection, but preclude the risk of the emergence of asymptomatic carriers of wild rabies virus after vaccination.

Similar results have been obtained with the raccoon and the skunk. The safety of the VVTGgRAB for administration to pregnant female raccoons has been evaluated, revealing an absence of epigenetic transmission to offspring, as well as an absence of the recombinant in the cerebrospinal fluid [22]. There was no evidence of active *in utero* or lactogenic transmission of the recombinant. These results are in contrast with those obtained with attenuated strains of rabies virus in the skunk [23].

Safety for non-target species

Field trials with attenuated strains of rabies virus have shown that several wildlife non-target species compete with foxes for bait consumption. It must also be taken into account that, within the orthopoxvirus group, vaccinia virus is a wide-host-range species. In fact, bait uptake surveillance and tetracycline (biomarker) detection controls, performed after vaccination campaigns, proved that mustelids, wild boars (*Sus scrofa*) and domestic canivora can ingest the vaccine baits. Moreover, a significant proportion of the baits are partially eaten by micromammals. It is important therefore to verify the safety of VVTGgRAB 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 behaviour and their presence in the areas where the vaccine must be distributed [24]. Safety of the vaccine has been tested in daubenton bat (*Myotis daubentoni*), wild boar, eurasian badger (*Meles meles*), wood mouse (*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*). Clinical signs and/or pox lesions were never observed in the vaccinated animals during the observation period (28 days minimum after vaccination).

Similar experiments have been carried out with wild species from North America [25], including meadow vole (*Microtus pennsylvanicus*), woodchuck (*Marmota monax*), grey squirrel (*Sciurus carolinensis*), ring-billed gull (*Larus delawarensis*), red-tailed hawk (*Buteo jamaicensis*), great horned owl (*Bubo virginianus*) and coyote (*Canis latrans*). Comparable results were obtained in these studies.

Tissue specificity and multiplication site of VVTGgRAB in foxes

When assessing a recombinant virus for use in vaccination, it is also of great importance to detect any variations in tissue-specificity compared with the parental vector strain. Experiments have been designed to determine the multiplication site in foxes of the recombinant virus compared with the parental strain of vaccinia virus, by virus isolation, titration and indirect immunofluorescence. The polymerase chain reaction was also used to detect specific virus DNA in several fox organs [26••]. Foxes were fed with 10^8 $TCID_{50}$ of either VVTGgRAB or vaccinia virus, and were destroyed 12, 24, 48 or 96 h after inoculation by the oral route.

Using these different techniques, VVTGgRAB or vaccinia virus could be detected during the first 48 h following vaccination by the oral route, but only in the tonsils, buccal mucosa and soft palate. Similar results have been obtained in raccoons using virus isolation [16]. Results of other experiments demonstrate that tonsillectomy of foxes does not completely impede seroconversion (I Thomas *et al.*, unpublished data).

As no virus could be detected in the salivary glands of foxes (parotid and maxillary), the risk of transmission from one animal to another through saliva can be neglected. Furthermore, the fact that VVTGgRAB only multiplies in restricted sites, minimizes the potential risk of recombination with other wild orthopoxviruses. In these experiments, no difference was observed between the multiplication sites of either VVTGgRAB or vaccinia virus, demonstrating that the recombination event did not modify the tropism of the virus. Additionally, virus was never detected in the brain. These results are consistent with those from studies on raccoons orally vaccinated with VVTGgRAB [22], that report the absence of detectable cytological abnormalities in the cerebrospinal fluid.

Field trials with the vaccinia–rabies recombinant virus

Taking into account all the available experimental data concerning the safety of the VVTGgRAB for target and non-target species and its efficacy in foxes, initial, limited field trials of fox vaccination were authorized first by the Belgian [27], and then by the French Public Health authority. In the Belgian trial (17–18 Oct 1987) a total of 250 vaccine-baits (chicken heads) were delivered manually on a 6 km^2 area situated in the central part of a military zone.

With the safety of the VVTGgRAB confirmed by this small trial, the Belgian authorities agreed for an enlarged open field trial [28••]. This was conducted in a 435 km^2 area in the southern part of the country, chosen because it has the lowest human population average density in the country (42 inhabitants km^{-2}), as well as a high rabies incidence in foxes. Furthermore, the region is characterized by various biotopes, including most of the animal species susceptible to bait consumption. Each bait contained a suspension of 10^8 $TCID_{50}$ of VVTGgRAB (2.2 ml by volume) within a plastic sachet and 150 mg tetracycline 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 vaccine and rabies antibodies.

Tetracycline was detected in foxes (61%), stone martens (*Martes foina*), domestic feral cats (*Felis catus*), wood mice (*Apodemus* sp.), wild boars and carrion crows, showing that wild boars, mustelids and feral cats are strong competitors of the foxes for bait uptake. Twelve months of monitoring failed to detect any ecological hazard or public health concern. The vaccine was very stable even following natural freezing and thawing cycles.

Three fox vaccination campaigns using VVT Gg-RAB were then carried out in Belgium in November 1989, April 1990

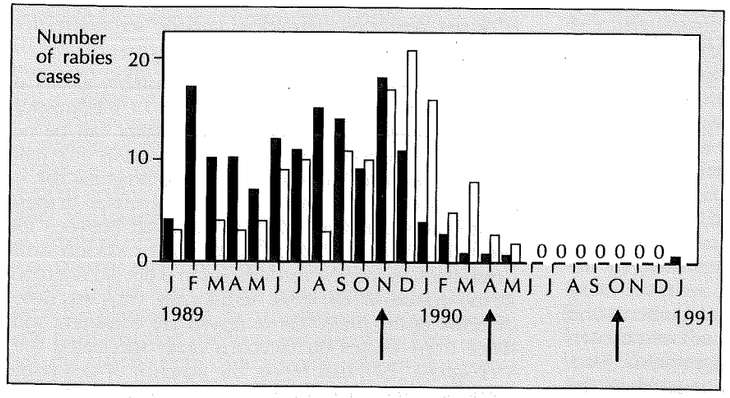

Fig. 1. The monthly evolution of animal rabies in a 2200 km² area treated three times (arrows) with the recombinant vaccinia–rabies virus, VVTGgRAB. ■, wild animals; □, domestic animals.

and October 1990, in order to check for efficacy in an area of 2200 km² with a mean baiting density of 15 baits km^{-2} [29]. Field controls of bait uptake performed after these releases revealed that 80–96% of the baits are taken by animals after 30 days. As shown in Fig. 1, rabies incidence has decreased severely in the treated area, despite the high density of fox population observed during the same period.

Perspectives

Because of its efficacy, safety and heat-stability, VVTGgRAB appears to be an excellent alternative to the attenuated strains of rabies virus currently used in the field. Local eradication of fox rabies may be achieved after three or four vaccination campaigns, if the following requirements are met: a potent, safe and thermostable vaccine; an efficient and resistant baiting system; a practical and efficacious method of bait dispersal; and an effective spatial and temporal pattern of bait distribution.

The first limited trial of raccoon vaccination against rabies has just been carried out in the United States [30]. This overall methodology could be extended to the control of other wildlife infections. Unfortunately, preliminary attempts to vaccinate harbour seals (*Phoca vitulina*) against phocine distemper with a vaccinia recombinant bearing the gene coding for a fusion protein of canine distemper morbillivirus, via the oral route, have failed (M-F van Bressem, personal communication). Thus, the only way to vaccinate seals against Phocine distemper remains the administration of inactivated vaccines by the parenteral route [31].

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Current Opinion in BIOTECHNOLOGY

Volume 2 Number 3 June 1991