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.


Introduction
Until recently, there have been few examples of biological control of wild animal infections. As far as biological control is concerned, new developments have mainly focused on bioposticides, 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 oral 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 convenience. Larger scale vaccination campaigns against rabies by oral 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 V198 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, and 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 antibodies against rabies virus-neutralizing antibodies (VNA) and has been shown to be capable of confering immunity to rabies.

Abbreviations
SAD—Street Alabama Dufferin; TCID50—median tissue culture infective dose; TK—thymidine kinase; VNA—virus-neutralizing antibody.
Thus, the ravies virus glycoprotein seems to be an ideal candidate for use in the production 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 recombinant viral proteins expressed in insect cells [10]. Expression of exogenous protein-coding sequences in vaccinia virus involves essentially two steps. First, the exogenous DNA is introduced into a vaccinia promoter and inserted into a site (a nonessential segment) of vaccinia DNA cloned into a suitable bac teriophage vector. The recombinant vaccinia sequences permit homologous recombination in vitro between the plasmid and the viral genome. Double recombinants, in which a segment of the DNA insert is transferred from the plasmid to the viral genome, wherein it is propagated and expressed. The raves 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 VVTGGRB.

Safety for non-target species

Field trials with attenuated strains of raves 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, bat uptake surveillance and tetracycline (biomarker) detection continued performed after vaccination campaigns, proved that mustelids, wild boars (Sus scrofa) and domestic carnivores can ingest the vaccine viruses. Moreover, a significant proportion of the bats are partially eaten by mico tammals. It is important therefore to verify the safety of VVTGGRB 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 shrew (Sorex araneus), water shrew (Neomys fodiens), carrion crow (Corvus corone), magpie (Pica pica) and Jay (Garrulus glandarius). Clinical signs and/or post-vaccination lesions were not observed in the vaccinated animals during the observation period (28 days minimum after vaccination).

Tissue specificity and multiplicity site of VVTGGRB 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 multiplicity of infection of the recombinant and non-recombinant vaccinia virus compared with the parental strain of vaccinia virus, by virus isolation, titration and indirect immunofluorescence. In these experiments, the recombinant raves virus was used to detect specific virus DNA in several fox organs [20,21]. Foxes were fed with 10^6 TCID50 of either VVTGGRB or vaccinia virus for 12, 24, 48 or 96 h after inoculation by the oral route.

Using these different techniques, VVTGGRB or vaccinia virus could be detected during the 1st week following vaccination in the muscles, brain, liver, spleen, lymph nodes, lungs, diaphragm, and buccal mucosa and soft palate. Similar results have been obtained in raves in tissues infected with virus isolation [16]. Results of other experiments demonstrate that tonsillolymph nodes of foxes does not completely impermeate tetracycline (20 mg/kg) in the liver, stomach and intestine. As no virus could be detected in the salivary glands of foxes (parotid and mandibular), the risk of transmission from one animal to another through saliva can be neglected. Furthermore, the fact that VVTGGRB only multiplies in restricted sites, minimizes the potential risk of recombination with other wild orthopoxviruses. In these experiments, no differences were observed between the multiplicity sites of either VVTGGRB or vaccinia virus, demonstrating that the recombination event did not modify the tropism of the virus, which was never detected in the brain. These results are consistent with those from studies on raccoons orally vaccinated with VVTGGRB [22], that report the absence of detectable cytological abnormalities in the cerebrospinal fluid.
and October 1990, in order to check for efficacy in an area of 2,000km² with a mean bathing density of 15 bats per
area. Field controls of bat uptake performed after these releases revealed that 80-90% of the bats are taken by
animals after 30 days. As shown in Fig. 1, rabbit inci-
dence 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 feasibility, VTVG-
G843 appears to be an excellent alternative to the attenu-
ated strains of rabies virus currently used in the field. Lo-
cal eradication of foxes may be achieved after three or
four vaccination campaigns, if the following require-
ments are met: a potent, safe and thermally stable vaccine; an efficient and reproducible vaccination system; a practical and
efficacious method of bat dispersal; and an effective spa-
tial and temporal pattern of bat distribution.

The first limited trial of this vaccine against rab-
ies has just been carried out in the United States [50].
This overall methodology could be extended to the con-
trol of other wildlife infections. Unfortunately, prelimi-
ary 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 multilines, via the oral route, have failed (M.F.van Hemmen, personal communication). Thus, the
only way to vaccinate seals against phocine distemper re-
lates the administration of inactivated vaccines by the
parenteral route [51].

References and recommended reading
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