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FIELD TRIALS OF A RECOMBINANT RABIES VACCINE

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ABSTRACT

To improve both safety and stability of the vaccines used in the field to vaccinate foxes against rabies by the oral route, a recombinant vaccinia virus, expressing the glycoprotein of rabies virus (VVTGgRAB) has been developed. VVTGgRAB innocuity was verified in target species and in domestic animals as well as in numerous wild animal species that could compete with the red fox in consuming vaccine baits in Europe. Oral immunization of foxes, by distributing VVTGgRAB vaccine-baits, was undertaken for the whole infected area in Belgium (10000 km 2). Five campaigns of fox vaccination, were carried out from autumn 1989 until 1991. Each time, 150000 vaccine-baits were dropped by air at a mean density of 15 per km^2. These campaigns induced a drastic decrease in the incidence of rabies and the elimination of the disease from 80 % of the initially infected area. Regarding the geographical evolution of rabies in Belgium and in adjacent regions in neighbouring countries, new spatial strategies for bait dispersal were planned for 1992, 1993 and 1994: successive confined campaigns were carried out along political borders only. These campaigns induced a new decrease of incidence; no rabid fox could be detected in 1993 in spite of an improved epidemiological surveillance. In 1994, rabies was again confirmed in 13 foxes collected in an area close to the French border. These cases demonstrate the persistence of a border rabies focus and justify further restricted vaccination campaigns.

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Introduction

Rabies is a greatly feared disease still prevalent in many countries in most parts of the world. It may be maintained in two, not necessarily inter-related cycles: urban and sylvatic. Urban rabies, affecting stray and feral dogs and cats, is by far the most dangerous to man, 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 species in particular locations, and this pattern remains stable over many years. The wild animal 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 terrestrial epizootic of rabies has spread some 1400 kilometers westward from Poland since 1939. Although it involves all susceptible species, both wild and domestic, the red fox is involved in more than 75 % of cases. In Western Europe, the fox seems 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, therefore, cease to be a problem for man.

The control of fox rabies is used as an example in the following review; nevertheless, many different epidemiological cycles exist in the world, either urban or sylvatic, 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 (Pastoret, Boulanger & Brochier, 1994).

Control of fox rabies

Prophylactic measures taken in the past, such as the destruction of foxes to reduce fox populations, 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 fox vaccination by the oral route, 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 allowing the immunization of a sufficient proportion (75 %) of wild foxes through the distribution of vaccine baits. Therefore, the only applicable vaccines were either attenuated strains of rabies virus or live vectored vaccines. (Aubert, 1994).

Even so, as far as safety and stability are concerned, the use of attenuated rabies virus remains controversial because these virus strains are still pathogenic for laboratory and wild rodents (Artois *et al.* 1992) wildlife species, such as the chacma baboon (*Papio ursinus*) (Bingham *et al.*

1992), or target species, such as the striped skunk (Rupprecht *et al.* 1990); moreover, these strains may still be pathogenic to man. Thus, humans exposed to SAD-derived attenuated strains of rabies must be treated with a conventional inactivated rabies vaccine. SAD-derived attenuated strains may also be inefficient in certain rabies vectors, such as the raccoon in North America (Rupprecht *et al.* 1986). Because of their residual pathogenicity, the use of attenuated strains of rabies virus for domestic animal vaccination in Western Europe has been discontinued.

Pathogenicity of attenuated rabies virus strains can be abolished by mutating arginine residues at position 333 of the rabies virus glycoprotein. This has led to the development of a new attenuated vaccine strain, which is already in use in the field (Leblois *et al.* 1990). Nevertheless another inconvenience of attenuated strains of rabies virus is their heat-sensitivity, which reduces their potential efficacy in field conditions.

Thus, in order to improve both safety and stability of the vaccines used for fox vaccination in the field, a recombinant vaccinia virus has been developed that expresses the immunizing glycoprotein of rabies virus. This virus vaccine has been tested in the field for oral vaccination of foxes against rabies (Blancou *et al.* 1986; Pastoret *et al.* 1992).

Development of a vaccinia-rabies vector vaccine for oral vaccination of wildlife against rabies

The glycoprotein of rabies virus is the sole viral protein present on the external surface of the viral membrane. It is the only viral antigen capable of eliciting the production of rabies virus-neutralizing antibodies and has been shown to be capable of conferring immunity to rabies. Thus, the rabies virus glycoprotein is an ideal candidate for use in the construction of a subunit marked vaccine. The rabies virus glycoprotein gene has been inserted into the thymidine-kinase (TK) gene of vaccinia virus (VV), generating a selectable TK-virus (Kieny *et al.* 1984 *;* Wiktor *et al.* 1984) known as VVTGgRAB, which is safer than the parental strain (Buller *et al.* 1985). VVTGgRAB has been tested for efficacy and safety in the main target species in Western Europe and North America ; fox, raccoon and striped skunk. The duration of protection conferred by VVTGgRAB (a minimum of 18 months in adult animals) corresponds to the length required for fox vaccination in the field due to the high turnover of the fox population.

The preclusion of epizoological risks, such as the emergence of asymptomatic carriers of wild rabies virus, is also of major importance. This situation could occur in the field by vaccination of naturally infected animals during the incubation period. The influence of vaccination with VVTGgRAB, both on the onset of the disease, and on the delay before death in foxes previously infected with wild rabies virus, has been investigated (Brochier *et al.* 1989a). The 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.

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It is also preferable that a vaccine virus used for oral vaccination of wildlife should not be horizontally transmitted to unvaccinated animals. Accordingly, no transmission of immumzing amounts of VVTGgRAB was found to occur in adult or young foxes. Changes in tissue tropism were also not observed (Thomas *et al.* 1990). In areas of Europe earmarked for vaccine distribution, several non- target wild species were chosen for safety testing, both because of their opportunistic feeding behaviour, and because of their presence in the area (Brochier *et al.* 1989b) ; similar experiments were carried out on wild species from North America. In every case, the recombinant virus was always perfectly safe. More recent experiments have also shown that the recombinant virus, administered either by scarification or by the oral route, is also safe for squirrel monkeys (*Saimiri sciureus*) and for chimpanzees (*Pan troglodytes*) (Rupprecht *et al.* 1992). Additional experiments were performed on several species (including cows) in contact with control animals to test for horizontal transmission of VVTGgRAB. Without exception, the results showed that no horizontal transmission took place.

The only remaining perceived risk to be investi- gated was the eventual recombination of the recombinant virus with a wild orthopox virus. For such an event to occur, both parental viruses must multiply during the same period of time in the same cells of the same animal. As no serological evidence for orthopox virus infection in the fox population has been found, however, this risk may be discarded in the main target species. Moreover, experimental inoculation of cowpox virus into foxes via the oral route results in viral multiplication only at a low level and for a short duration in the mouth cavity (Boulanger *et al.* in press).

Taking into account these epidemiological and experimental data, it is most unlikely that recombination between VVTGgRAB and another orthopox virus could occur in the vaccinated foxes. It is, therefore, preferable to choose a recombinant virus that has no counterpart in the wild (e.g. vaccinia virus) and that, besides a long history of use in uncontrolled conditions, has never been established in wildlife. Thus, a vector virus previously unencountered by wildlife, but with a wide host range, is, for safety reasons, better than another virus isolated from a target species (e.g. raccoonpox virus) that is still prevalent in the wild. The fact that vaccinia virus has been used for more than 150 years without any undesirable ecological impact, such as installation in wildlife, also argues strongly for its choice.

Development of a vaccine bait system

The development of an efficient baiting system is important since an attractive bait permits the self-vaccination of the target species. The VV.TGgRAB vaccine suspension consists of the supernatent of a Baby Hamster Kidney (BHK) cells culture infected with VVTGgRAB. The viral suspension medium is a saline solution added with gentamycine (125 µg/dose). The baiting-system is formed from an appetant mixture of fish meal (SO %) and fish oil (11 %) aggregated using an hydrophobic synthetic polymer (11 %). A sealed polyethylene sachet containing 2·5 ml liquid vaccine (titrating approximately 108 CCIDSO) is fixed into the bait with a binding agent (11 %). Tetracycline hydrochloride, introduced into the appetant mixture bait (150 mg/bait), serves as a

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biomarker for bait uptake. This machine- made vaccine-bait system (RABORAL®) forms a rigid 5 x 3 x 2 cm parallelipiped weighing from 34 to 40 g. The RABORAL® vaccine-bait can be stored without freezing (at 4 °C) and because of its mechanical resistance can be droppedd by air.

Deliberate release of the vaccinia rabies recombinant virus for oral vaccination of foxes against rabies

On the basis of all the available experimental data concerning the safety of the VVTGgRAB 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 (Pastoret *et al.* 1988) and then by the French public health authorities.

The Belgian authorizations were preceded by safety assessment (i.e. risk versus benefit) of the use of recombinant vaccinia-rabies virus for fox vaccination against rabies. It was concluded that there was considerable risk of exposure to rabies infection in the target area and that this risk could be reduced through the use of a vaccine (i.e. VVTGgRAB) more efficacious (in terms of immunogenicity and stability) than the vaccines already in use. As far as safety was concerned, clear and identified risks were associated with the use of conventionally attenuated rabies virus strains, such as the SAD B19 strain. It was possible to abolish the risk associated with vaccination by substituting the attenuated virus either with recombinant vaccinia-rabies virus or with rabies virus strains in which arginine at position 333 of the glycoprotein had been modified.

With the safety of the VVTGgRAB confirmed by these small trials, the Belgian authorities agreed to an enlarged open field trial. The vaccine was subsequently shown to be very stable, even following natural freezing and thawing cycles (Brochier *et al.*) 1990). The VVTGgRAB vaccine retained its capacity to immunize for at least one month in field conditions, a period that corresponds to the longest delay of uptake that baits may undergo in the field. Following this enlarged trial, three fox-vaccination campaigns using VVTGgRAB were carried out in Belgium in November 1989, April 1990 and October 1990 in order to check for efficacy in an area of 2200 km 2 (Brochier *et al.* 1991).

The above trials, in which the VVTGgRAB was deliberately released over a 2200 km^2 area of Southern Belgium, were intended to test feasibility of rabies elimination over a large area. On this occasion, the economics of the vaccine-bait dispersal programme were also investigated. The average yearly cost of rabies infection in Belgium (in the period 1980-89), including post-exposure treatments of humans, animal diagnosis, compensation to farmers for the culling of infected livestock and the culling of wild foxes, was estimated to be 400000 ECUs (10000 km^2) , or 88000 ECUs per annum for the area under study. (These figures do not include the cost of vaccination of domestic animals nor the salaries of civil servants.) In comparison, the overall expenditure during the three campaigns of vaccine- bait distribution in Belgium was estimated to be 118000 ECUs. In addition, as 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 VVTGgRAB is econ- omically justifiable. The use of VVTGgRAB has now

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been extended to all contaminated areas in Belgium and the grand duchy of Luxembourg as well as to large areas of France. The vaccine IS presently being tested in the United States.

Towards elimination of rabies in Belgium?

As shown in Fig. 1, Belgium was heavily infected before the campaigns of fox vaccination. The rabies infected area covered 10000 km2 in the southern part of the country. Five campaigns of fox vaccination, covering the whole infected area, were carried out from autumn 1989 until 1991. The two first campaigns (autumn 1989, spring 1990) were carried out using both attenuated rabies virus strain (SAD B19) and VVTGgRAB as vaccines. Since autumn 1990, the VVTGgRAB was used exclusively. Each time, 150000 vaccine-baits were dispersed by air (helicopter or plane) according to a grid resulting in a mean density of 15 baits/km^2.

Five 'full ' campaigns induced a drastic decrease in the incidence of rabies and the elimination of the disease from 80 % of the initial infected area (Fig. 2A). Regarding the geographical evolution of rabies in Belgium and in adjacent regions in neighbouring countries, new spatial strategies for bait dispersal were planned for 1992, 1993 and 1994 ; successive confined campaigns were carried out along political borders only. These campaigns, combined with vaccination operations in neighbouring countries, induced a new decrease of incidence ; no rabid fox could be detected in 1993 in spite of an improved epidemiological surveillance (488 collected foxes were shown to be rabies negative). Nevertheless, rabies was observed in a badger and a domestic cat found close to the international borders (Brochier *et al.* 1994a) (Fig. 2 B). Both these rabies cases were confirmed in the laboratory and nucleotide sequencing obtained from these rabies virus isolates indicated that these rabies cases were from fox origin, demonstrating the persistence of undetected rabies foci of fox origin in Belgium (Bourhy H., personal communication). In 1994 (data until 15 August), rabies was confirmed in 13 foxes collected in a region situated close to the border with France (Fig. 2 C). These cases demonstrate the persistence of a border rabies focus and justify further restricted campaigns of vaccination in both countries. Positive consequences of fox rabies control were obtained in the field of public health, domestic animal health and environ- mental monitoring. Because notification of cases of rabies in cattle is mandatory in Belgium, the incidence of rabies in domestic livestock provides a reliable indicator of the prevalence of rabies in the wild. No case of cattle rabies has been recorded in Belgium since December 1992. Similarly, as a second consequence of fox rabies control, only one rabies case was reported in domestic carnivores in two years. The number of people who received medical treatment (curative vaccination schedule) after con- tact with a suspected animal has also decreased markedly. 71 people were treated in 1993 ; only 6 of them after being actually exposed to an infected animal case confirmed during that year (Brochier *et al.* 1994b).

The diminution of the incidence of rabies in wildlife has had a beneficial effect on the survival of threatened wild species, such as the Eurasian badger (*Meles meles*), in the contaminated area. Estimation of the badgers' population in the treated area shows a gradual increase in number. In fact, Belgium is slowly recovering badger numbers similar to those before 1966, when rabies was

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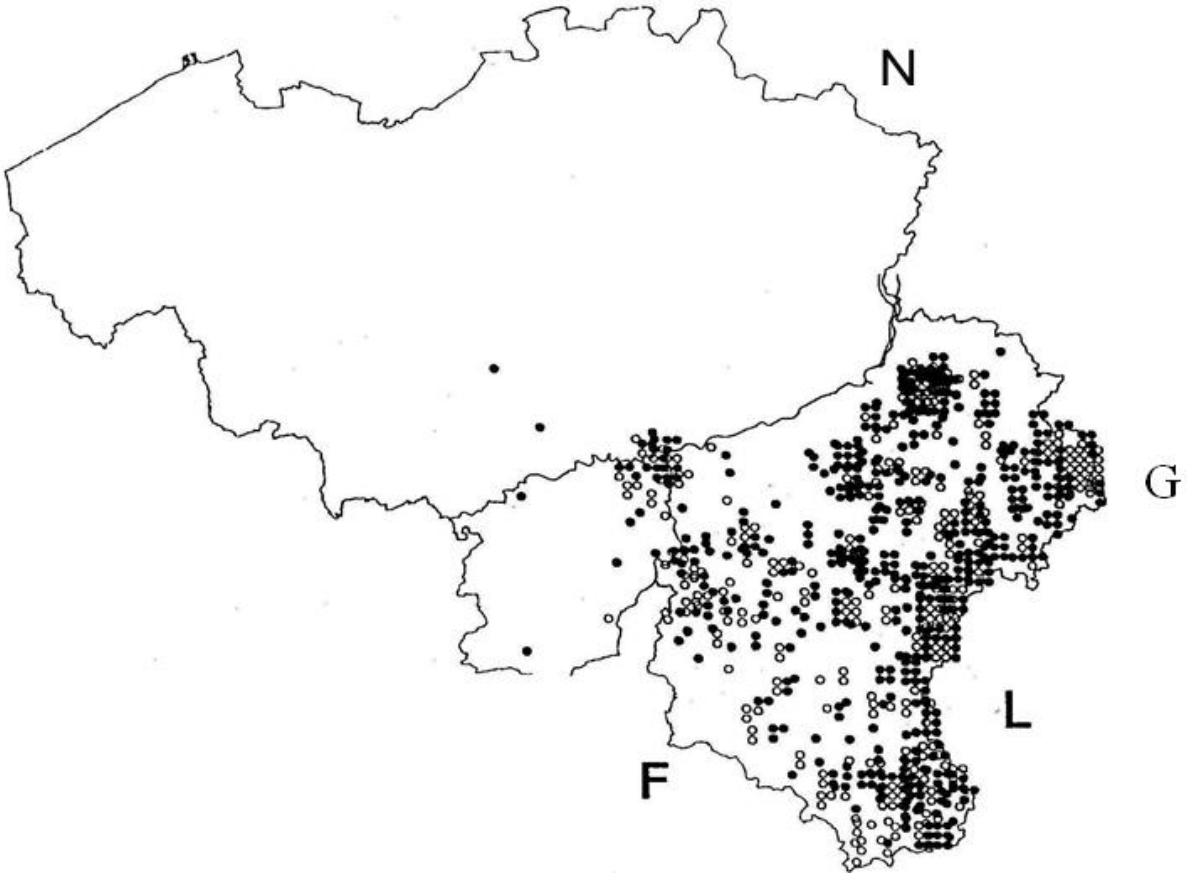
reintroduced from Germany. Finally, elimination of rabies will help to authorize free movement of pets within the European Union.

Figure 1. *Geographical distribution of 841 animal rabies cases in Belgium in 1989*



Légende de la figure. Black dots: 520 wild animals; white dots: 321 domestic animals. Infected area : 10000 km 2 • F: France, G: Germany, L: grand duchy of Luxemburg, N : the Netherlands.

Figure 2. *Geographical distribution of animal rabies cases*

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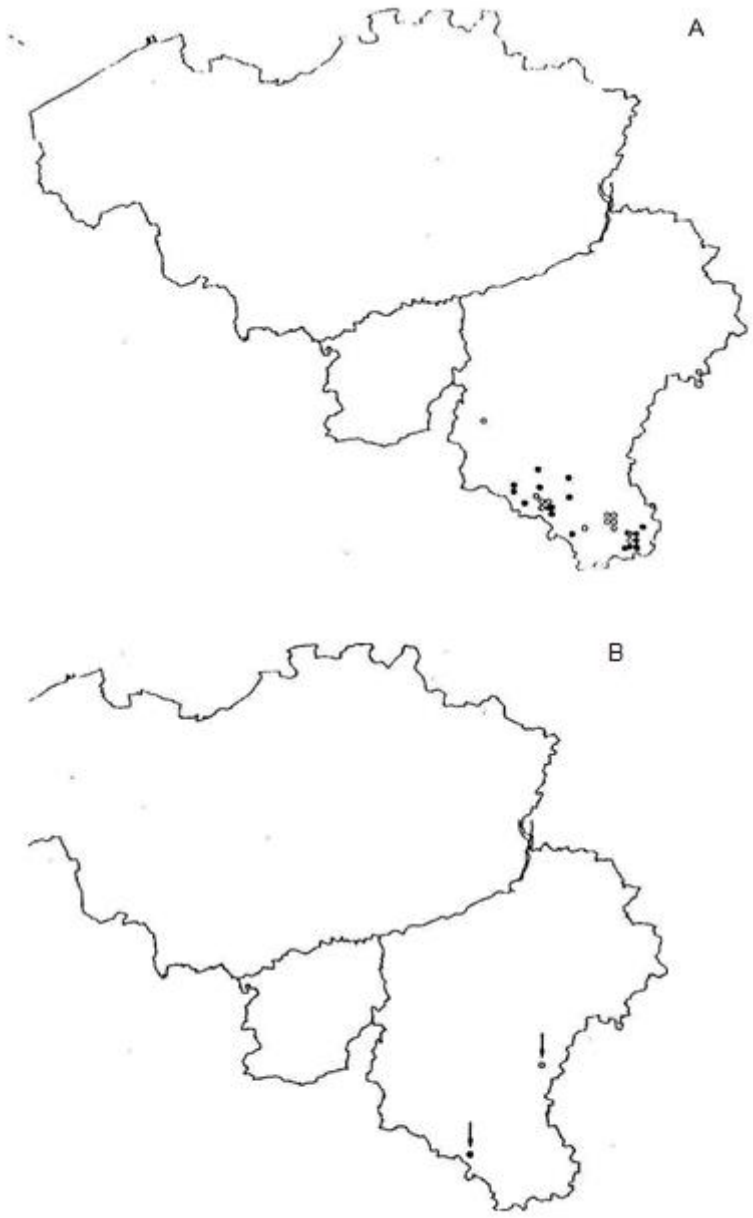

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Légende de la figure. (A) in 1992. (B) in 1993. (C) in 1994 (data until 15 August). Black dots: wild animals; white dots: domestic animals.

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