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ELIMINATION OF FOX RABIES FROM BELGIUM USING A RECOMBINANT VACCINIA-RABIES VACCINE : AN UPDATE

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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 in the whole of the infected area of Belgium (10 000 km^2). Five campaigns of fox vaccination covering the whole infected area were carried out from the autumn of 1989 until 1991. Each time, 150 000 vaccine-baits were dispersed 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 initial 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 restricted campaigns were carried out along political borders only. These campaigns induced a new decrease of incidence; no rabid foxes could be detected in 1993 in spite of an improved epidemiological surveillance. In 1994, rabies was confirmed again in 13 foxes collected in a region situated close to the French border.

These cases demonstrate the persistence of a focus of rabies on the border and justify further restricted campaigns of vaccination.

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Introduction

The main vector of the current epidemic of sylvatic rabies in Europe is the red fox. Here, the term vector means the animals most susceptible to rabies in a region at a given time, solely responsible for maintaining the infection. All other species are victims, even if they are able to transmit rabies. Consequently their destruction or immunization have no effect on the disease cycle. Whereas rabies among domestic animals can be controlled by appropriate prophylactic measures, it poses a bigger problem in wildlife, and until 1960 the only possible available means was considered to be the reduction of vector populations.

As in most other European countries, control measures by reducing the fox population were only temporarily effective and did not stop the spread of the disease. For this reason, other methods such as oral immunization of foxes against rabies needed to be assessed. The principle of this control method consists of the immunization of a fraction of the fox population sufficient to reduce the efficacy of rabies transmission, thus disrupting the viral infection chain (Anderson et al., 1981). Research focussed on oral vaccination, the only procedure appropriate for the immunization of wild foxes in the field (distribution of vaccine baits). There have been numerous experiments in which procedures varied according to the type of vaccine and the type of bait used as vaccine vehicle. The method of vaccinating wild animals against rabies was developed first in the USA (Baer, 1975), then in Europe (Steck et al., 1982), and used for the first time in the field in October 1978 in Switzerland. Since 1978, several European countries have conducted at different times, large scale field trials of oral vaccination of foxes using the SAD, standard or B 19 modified (Schneider and Cox, 1983), attenuated strain of rabies virus. The promising results obtained with these vaccination campaigns attest to the feasibility and efficacy of the method. However, the use of attenuated rabies virus still remains controversial as far as innocuity and stability are concerned, since these virus strains retain pathogenicity for some non target species (Artois et al., 1992; Bingham et al., 1992) and are heat-sensitive. Moreover attenuated strains of rabies virus may still be pathogenic for man; therefore humans exposed to those strains must be treated using standard procedures. Therefore, recent work has focused on technical adjustments, particularly the type of vaccine. 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 has been developed and extensively tested in the laboratory as well as in the field. This vaccine is currently being used on a large-scale in France, the Grand Duchy of Luxemburg and Belgium. Field trials are also in progress in the USA for oral vaccination of raccoons.

Development of a vaccinia-rabies vector vaccine

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 the construction of a subunit marked vaccine.

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The rabies virus glycoprotein gene (ERA strain) has been inserted into the thymidine-kinase (TK) gene of vaccinia virus (Copenhagen strain) (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 (Blancou et al., 1986) and North America: fox, raccoon and striped skunk. The duration of protection conferred by VVTGgRAB (a minimum of 18 months in adult foxes) corresponds to the length required for fox vaccination in the field due to the high turnover of the fox population.
The preclusion of epizootiological 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' deaths occur as a consequence of interaction between oral vaccination with VVTGgRAB and rabies infection, but preclude the risk of the emergence of asymptomatic carriers of wild rabies virus after vaccination.
It is also preferable that a vaccine virus used for oral vaccination of wildlife should not be horizontally transmitted to unvaccinated animals. No transmission of immunizing amounts of VVTGgRAB was found to occur in adult and young foxes. Changes in tissue tropism were 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 (Brochier et al., 1989b); similar experiments were carried out on wild species from North America. In every case, the recombinant virus was 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 domestic and laboratory species in contact with control animals to test for horizontal transmission of VVTGgRAB. Without exception, the results showed that no horizontal transmission took place (Pastoret et al., 1992).
The only remaining perceived risk to be investigated 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 orthopoxvirus infection in the fox population has been found, the 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., 1994).

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 VVTGgRAB vaccine suspension consists of the supematent fluid from a BHK cell culture infected with VVTGgRAB. The viral suspension medium is a saline solution with gentamycin added (125 µg/dose).

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The baiting system is formed from an appetent mixture of fish meal (50%) and fish oil (11%) aggregated using a hydrophobic synthetic polymer (11%). A sealed polyethylene sachet containing 2.5 ml liquid vaccine (titrating 108 to 109 CCID50) is fixed into the bait with a binding agent (11%).
Tetracycline hydrochloride, introduced into the appetent mixture bait (150 mg/ bait) serves as a biomarker of uptake of bait.
This machine-made vaccine bait system (RABORAL®) forms a rigid 5 X 3 X 2 cm parallelipiped (a solid body of which each face is a parallelogram) weighing from 34 to 40g.
The RABORAL® vaccine bait can be stored without freezing (at 4°C) and because of its mechanical resistance can be dropped by air. The efficacy and especially the attractive power of this baiting system to the red fox was established in an experimental station (Brochier et al., 1990a) and in the field (Brochier et al., 1990b).

Deliberate release of the vaccinia-rabies recombinant virus

PRELIMINARY LIMITED FIELD TRIAL

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, an initial limited field trial of fox vaccination was authorized by the Belgian Public Health Authority. The site chosen was a military field, isolated from public and domestic animals (Marche-en-Famenne). In this trial, each capsule containing a suspension of 10^8 $TCID_{50}$ of VVTGgRAB was introduced into a chicken head. On 17 and 18 October 1987, 250 vaccine-baits were manually delivered over a 6 km^2 area situated in the centre of the military field and surrounded by a non-vaccinated buffer zone. By day 15, the bait uptake was 64%. Of 145 rodents trapped in the baited area, only four had eaten baits and none showed any lesion that could be related to poxvirus infection (Pastoret et al., 1988).

ENLARGED SCALE FIELD TRIAL

The VVTGgRAB safety having been confirmed, the Belgium Ministry of Public Health on 21 September 1988 gave authorization for an enlarged open field trial. This latter was conducted in a 435 km^2 area covering four administrative districts in the Province of Luxembourg (South Belgium) (Brochier et al., 1990b). This vaccination zone was chosen because it has the lowest human population density in the country (42 inhabitants/km^2) combined with a high incidence of rabies. Furthermore, the region is characterized by various habitats including most of animal species which could consume baits.
The vaccine used was a suspension of 10^8 $TCID_{50}$ of VVTGgRAB contained in a plastic sachet. After the distribution of baits, field and laboratory controls confirmed the efficiency and stability of the vaccine bait association. Despite important variations of environmental temperatures (occurrence of natural freezing-thawing cycles), the VVTGgRAB bait system remained stable and attractive after one month in the field.

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As far as the safety of this vaccination campaign is concerned, no abnormal morbidity or mortality was observed in wildlife or in domestic animals. Moreover, 12 months' monitoring failed to detect any ecological hazard or problem of public heath.

Figure 1. *Geographical distribution of 841 animal rabies cases in Belgium in 1989.* Black dots: 520 wild animals; white dots: 321 domestic animals. Infected area: 10,000 km 2• F: France; G: Germany; L: Grand Duchy of Luxemburg; N: the Netherlands.

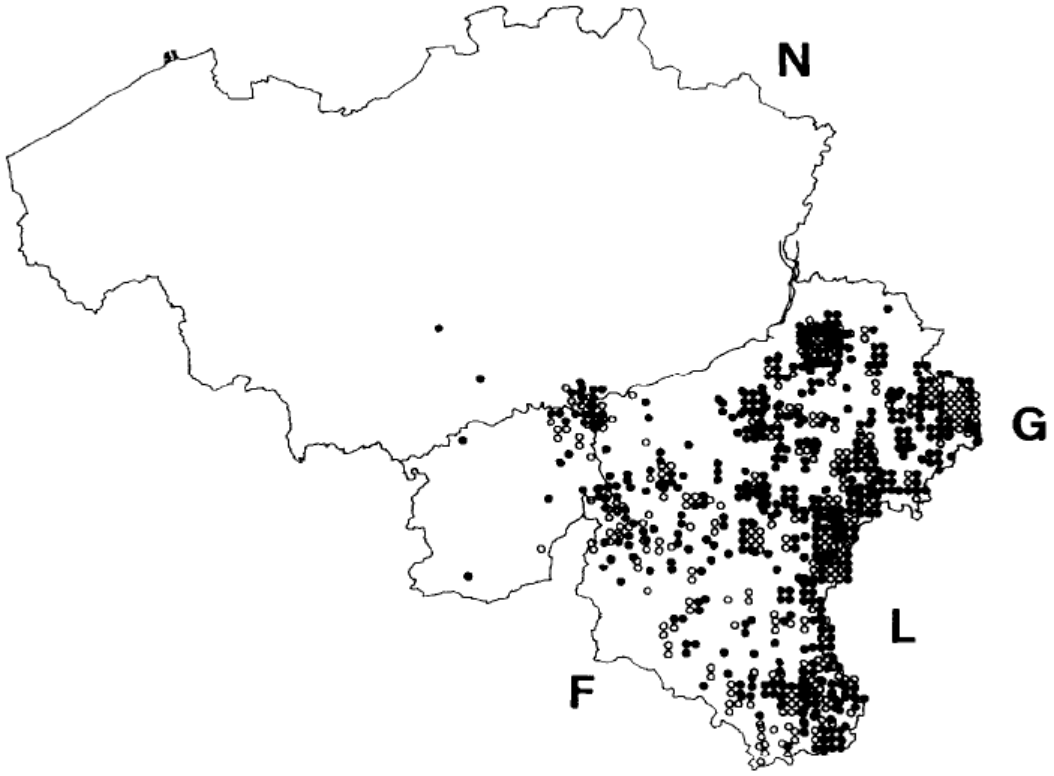

Table 1*. Protocol of fox vaccination campaigns in belgium*

No. Campaign	Year	Season	Vaccine	Treated Area (km²)	Dispersal Method
1	1989	Autumn	SAD B19[a]	8000	Manual
			VVTGgRAB[b]	2000	Helicopter
2	1990	Spring	SAD B19	8000	Helicopter
			VVTGgRAB	2000	Helicopter
3	1990	Autumn	VVTGgRAB	10000	Plane
4	1991	Spring	VVTGgRAB	10000	Plane
5	1991	Autumn	VVTGgRAB	10000	Plane
6	1992	Spring	VVTGgRAB	8500	Plane
7	1992	Autumn	VVTGgRAB	8000	Plane
8	1993	Spring	VVTGgRAB	8000	Helicopter
9	1993	Autumn	VVTGgRAB	5600	Helicopter
10	1994	Spring	VVTGgRAB	2500	Helicopter
11[c]	1994	Autumn	VVTGgRAB	2500	Helicopter

[a]Attenuated strain of rabies virus - [b]Vaccinia-rabies glycoprotein recombinant virus - [c]Not yet performed.

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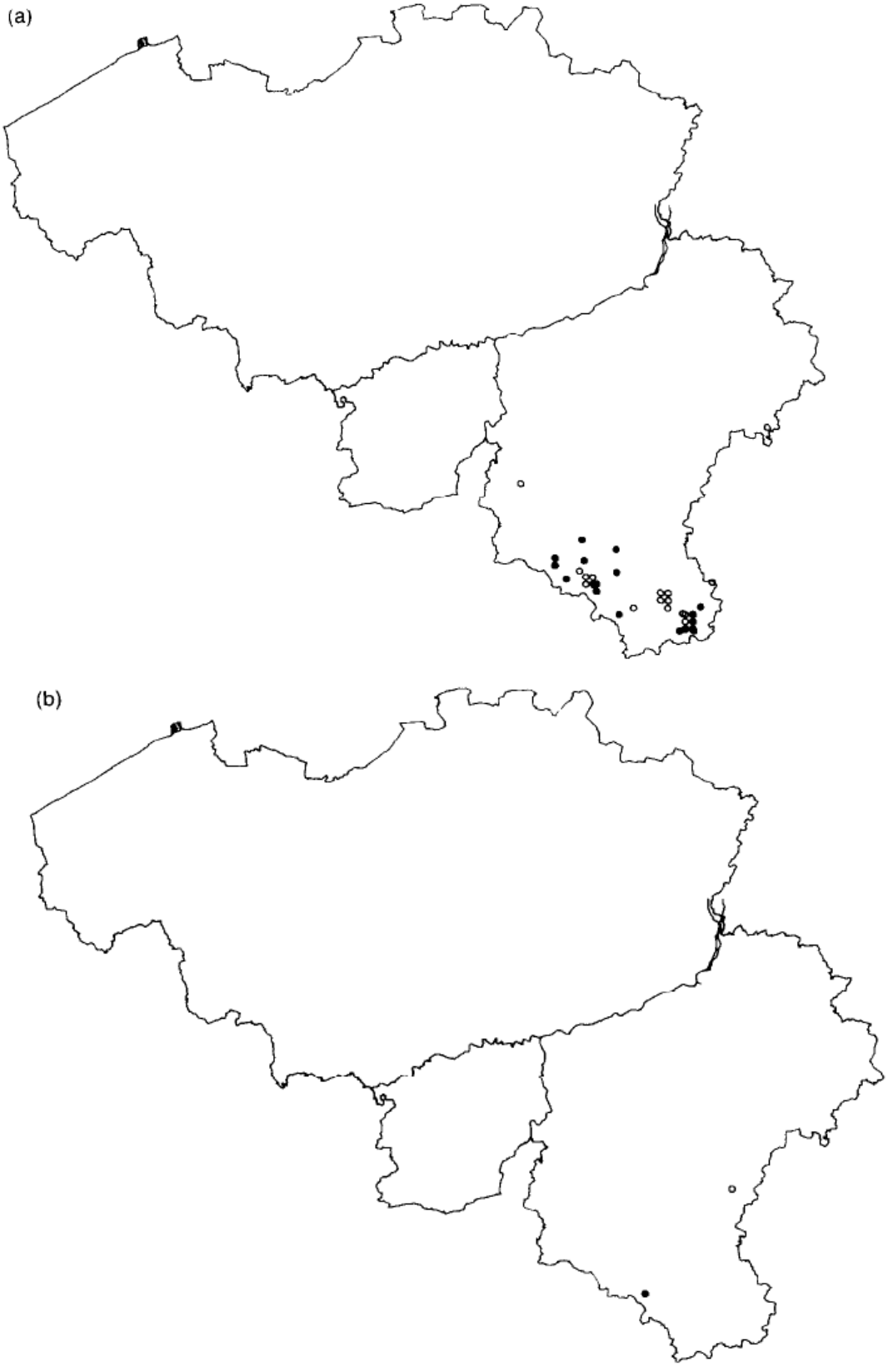
Figure 2. *Geographical distribution of animal rabies cases: a. in 1992, b. in 1993. c. in 1994 (data until 15 August). Black dots: wild animals; white dots: domestic animals.*



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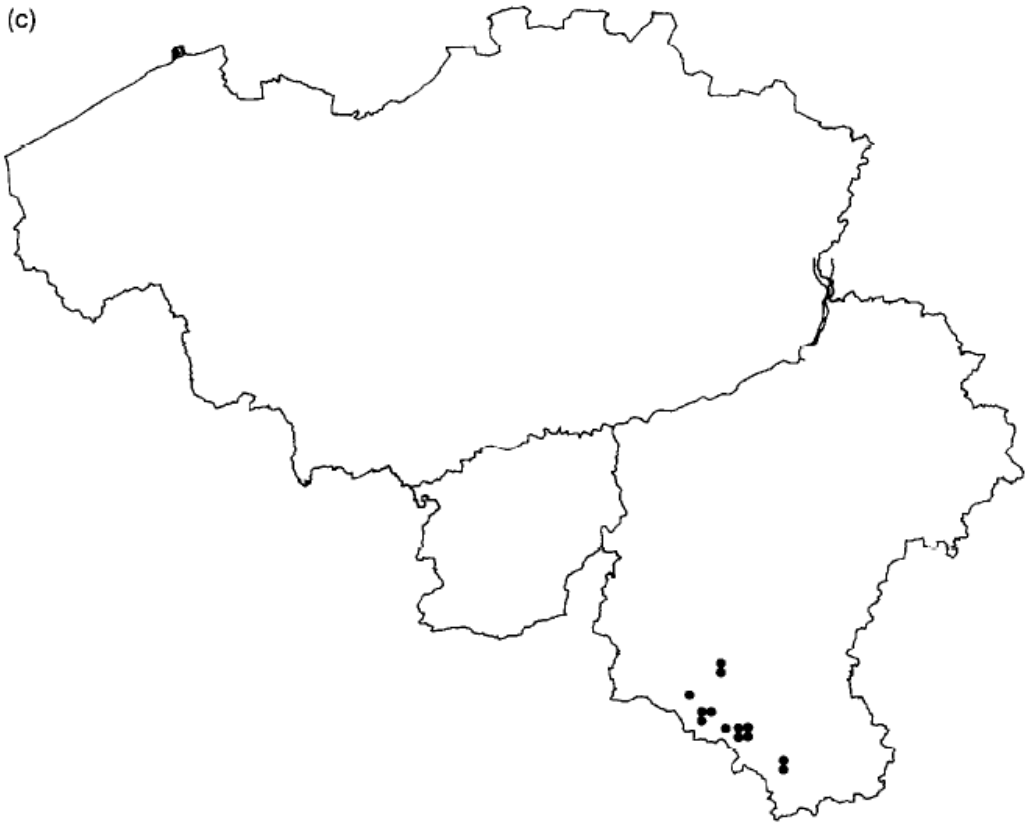

EXPERIMENTAL LARGE-SCALE CAMPAIGNS OF FOX VACCINATION

For evaluating the efficacy of the method, three fox vaccination campaigns using the VVTGgRAB were carried out in Belgium in November 1989, April 1990 and October 1990 (Brochier et al., 1991a, b).

Twenty-five thousand machine made baits each containing 10^8 $TCID_{50}$ of VVTGgRAB were each time dropped by helicopters in a 2200 km^2 area in south Belgium (province of Luxembourg). Baits were dispersed in the field at a mean density of 15 baits/km^2 • After bait dispersal, 81% of foxes inspected were positive for the tetracycline biomarker and only one rabid fox was detected close to the periphery of the treated area. No case of rabies, either in foxes or in domestic livestock, has been reported in the area.

National programme of fox rabies elimination

As shown in Fig. 1, Belgium was heavily infected before the campaigns of fox vaccination. The rabies infected area covered 10 000 km^2 in the southern part of the country. Rabies has been endemic since 1981 and its incidence remained high, especially during 1989 when 841 animal rabies cases were recorded. Five campaigns of fox vaccination, covering the whole infected area, were carried out from autumn 1989 until 1991 (Table 1) (Brochier et al., 1991b; Coppens et al., 1992). The two first campaigns (Autumn 1989, Spring 1990) were carried out using both attenuated rabies virus strain (SAD B 19) and recombinant vaccinia-rabies virus as vaccines. Since autumn 1990, the VVTGgRAB was used exclusively. Each time, 150 000 vaccine baits were dispersed by air (helicopter or plane) according to a grid resulting in a mean density of 15

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baits/km^2 ; urban areas and stretches of water were not seeded.
After each vaccination campaign, foxes found dead or shot by hunters were collected for bone tetracycline analysis (bait uptake control) and rabies diagnosis. Tetracycline was detected in animal bones (left jaw) by ultraviolet fluorescence microscopy of a 400 µm transverse section (diamond saw, Isomet-Buchler). The presence of brain rabies virus was determined by immunoftuorescence and inoculation of murine neuroblastoma cells as recommended by the World Health Organization (1992).
These 5 "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. 2 and Fig. 3a). 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 restricted campaigns were carried out along political borders only (Table l) (Brochier et al., 1993, 1994). These campaigns combined with vaccination operations in neighbouring countries induced a new decrease of incidence (Fig. 3a) ; no rabid foxes 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 international borders (Fig. 2b). Both of these rabies cases confirmed in the laboratory and nucleotide sequencing of these isolates demonstrated that they originated from the enzootic fox virus, demonstrating the persistence of undetected rabies foci of fox origin in Belgium (Bourhy, personal communication).
In 1994 (data until 15 August), rabies was confirmed again in 13 foxes collected in a region situated close to the border with France (Fig. 2c). These cases demonstrate the persistence of a border rabies focus and justify further restricted campaigns of vaccination in both countries.
Positive ethical and economical consequences of fox rabies control were obtained in the fields of public health, domestic animal health and environmental 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. Fig. 3b plots the number of notified cases of rabies in cattle over a 12 year period before and during the vaccination campaigns. 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 for two years (Fig. 3b). This could have a beneficial effect on the free movement of pets within the European Union by abolishing quarantine containment in some disease-free countries.
The number of people who received medical treatment (curative vaccination schedule) after coming in contact with a suspected animal has also decreased markedly (Fig. 3c). 71 people were treated in 1993, only 6 of whom were confirmed to have been exposed to an infected animal during that year.

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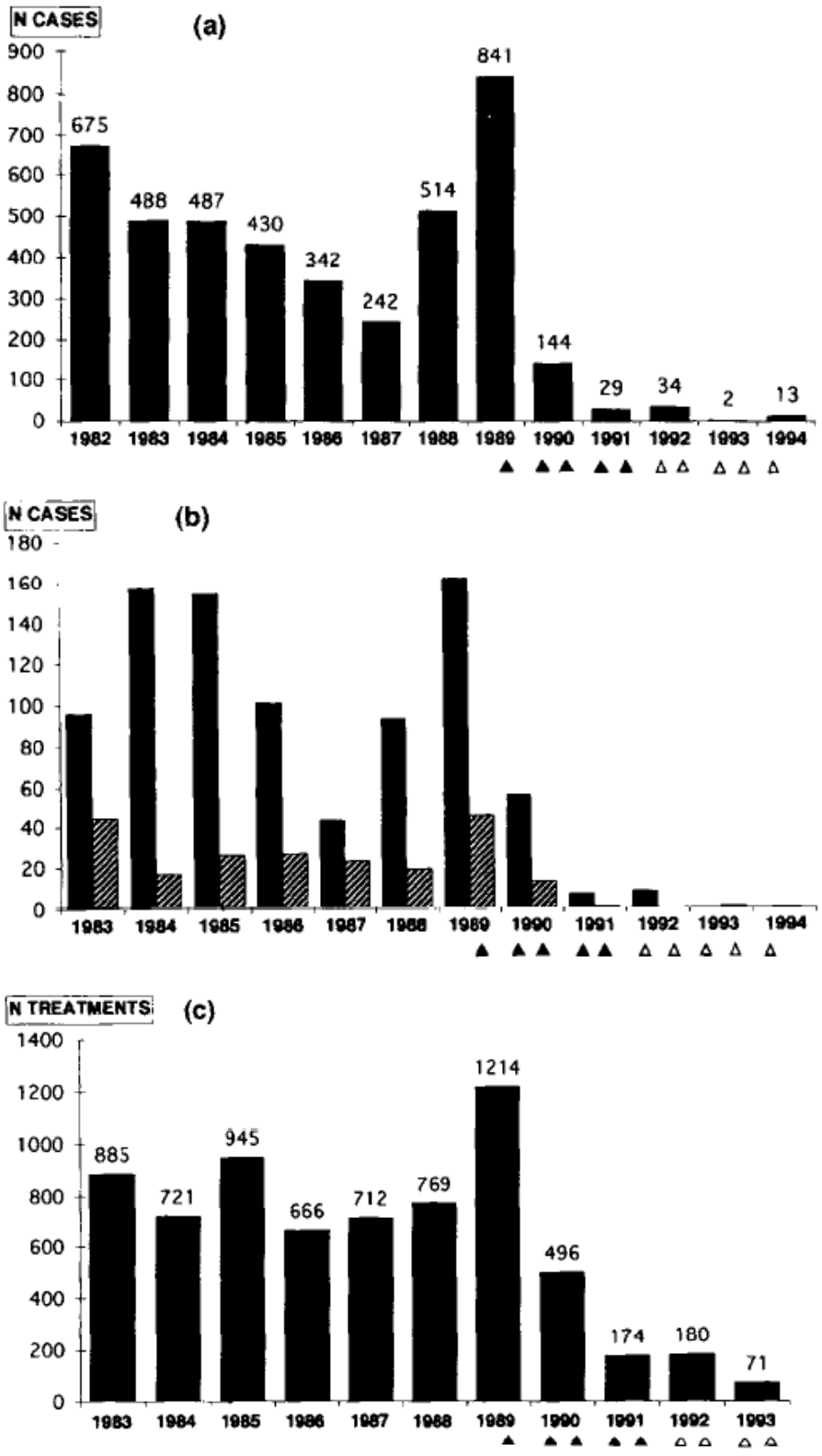
Figure 3. *a. Animal rabies incidence in Belgium; data until 15 August 1994, b. Rabies in cattle (full columns) and domestic carnivores (hatched columns); data until 15 August 1994. c. Evolution of human vaccinations (postexposure schedule); data until 31 December 1993. Black arrows: "full" campaigns of fox vaccination (10,000 km²) white arrows: "restricted" campaigns (8500 km² --> 2500 km 2).*



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Conclusions

Because of its efficacy, innocuity and heat-stability, the VVTGgRAB seems to offer an excellent alternative to the attenuated strains of rabies virus currently used in the field. Furthermore, the bait used is attractive, efficient and stable. In addition to the efficiency of a vaccine bait system, the strategy of bait delivery is of major importance for achieving the immunization of the required fraction of the fox population and, subsequently, the disruption of the viral infection cycle. Both spatial and temporal patterns of the bait distribution must be planned according to several natural factors. In this sense, the heat-stability of the VVTGgRAB bait system provides flexibility in planning a campaign.

Sylvatic rabies has been eliminated from the majority of the initial infected area of Belgium after five fox vaccination campaigns. Nevertheless, the creation of immune belts along borders was required to prevent spread of rabies from infected regions in neighbouring countries. A surveillance area, including Belgium and adjacent regions in neighbouring countries 50 km around Belgium, has been defined. Further spatial strategies or potential cessation of fox vaccination will depend on the rabies evolution in this surveillance area. A complete cessation will be justifiable when this zone will have been rabies-free for one year.

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