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Rabies eradication in Belgium by fox vaccination using vaccinia-rabies recombinant virus

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ABSTRACT

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Oral immunization of foxes against rabies, by distributing vaccine-baits in the field, has been in progress since 1993 in the whole of the infected area of Belgium (10 000 km^2). A vaccinia-rabies recombinant virus (VR-G) was used as vaccine because of its efficacy, safety and heat-stability.

The successive campaigns of fox vaccination have induced a drastic decrease in rabies incidence and in 1993 there were no cases of rabies detected in the fox population.

A marked decrease of human post-exposure treatments and the elimination of the disease in domestic animals have been the consequence of fox rabies control.

INTRODUCTION

In Belgium, as in other countries of Western Europe, the red fox is the vector and reservoir of the current endemic of sylvatic rabies.

Since rabies control by fox population reduction was not successful, oral immunization of foxes, by the distribution of vaccine-baits, has been experimentally assessed and subsequently used throughout the infected area of Belgium. The three main requirements of a live virus vaccine to be used in the field are efficacy, safety and heat-stability. A vaccinia recombinant virus expressing the immunogenic glycoprotein of rabies virus (V-RG) has been developed (Kiény, Lathe, Drillen, Spehner, Skory, Schmitt, Wiktor, Koprowski & Lecocq 1984) and extensively tested in the laboratory as well as in the field (Brochier, Thomas, Baudin, Leveau, Pastoret, Languet, Chappuis, Desmettre, Plancou & Artois 1990a; Pastoret, Brochier, Blancou, Artois, Aubert, Kieny, Lecocq, Languet, Chappuis & Desmettre 1992). The efficacy of this recombinant strain was tested by the oral route, primarily in foxes and subsequently in other wild vectors of rabies (Blancou, Kieny, Lathe, Lecocq, Pastoret, Soulebout & Desmettre 1986; Ruprecht, Wiktor, Johnston, Hamir, Dietzschold, Wunner, Glyckman & Koprowski 1986; Brochier, Languet, Blancou, Kieny, Lecocq, Costy, Desmettre & Pastoret 1988; Blancou, Artois, Brochier, Thomas, Pastoret, Desmettre, Languet & Kieny 1989). V-RG innocuity was tested in foxes and in numerous target and non target animal species (Table 1) (Artois, Charlton, Tolson, Casey, Knowles & Campbell 1990; Pastoret *et al.* 1992; Ruprecht, Hanlon, Cummins & Koprowski 1992).

TABLE 1 Development of vaccinia rabies recombinant vaccine: safety trials conducted between 1983 and 1992 in 54 target and non-target animal species

Species			Europe	Europe/North America	North America
Wild target species	6	*Mammalia*	Raccoon dog	Red fox Arctic fox	Raccoon Striped skunk Vampire bat
Wild non-target species	36	*Mammalia*	Carnivora 2 Rodentia 6 Artiodactyla 1		Carnivora 6 Rodentia 10 Artiodactyla 1 Marsupiala 1 Insectivora 1 Charadriiformes 1 Falconiformes 1 Stringiformes 1
Domestic species	6	Cattle; sheep; pig; horse; dog; cat			
Laboratory species	4	Mouse; ferret; rabbit; hamster			
Primates	2	Chimpanzee; squirrel monkey			

A vaccine-bait system has been developed and successive field trials demonstrated its immunogenicity, attractive power, safety, heat-stability and resistance (Brochier *et al.* 1990a; Brochier, Languet, Artois, Zanker, Guittre, Blancou, Chappuis, Desmettre & Pastoret 1990b; Brochier, Kieny, Costy, Coppens, Bauduin, Lecocq, Languet, Chappuis, Desmettre, Afiademanyo, Libois & Pastoret 1991a).

As shown in Fig. 1 Belgium was heavily infected before fox vaccination was embarked upon. 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 the year 1989 in which 840 animal rabies cases were recorded.

MATERIALS AND METHODS

Vaccines

In the autumn of 1989 and the spring of 1990, SAD B19 and V-RG vaccines were both used to treat the whole of the infected area: since the autumn of 1990, the V-RG vaccine alone was used.

SAD B19 vaccine

SAD B19 is an attenuated strain of rabies virus adapted to and grown in cloned baby hampster kidney cells (Schneider & Cox 1983). It was produced by the Rabies Centre of the Federal Research Institute in Tübingen, Germany. One dose of vaccine containing this virus consisted of 1,8 mℓ of virus suspension contained in a hermetically sealed capsule.

VR-G vaccine

VR-G is a vaccinia (Copenhagen strain) recombinant virus expressing the immuniogenic glycoprotein of rabies virus (ERA strain). V-RG, propogated on cultured green monkey kidney cells (VERO), was used to establish a freeze-dried master stock from which all vaccine preparations derive (< 5 passages).

Baiting system

"Tübingen fox" bait

The "Tübingen fox" bait consisted of a machine-made mixture of fat, bone and fishmeal enclosing a vaccine capsule. The vaccine-bait stock was stored at –20 °C until distribution in the field.

"Raboral®" vaccine-bait

The baits were formed from a mixture of plant and animal protein and fish oil aggregated using a synthetic polymer. A sealed plastic sachet containing 2,5 mℓ liquid vaccine (> 10^8 $TCID_{50}$) was introduced into the bait. The vaccine bait was stored at 4 °C prior to distribution in the field.

Tetracycline hydrochloride (150 mg per bait) was introduced into both "Tübingen Fox" and "Raboral®" baits for long term bio-marking of bait uptake.

Vaccine-baits were dropped by helicopter or aeroplane according to a grid resulting at a mean density of 15 baits/km^2; urban areas and stretches of water were avoided.

Protocol for vaccination campaigns

The first fox vaccination campaign was carried out in the autumn of 1989 over the whole of the infected area. Subsequently, similar "full" campaigns were performed four times: two in the spring and autumn of 1990 and two in the spring and autumn of 1991 (Brochier, Cost, Hallet, Duhaut, Réharpré, Afiademanyo, Bauduin & Pastoret 1991b; Coppens, Brochier,

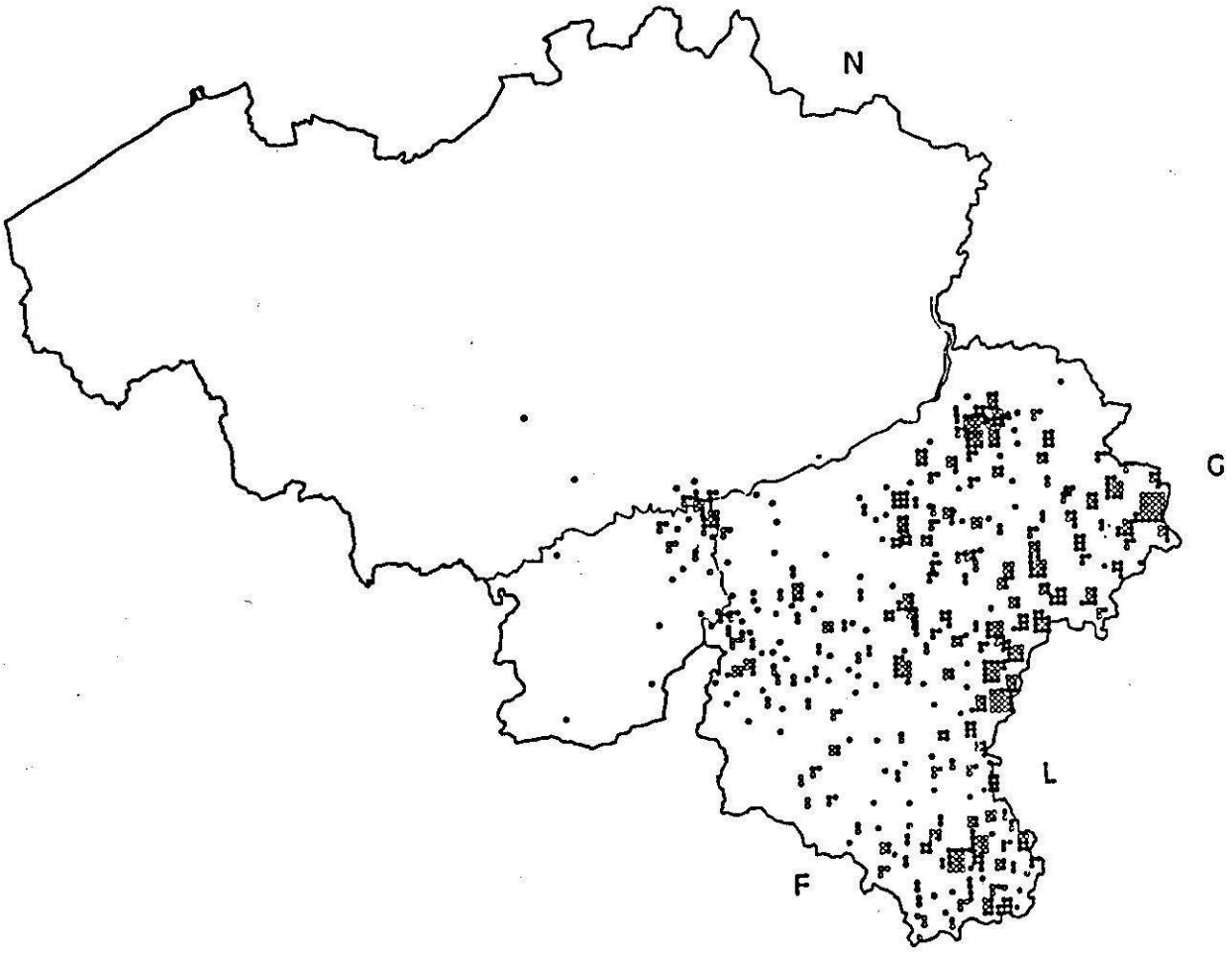

FIG. 1 Distribution of 841 animal rabies cases in Belgium in 1989. Black dots: 520 wild animals; white dots: 321 domestic animals. Infected area: 10 000 km^2

Hallet, Péharpé, Duhaut, Costy, Marchal, Libois, Afiademanyo, Bauduin & Pastoret 1992).

On each occasion 150 000 V-RG baits were dispersed by air. In 1992 and 1993 a revised strategy was employed in Belgium and adjacent regions in neighbouring countries. This involved three successive "defence" campaigns conducted over a reduced vaccination area which formed an immune belt along political borders (Brochier, Coppens, Costy, Péharpré, Marchal, Hallet, Duhaut, De Koninck, Bauduin & Patoret 1993). The campaigns of bait dispersal, carried out by the Veterinary Inspectorate of the Ministry of Agriculture, were supported financially by the Ministry of Agriculture and the Commission of the European Communities.

Efficacy controls

After each vaccination campaign, foxes found dead (or shot by hunters) were collected for tetracycline analysis of bone sections and rabies diagnosis.

Tetracycline uptake was detected in animal bones (left mandible) using ultraviolet flourescence microscopy of a 400 µm transverse section (diamond saw, Isomet-Büehler®). The presence of rabies virus in the brain of foxes was determined by immunofluorescence and inoculation of murine neuroblastoma cells as recommended by the World Health Organization.

RESULTS

The five "full" campaigns covering the whole of the infected area resulted in a drastic decrease in rabies incidence (Fig. 2) and the elimination of the disease from most of the initial infected area.

As shown by the geographical distribution of rabies cases in 1992 (Fig. 3), the spatial pattern of rabies changed: all fox cases recorded were within 20 km of the border with France and more than 70 % were less than 5 km from the border. At that time France

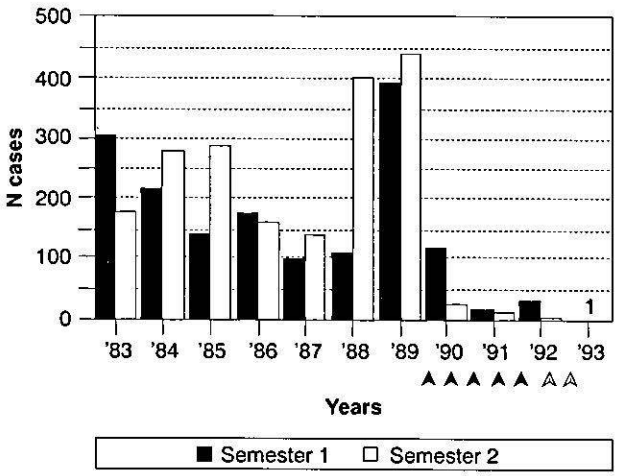

FIG. 2 Bi-annual development of animal rabies in Belgium; data from 1 January 1983 to 30 April 1993. Black arrows: "full" campaigns of fox vacination; white arrows: "defence" campaigns along borders

reported numerous rabies cases in the contiguous area.

The successive "defence" campaigns carried out in 1992 and 1993 combined with vaccination operations in neighbouring countries induced the almost complete disappearance of fox rabies in 1993 (data up to 30 April) (Fig. 4).

In accordance with the recommendations of the World Health Organization, surveillance of rabies in foxes was increased. Fig. 5 shows the geographical distribution of 420 foxes collected in 1992 and shown to be free of rabies.

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. The number of notified cases of rabies in cattle decreased during the

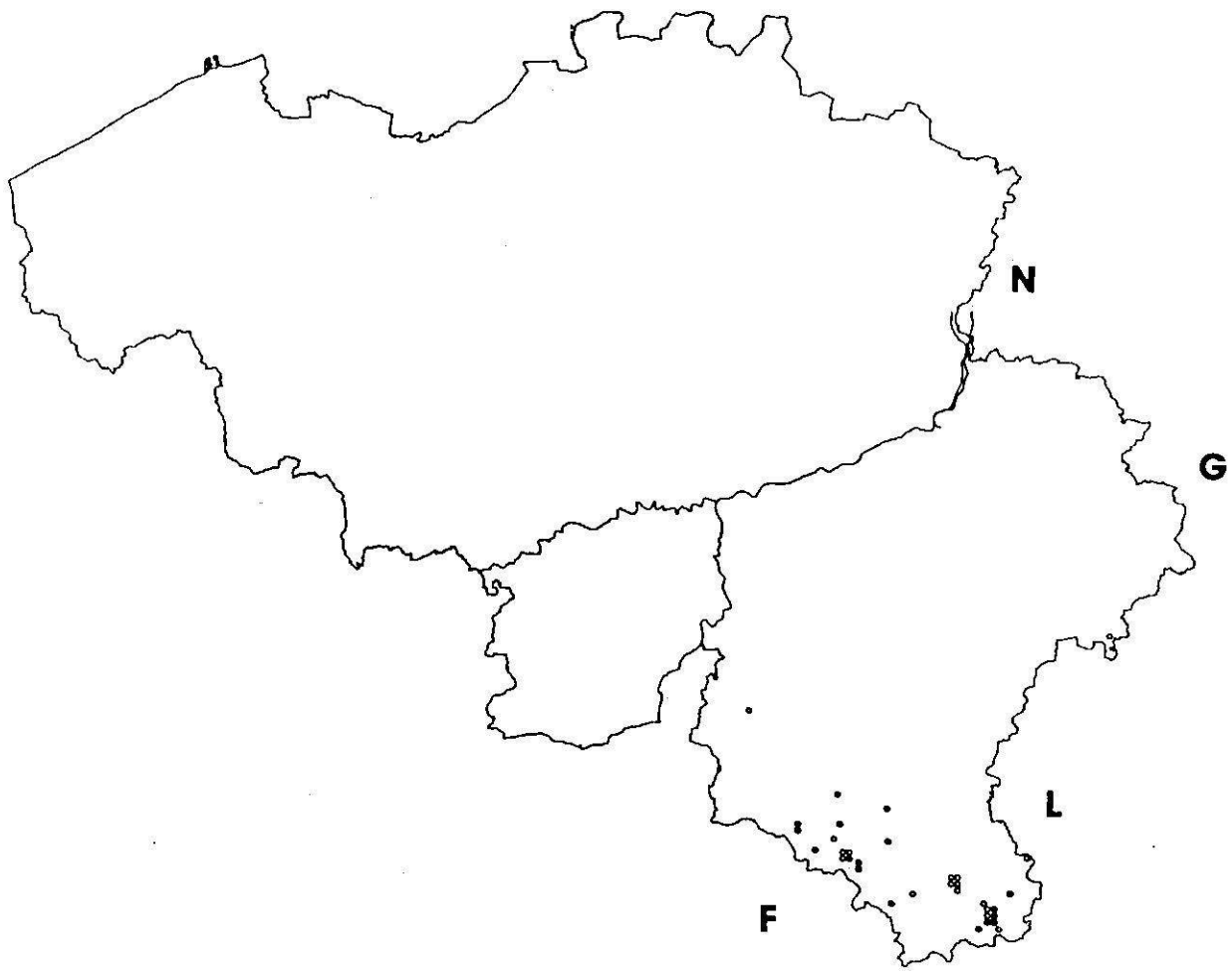

FIG. 3 Geographical distribution of 34 animal rabies cases in Belgium in 1992. Black dots: 17 wild animals; white dots: 17 domestic animals. Infected area 2 000 km^2 along the French border

first vaccination campaigns and no cattle rabies has been recorded in Belgium since December 1992.

Similarly, as a second consequence of fox rabies control, no rabies cases were reported in domestic carnivores for two years (Fig. 6).

The number of people who received medical treatment (post exposure immunization) after coming into contact with suspected rabid animals also decreased markedly (Fig. 7)—47 people were treated in 1993 (data up to 30 April); three of them after being exposed to the only animal diagnosed as rabid during that year (a badger).

At the beginning of 1993, the immune status of the fox population was estimated from data available from tetracycline biomarking. In the area that was treated seven times between 1989 and 1992 and which formed an immune belt along the international borders, the bait-uptake rate was 82%. From a fox population density estimate of two individuals per km^2 it was possible to infer the elimination of rabies virus from the fox population (Anderson, Jackson, May & Smith 1981). In the area in which fox vaccination was discontinued in 1992 (five campaigns of fox vaccination) only 51% of foxes were shown to be positive for tetracycline. This lower value may be explained by the turnover of the fox population in the spring of 1992 (recruitment of unvaccinated offspring). Thus, the vaccine coverage observed in this area was no longer sufficient to break the cycle of rabies infection and transmission. In spite of this, the continued absence of rabies in this region provides proof that rabies had been eliminated from the fox population by the five previous campaigns.

FIG. 4 Location of 1 animal rabies case (badger) in 1993 (data up to 30 April)

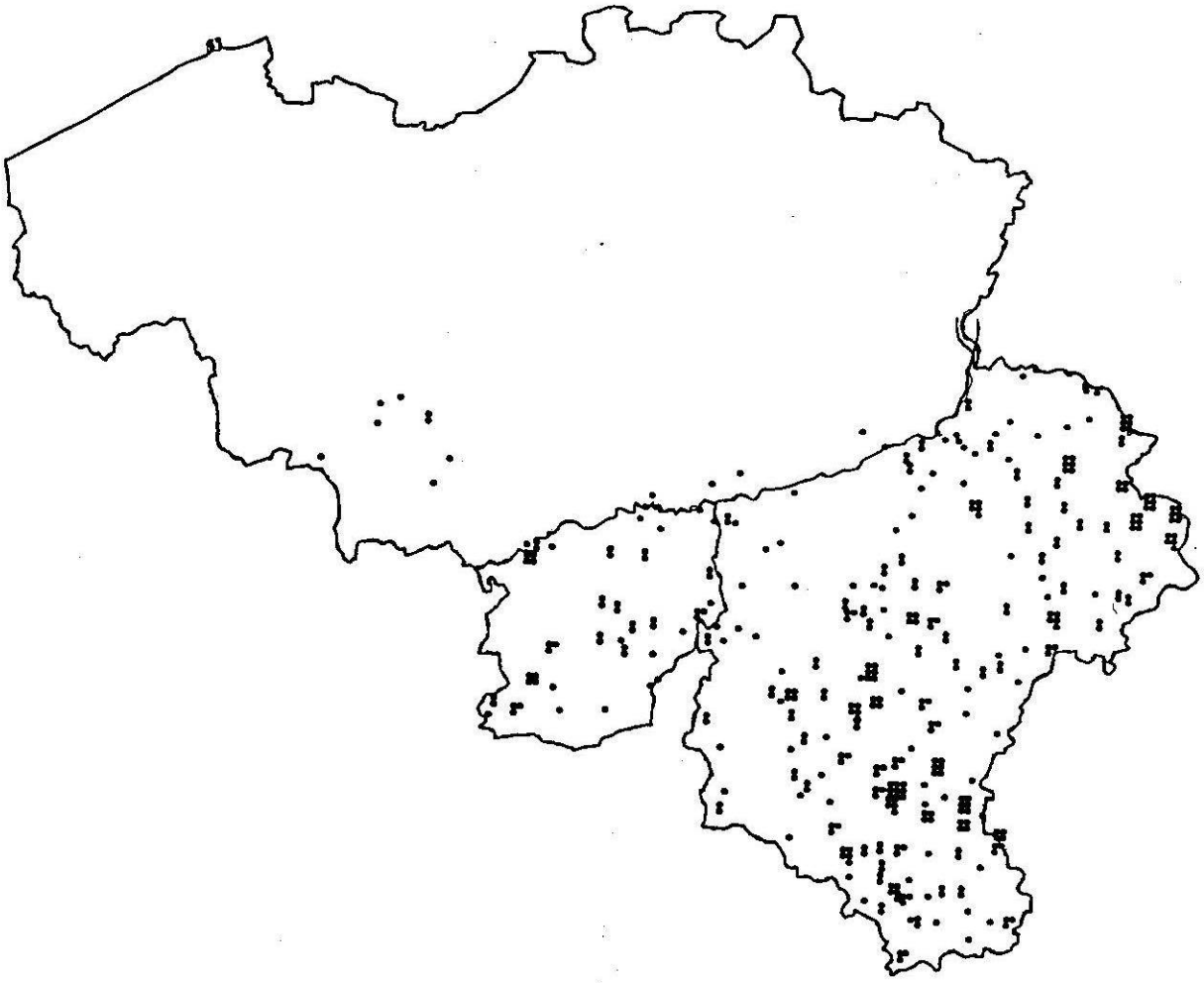

FIG. 5 Distribution of 430 rabies-negative foxes collected in Belgium in 1992

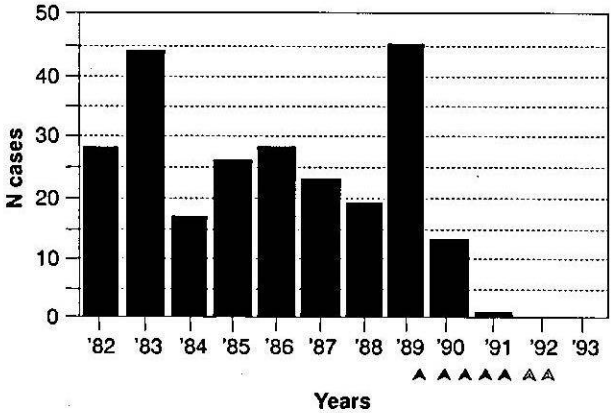

FIG. 6 Annual incidence of rabies in domestic carnivores; data from January 1982 to 30 April 1993. Black arrows: "full" campaigns of fox vaccination; white arrows: "defence" campaigns along borders

DISCUSSION

Sylvatic rabies has been eliminated from most of the initially infected areas of Belgium by conducting five "full" fox vaccination campaigns. This was achieved within two years by using an efficient and heat-stable bait vaccine. Nevertheless, the creation of immune belts along the borders of Belgium was required to prevent re-introduction from infected regions in neighbouring countries. A 50 km wide surveillance area along the borders of Belgium and adjacent countries was defined. Further action, including the possible discontinuation of fox vaccination will depend on the incidence of rabies in this surveillance area. A complete cessation of immunization may be justifiable if this zone is rabies-free or one year.

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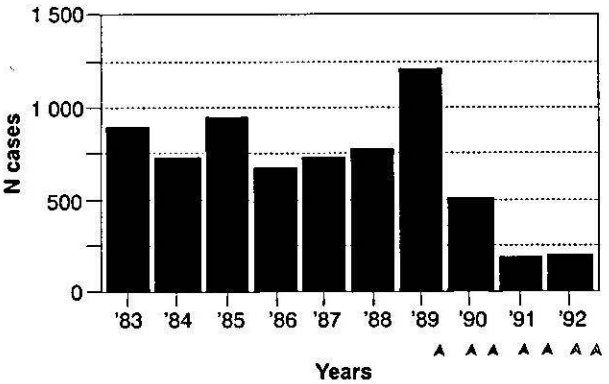

FIG. 7 Annual human vaccination against rabies in Belgium (post-exposure schedule); data from 1 January 1983 to 31 December 1992. Black arrows: "full campaigns of fox vaccination; white arrows: "defence" campaigns

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