Large-scale eradication of rabies using recombinant vaccinia–rabies vaccine

B. Brochier*, M. P. Kieny†, F. Costy‡, P. Coppens*, B. Bauduin*, J. P. Lecocq†, B. Languet§, G. Chappuis§, P. Desmettre§, K. Afiademanyo||, R. Libois|| & P.-P. Pastoret*

* Department of Virology, Faculty of Veterinary Medicine, University of Liège, 45 rue des Vétérinaires, 1070 Brussels, Belgium
† Transgène SA, 11 rue de Molsheim, 67000 Strasbourg, France
‡ Rabies Department, Pasteur Institute of Brabant, 642 rue Engeland, 1180 Brussels, Belgium
§ Rhône Mérieux, Laboratoire Iffa, 254 rue Marcel Mérieux, 69342 Lyon, France
|| Institute of Zoology, Faculty of Sciences, University of Liège, 22 Quai Van Beneden, 4020 Liège, Belgium

RABIES infection of domestic and wild animals is a serious problem throughout the world. The major disease vector in Europe is the red fox (*Vulpes vulpes*) and rabies control has focused on vaccinating and/or culling foxes. Culling has not been effective, and the distribution of live vaccine baits is the only appropriate method for the vaccination of wild foxes[1]. Although some European countries have conducted field vaccination campaigns using attenuated rabies virus strains[2–5], their use has not been extensively approved because they retain pathogenicity for rodents and can revert to virulence[6,7]. These strains cannot be used in North America because they are pathogenic for the striped skunk (*Mephitis mephitis*)[8] and are ineffective in the racoon (*Procyon lotor*)[9]. We have constructed a recombinant vaccinia virus[10], VVTGgRAB, expressing the surface glycoprotein (G) of rabies virus (ERA strain)[11–13]. The recombinant was a highly effective vaccine in experimental animals[11–13], in captive foxes[14,15] and in racoons[9]. We report here the results of a large-scale campaign of fox vaccination in a 2,200 km² region of southern Belgium, an area in which rabies is prevalent. After distribution, 81% of foxes inspected were positive for tetracycline, a biomarker included in the vaccine bait and, other than one rabid fox 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.

The field vaccine-bait system[15] containing VVTGgRAB is safe for laboratory, domestic and European wild animals[15–23], and the duration of immunity elicited (more than 12 months in cubs and 18 months in adult foxes) corresponds to the length of protection necessitated by the life expectancy of foxes (1.5–2.5 years)[16,17]. Small-scale field trials of fox vaccination[24–26] were therefore conducted in southern Belgium for safety assessment (data not shown). No abnormal morbidity or mortality was recorded in wild animals by hunters and forestry rangers nor in domestic animals by farmers and veterinary practitioners. But the true efficacy of the vaccination procedure could not be evaluated because of the small size of the areas, the minimum size recommended by the WHO for this purpose being 2,000 km² (ref. 27). A 2,200 km² region of southern Belgium (Fig. 1) was approved for full-scale vaccination in September 1989. The 25,000 baits containing VVTGgRAB and a tetracycline biomarker were dropped by helicopter on three occasions (November 1989, April 1990 and October 1990). After each vaccination campaign, foxes found dead (or shot by hunters) were collected for rabies diagnosis and bone tetracycline analysis. The three collection periods (Jan–Mar 1990; Apr–Oct 1990; Nov 1990–Apr 1991) permitted the collection of 10 rabid and 178 healthy foxes. The bait uptake rate determined from tetracycline status in 23 adult foxes (9 rabid, 14 healthy) during the first period was 74%; six of the 9 rabid and 11 of the 14 healthy foxes were positive for tetracycline. During the second collection period bait uptake rates were 80% (25/31) in adult foxes while only 49% (27/55) in juveniles; during spring dispersal of baits most cubs (born principally in March) would be expected to be confined to the immediate surroundings of the breeding den and thus less likely to ingest baits. No rabid fox was recorded during this period (0/86). After the third phase of vaccination (October 1990) 81% (64/79) of inspected animals were tetracycline-positive. The one rabid animal recorded, at the periphery of the baited area, was tetracycline-negative. The geographical distribution of these animals is given in Fig. 2.

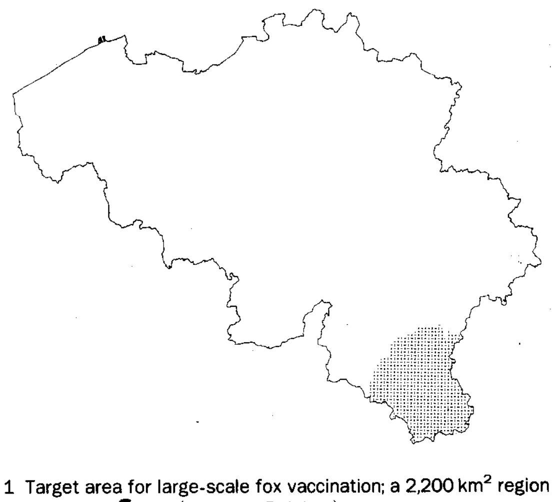

FIG. 1 Target area for large-scale fox vaccination; a 2,200 km² region in the province of Luxembourg (southern Belgium).

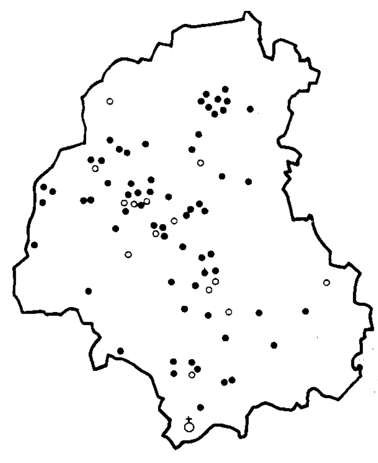

FIG. 2 Geographic distribution of 79 foxes (78 healthy, 1 rabid) shot or found dead in the area vaccinated three times (collection period: October 15, 1990 to April 30, 1991). ●, Animals tetracycline-positive; ○, animals tetracycline-negative; ♀, rabid tetracycline-negative fox.

METHODS. VVTGgRAB propagated on cultured green monkey kidney cells (VERO) was used to establish a freeze-dried master stock from which all vaccine preparations derive (≤5 passages). Vaccine-baits[15] were formed from a mixture of plant and animal proteins and fish oil aggregated using a synthetic polymer and contained tetracycline hydrochloride (150 mg per bait) as a biomarker. A sealed plastic sachet containing 2.5 ml liquid vaccine (≥10^8 $TCID_{50}$) was introduced into the bait, stitched closed, and baits were stored at 4 °C until distribution. Baits were dropped from helicopter (average height 80 m) according to a grid resulting in a mean density of 15 km^{-2}; urban areas were not seeded. Tetracycline was detected in the left jaw of culled animals or animals found dead using ultraviolet fluorescence microscopy of a 400-μm transverse section (diamond saw, Isomet-Buehler). The presence of brain rabies virus was determined by immunofluorescence and intracerebral inoculation of mice as recommended by the WHO (ref. 31).

Despite the dramatic decrease in the number of rabid foxes recorded after vaccine-bait distribution, the efficacy of the vaccination campaign is difficult to evaluate because systematic collection of foxes is not logistically feasible. But transmission of rabies to domestic animals occurs through the bite of a rabid wild animal. Because notification of cases of rabies in cattle and sheep is mandatory in Belgium the incidence of rabies in domestic livestock provides a reliable indicator of the prevalence of rabies in the wild. Figure 3 plots the number of notified cases of rabies in livestock over a 7-year period before, during and after the vaccination campaigns. No case of livestock rabies has been recorded in the study zone since the second phase of vaccination.

Our results indicate that the dispersion of live recombinant vaccinia virus VVTGgRAB can be an effective means of controlling rabies. But a number of issues remain to be addressed. First, after the first phase of vaccination (but not subsequently) a number of foxes were recorded that were positive for both tetracycline and rabies infection. Because the efficacy of the vaccine-bait system was previously demonstrated in captive foxes[14,15] when animals resisted severe challenge 30 days post-vaccination, we surmise that the animals may have been incubating rabies at the time of vaccination. Second, the uptake rate of vaccine by cubs (49%) compared with adults (80%) when vaccine dispersal took place in the spring falls below the critical fraction (p) of immunized animals required to prevent spread of rabies. This fraction depends on the mean density of target animals, and for foxes can be estimated from the relation $p \geq 1 - K_T/K$, where K_T is the density of foxes necessary to maintain the endemic persistence of rabies and K is the actual fox density in the absence of rabies[28,29]. K_T is estimated to be roughly 0.4 foxes per km^2 and the average K value in Europe is 1 fox per km^2 (ref. 29), although this figure can vary from 0.1 in poor biotic areas to over 4 in some suburban areas[30]. Thus, for a K value of 2 foxes per km^2, a probable overestimation of the mean density in the rural area under study, the proportion of the population (p) which must be vaccinated is 0.8. This indicates that vaccination campaigns might be more effective in the autumn when young foxes forage independently. But our observations indicate that an overall bait uptake rate of 74–81% is sufficient to break the cycle of rabies infection and retransmission in the area under study.

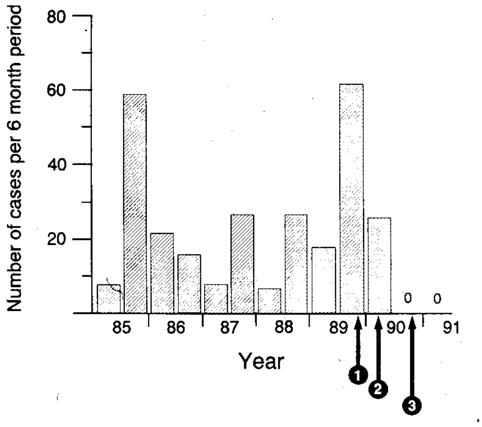

FIG. 3 Seven-year evolution of the incidence of rabies in domestic livestock. Histogram boxes plot the half-yearly (Jan–Jun and Jul–Dec) numbers of cases of rabies notified in sheep and cattle in the target area before and following vaccination. The arrows indicate the rough timing of the three large-scale campaigns of vaccine-bait dispersal.

We have also investigated the economics of the vaccine-bait dispersal programme. The average yearly cost of rabies in Belgium (1980–89), including treatment of humans, animal diagnosis, compensation to farmers for the culling of infected livestock, and the culling of wild foxes, is estimated to be 400,000 ECUs 10,000 km^{-2}, or 88,000 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 we estimate the overall expenditure during the three campaigns of vaccine-bait distribution (bait, helicopter and personnel costs) to be 118,000 ECUs. Because vaccination following eradication can, in principle, be interrupted or subsequently limited to the borders of vaccinated zone, long-term maintenance of a rabies-free area by peripheral vaccination with live recombinant vaccinia virus VVTGgRAB may well be economically justifiable. □

Received 28 February; accepted 28 October 1991.

1. Baer, G. M. in *The Natural History of Rabies* Vol. 2 (ed. Baer, G. M.) 261–266 (Academic, New York, 1975).
2. Steck, F., Wandeler, A., Bichsel, P., Capt, S. & Schneider, L. *Z. Vet. Med.* **29**, 372–396 (1972).
3. Kappeler, A., Wandeler, A. I. & Capt, S. in *Vaccination to Control Rabies in Foxes* (eds Pastoret, P.-P., Brochier, B., Thomas, I. & Blancou, J.) 55–60 (Office for Official Publications of the European Communities, Brussels-Luxembourg, 1988).
4. Schneider, L. G. & Cox, J. H. *Tierärstl. Umsch.* **38**, 315–324 (1983).
5. Brochier, B. *et al. Vet. Rec.* **123**, 618–621 (1988).
6. Wandeler, A., Bauder, W., Prochaska, S. & Steck, F. *Comp. Immun. Microbiol. Infect. Dis.* **5**, 173–176 (1982).
7. Leblois, H. & Flamand, A. in *Vaccination to Control Rabies in Foxes* (eds Pastoret, P.-P., Brochier, B., Thomas, I. & Blancou, J.) 101–104 (Office for Official Publications of the European Communities, Brussels-Luxembourg, 1988).
8. Rupprecht, C. E. *et al. J. Wildl. Dis.* **26**, 99–102 (1990).
9. Rupprecht, C. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 7947–7950 (1986).
10. Moss, B. & Flexner, C. *A. Rev. Immun.* **5**, 305–324 (1987).
11. Kieny, M. P. *et al. Nature* **312**, 163–166 (1984).
12. Wiktor, T. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 7194–7198 (1984).
13. Wiktor, T. J., Kieny, M. P. & Lathe, R. in *Applied Virology Research* Vol. 1 (eds Kurstak, E., Marusyk, R. G., Murphy, F. A. & Van Regenmortel, M. H. V.) 69–90 (Plenum, New York, 1988).
14. Blancou, J. *et al. Nature* **322**, 373–375 (1986).
15. Brochier, B. M. *et al. Vet. Rec.* **127**, 165–167 (1990).
16. Brochier, B. *et al. Vet. Microbiol.* **18**, 103–108 (1988).
17. Blancou, J. *et al. A. Rech. Vét.* **20**, 195–204 (1989).
18. Tolson, N. D. *et al. Archs Virol.* **102**, 297–301 (1988).
19. Wiktor, T. J., MacFarlan, R., Dietzschold, B., Rupprecht, C. & Wunner, W. H. *Annls Inst. Pasteur/Virol.* **136E**, 405–411 (1985).
20. Soria Baltazar, R., Blancou, J. & Artois, M. *Ann. Méd. Vét.* **131**, 481–486 (1987).
21. Desmettre, P. *et al. Vet. Microbiol.* **23**, 227–236 (1990).
22. Brochier, B. *et al. J. Wildl. Dis.* **25**, 540–547 (1989).
23. Thomas, I. *et al. J. gen. Virol.* **71**, 37–42 (1990).
24. Pastoret, P.-P. *et al. Vet. Rec.* **123**, 481–483 (1988).
25. Newman, P. *Nature* **336**, 416 (1988).
26. Brochier, B. *et al. Vaccine* **8**, 101–104 (1990).
27. WHO Report. *Consultation on Requirements and Criteria for Field Trials on Oral Rabies Vaccination of Dogs and Wild Carnivores* Geneva, 1–2 March 1989 (WHO/Rab. Res./89.32).
28. Anderson, R. M., Jackson, H. C., May, R. M. & Smith, A. D. *Nature* **289**, 765–771 (1981).
29. Anderson, R. M. *Nature* **322**, 304–305 (1986).
30. Harris, S. & Rayner, J. M. V. *J. Anim. Ecol.* **55**, 575 (1986).
31. Dean, D. J. & Abelseth, M. K. in *Laboratory Techniques in Rabies* (eds Kaplan, M. M. & Koprowski, H.) 75–83 (WHO, Geneva, 1974).

ACKNOWLEDGEMENTS. We thank Richard Lathe and Peter Nettleton for revising the manuscript, the National Council of Hygiene of the Ministry of Public Health for legal authorization, the General Inspection of Environment and Forestry and the Veterinary Inspection of the Ministry of Agriculture for collaboration in the organization of the campaigns and the Ministry of Agriculture for providing figures for our economic assessments. This work is supported by a grant from the Commission of the European Communities and the Ministère de la Région Wallonne pour la Conservation de la Nature.