# Use of a vaccinia–rabies recombinant virus for the oral vaccination of foxes against rabies

*The vaccination of wild animals against rabies has been developed most extensively in Europe. Experiments have demonstrated the efficacy of a vaccinia–rabies recombinant virus administered by the oral route in foxes. The innocuity of this vaccine was tested in the target species as well as in several non-target wild and domestic species. Because of its safety and heat-stability, this recombinant virus should offer an excellent alternative to the attenuated strains of rabies virus currently used in the field. A large scale field trial was conducted in Belgium in October 1988 to assess the efficacy of this new vaccine-bait systems.*

Sylvatic rabies invaded Belgium from Germany in 1966. The epizootic moved to the west and the south of the country and reached the Meuse and Sambre valleys, which appear to constitute a natural barrier to the spread of the disease. The evolution of the epizootic has been characterized by a succession of peaks which, until 1982, had a mean periodicity of 4 years. However during the last 6 years the annual numbers of rabies cases remained abnormally high (514 animal cases reported in 1988 in a 9500 $km^2$ area). It may therefore be assumed that rabies persists enzootically behind the leading edge of the invasion.

As in most other European countries, control measures of reducing fox population were only temporarily effective and did not stop the spread of the disease. Other methods such as oral immunization of foxes against rabies needed to be assessed[1]. Oral administration is the only route appropriate for the vaccination of a sufficient number of wild foxes (distribution of vaccine baits). Since 1978, several European countries have conducted at different times, large scale field trials of oral vaccination of foxes (*Vulpes vulpes*) using the SAD, standard or B19 modified, attenuated strain of rabies virus[2–6]. The promising results obtained from 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 laboratory and wild rodents[7,8] and are heat-sensitive.

## New candidate vaccine: the vaccina–rabies recombinant virus

To improve both safety and stability of the vaccine used in the field, a recombinant vaccinia virus, expressing the immunizing G glycoprotein of rabies virus (VVTGgRAB-26D3 187XP strain) has been developed[9]. The c-DNA corresponding to the glycoprotein of the Era, strain of rabies virus has been inserted into the thymidine kinase (TK) gene of the vaccinia virus (Copenhagen strain). VVTGgRAB is propagated in a Vero green monkey kidney cell line and a master stock is preserved by freeze drying. All the vaccine preparations originate from this stock, within the limit of a maximum of five cell passages.

## Immunogenicity

When directly fed $10^7$ $TCID_{50}$ of VVTGgRAB, 100% of adult foxes are protected against a severe rabies challenge. Nevertheless, a dose of $10^8$ $TCID_{50}$ is required when the vaccine is enclosed in a bait. The liquid form of VVTGgRAB presentation seems to be more efficient than the freeze-dried one[10,11]. When administered by the oral route (direct instillation) to fox cubs, a dose of $10^{7.2}$ $TCID_{50}$ of VVTGgRAB induces significant levels of rabies virus antibody and protects 92% of them from rabies[12]. The duration of immunity conferred by the VVTGgRAB, a minimum of 12 months in cubs and 18 months in adult animals, corresponding to the length of protection required for fox vaccination in the field[3,11,12]. The effect of maternal antibodies, cubs born from vaccinated vixen, on active immunization of the young animals with the VVTGgRAB is now under study.

## Safety

The use of a recombinant vaccinia virus may help to solve some of the problems related to the use of a live modified rabies virus. Nevertheless, the safety problems of live vaccines (required for oral immunization) must still be investigated when using a recombinant orthopox virus. Therefore, it is important to verify the absence of pathogenicity, excretion and transmission of VVTGgRAB in both target and non-target animal species.

No clinical signs or lesions were recorded in any of the inoculated foxes during 1, 6, 12 and 18 months after vaccination. The absence of pathogenicity was observed whatever the dose inoculated ($10^2$–$10^{10}$ $TCID_{50}$) or route of administration (oral, intramuscular, intraduodenal, subcutaneous, intradermic (scarification), ocular and intranasal)[10–13]. The absence of pathogenicity of VVTGgRAB administered by the oral route also has been demonstrated in the laboratory mouse, rabbit, ferret (*Putorius furo*), cattle, cat, dog, pig and sheep[11,14–17]. Moreover, VVTGgRAB innocuity was tested in several wild animal species that could compete with the red fox in consuming vaccine baits in Europe[18]. The following species were fed: wild boar (*Sus scrofa*), 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 buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), carrion crow (*Corvus corone*), magpie (*Pica pica*) and jay (*Garrulus glandarius*). Postmortem examination did not result in the detection of pox lesions in the oral mucosa or the skin in mammals or the unfeathered portions of birds. During a minimum of 28 days postvaccination, neither clinical signs nor lesions were observed in any of 107 vaccinated animals[18]. The absence of mortality,

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especially in rodents, is a notable advantage of the recombinant virus compared with the SAD live-modified strains of rabies virus. Our results confirm the predicted safety linked to this recombinant virus. Inactivation of the thymidine kinase (TK) gene by the insertion of a c-DNA sequence coding for rabies glycoprotein affects the pathogenicity of vaccinia virus and markedly attenuates its virulence[19].

For testing the excretion and horizontal transmission of the VVTGgRAB, unvaccinated control animals have been held in close contact with vaccinated ones. Seroconversion has never been observed in these 'contact' animals. When using this assessment method, no transmission of immunizing amounts of VVTGgRAB occurred in the red fox (except one fox bitten by a freshly inoculated animal), dog, cat, cattle, ferret, badger and wild boar[10,12,17,18]. Several laboratory techniques failed to detect any virus in the saliva, salivary glands, the blood and internal organs of freshly inoculated foxes. The highly sensitive method of polymerase chain reaction could detect the VVTGgRAB at very low levels in tonsils, buccal mucosa and soft palate only[20]. Finally, the genetic stability of this recombinant virus, a second indication of safety, could be attested after serial passages *in vitro* (cell lines) as well as *in vivo* (red fox, laboratory mouse).

It is also of major importance to preclude some epizootical risks such as the emergence of asymptomatic carriers of rabies virus. This situation could occur in the field by vaccinating naturally infected animals during the incubation period. An experimental study has shown that 'early' or 'late' death phenomenon, as consequences of interactions between oral vaccination with VVTGgRAB and rabies infection, can occur in foxes[21]. Both 'death phenomena' are thus likely to impede the emergence of survivors to rabies infection and subsequently the creation of asymptomatic carriers of rabies virus. Therefore, there do not seem to be peculiar epidemiological risks in similar natural conditions.

## Field trials in Belgium

Taking into account all the available experimental data concerning the safety of this recombinant virus for target and non-target species and its efficacy in foxes, a first preliminary field trial of fox vaccination using this VVTGgRAB was carried out in Belgium on 24 October 1987 (Ref. 22). In this trial, each capsule containing a suspension of $10^8$ $TCID_{50}$ of VVTGgRAB was introduced in a chicken head. Vaccine-baits, 250, were delivered in an area of $6\,km^2$ situated within a closed military zone (mean density: 40 baits $km^{-2}$). No adverse phenomena were observed in wildlife after this first VVTGgRAB release.

Nevertheless, the efficacy of the method could not be evaluated because of the small size of the area. Therefore, a large trial has been conducted in a $435\,km^2$ area covering four administrative districts in the province of Luxembourg (south Belgium). This vaccination zone was chosen because it has the lowest human population density (42 inhabitants $km^{-2}$) in the country combined with a high incidence of rabies. Furthermore, the region is characterized by various habitats including most of animal species liable to consume baits. The vaccine used was a suspension of $10^8$ $TCID_{50}$ of VVTGgRAB (volume 2.2 ml) contained in a plastic sachet. Each vaccine sachet was enclosed in a bait consisting of a mixture of fishmeal and fishoil. The characteristics of this bait system are given in *Table 1*. Each bait also contained 150 mg tetracycline used as a long-term biomarker of bait uptake. Tetracycline can be detected in animal bones by ultraviolet microscopy. The vaccine-bait stock was stored at 4°C until the day of distribution in the field. The distribution was performed by 26 forestry rangers and lasted about 2 weeks (24 October to 10 November 1988). It was done by hand at a mean density of 15 baits $km^{-2}$. After the baiting campaign, both efficacy and innocuity of the method were monitored in the field as well as in laboratory.

For the bait uptake control, 238 baits were placed and identified in a $20\,km^2$ zone situated close to the centre of the vaccination area. Each bait location was then visited at different times after the distribution. Sixty five (27%), 128 (54%), 163 (68.5%) and 223 (94%) baits were either consumed or at least removed by animals on days 4, 8, 15 and 30, respectively.

**Table 1** Characteristics of the baiting system used during the first large scale campaign of fox vaccination with the VVTGgRAB

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|---|
| Attractive power to the fox (but also to non-target species such as cats, wild boars, rodents, mustelids) |
| Storage without cold chain |
| Aerial dropping (mechanical resistance) |
| High heat-stability (no melting) |
| Absence of vaccine inactivation |
| Availability (machine-made) |
| Good delivery of the vaccinia liquid in the oral cavity |
| Good ratio: bait/vaccine |
| Vaccine container easily ruptured (sachet) |
| Bait sufficiently hydrophobic |

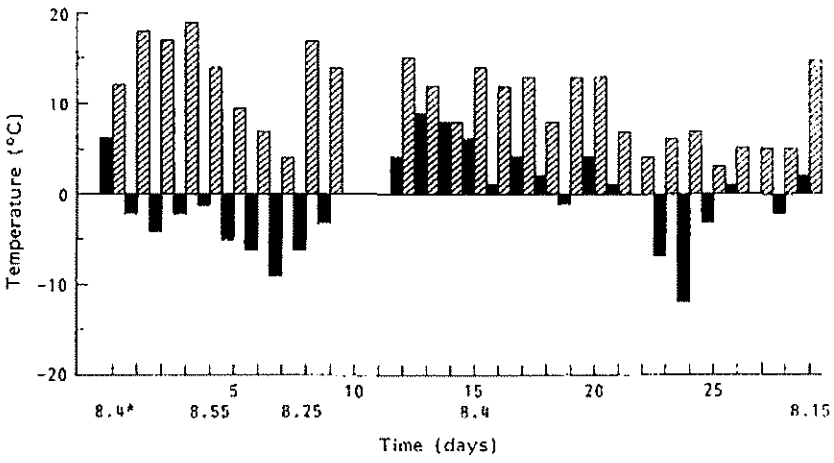


**Figure 1** Minima and maxima of environmental temperatures recorded after bait distribution (day 0). VVTGgRAB mean titre of two baits removed on days 0, 4, 8, 15 or 29 is given below the axis line. *, Expressed as the log 10 $TCID_{50}$ $ml^{-1}$

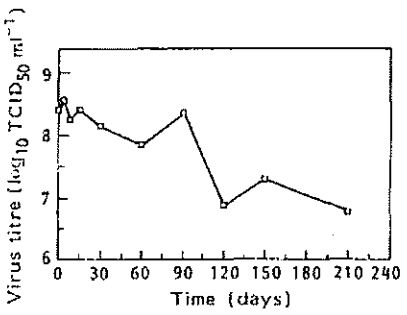


**Figure 2** Stability of the VVTGgRAB titre under local field conditions (winter 1988–1989 and spring 1989)

These results confirm the efficiency of the baiting system. A significant proportion of baits was partially eaten by rodents and insects (*Necrophorus verspillo* and *Geotrupes* sp.). Despite this competition, further uptake foxes was generally not precluded. However, in some cases, rodents or beetle perforated the plastic sachet and entered into contact with the vaccine liquid. Despite important variations of environmental temperatures (occurrence of natural freezing–thawing cycles), see *Figure 1*, the VVTGgRAB titre remained stable after 1 month in the field. A first significant decrease of the VVTGgRAB titre was observed after 4 months (*Figure 2*).

After the vaccination campaign, 222 dead wild animals of 19 species were collected within the vaccination area for postmortem examination and laboratory testing. No pox-like lesion could be detected in any specimen. Every suspected lesion would have been submitted to three methods of virus identification: virus isolation on cell culture, polymerase chain reaction and electron microscopy. After necropsy, the following organs were removed: brain for rabies diagnosis, jaw for tetracycline detection and blood for the determination of vaccinia and rabies antibodies.

Until 1 June 1989, 16 out of 26 foxes (61%) collected in the vaccination area were found to be tetracycline positive. This result can be considered as a good score, especially after a first campaign of bait delivery. This biomarking is also helpful for the detection of bait consuming non-target species. Among 222 collected specimens, tetracycline was detected in 3/7 stone martens (*Martes foina*), 3/11 domestic or feral cat (*Felis catus*), 5/50 wood mice (*Apodemus* sp.), 15/32 wild boars (*Sus scrofa*) and 1/10 carrion crows (*Corvus corone*). Wild boars, mustelids and feral cats are thus strong competitors of the foxes for the bait uptake. As far as the safety of this vaccination campaign is concerned, no abnormal morbidity or mortality was noted in wildlife or in domestic animals. Moreover, 7 months monitoring failed to detect any ecological hazard or problem of public health.

Serological data are currently misunderstood because of strong discordances in the results obtained with the following tests: rapid fluorescent foci inhibition test (RFFIT; macromethod and micromethod), ELISA techniques using either the rabies virus glycoprotein or the vaccinia virus (Copenhagen strain) as antigens. Blood samples collected from dead animals are of poor quality. When using virus-neutralization serological techniques, such as the RFFIT, non-specific reactions are generally observed. Therefore, ELISA techniques are likely to be more appropriate tools for serological examinations of blood samples collected in field conditions. Nevertheless, these techniques of antibody detection directed against vaccinia virus and the rabies virus glycoprotein still need to be improved and adapted.

Further trials carried out with captive foxes should precisely determine: the concordance between production of antivaccinia antibodies and the production of antibodies directed against the rabies virus glycoprotein by using ELISA tests; the concordance between results of tetracycline detection and results of serological ELISA tests; the concordance between results of both serological ELISA tests and results of the test of protection against an experimental rabies infection (determination of the thresholds of positivity). Serological controls are currently insufficient for the evaluation of the efficacy of a fox vaccination campaign. This latter would have to be established by the bait uptake rate (using a reliable biomarker) and the evolution of rabies epizootic in the treated area. The ideal control of efficacy of a vaccination campaign would require the capture of live foxes. Serological results would be totally reliable, whatever the technique used. Furthermore, the possibility to perform a rabies challenge would permit confirmation of the protection against rabies in seropositive foxes and detection of animals that are protected despite the absence of detectable rabies virus-neutralizing antibodies.

## Conclusions

Because of its efficacy, innocuity and heat-stability, the VVTGgRAB seems to offer an excellent alternative to the attenuated strains of rabies virus

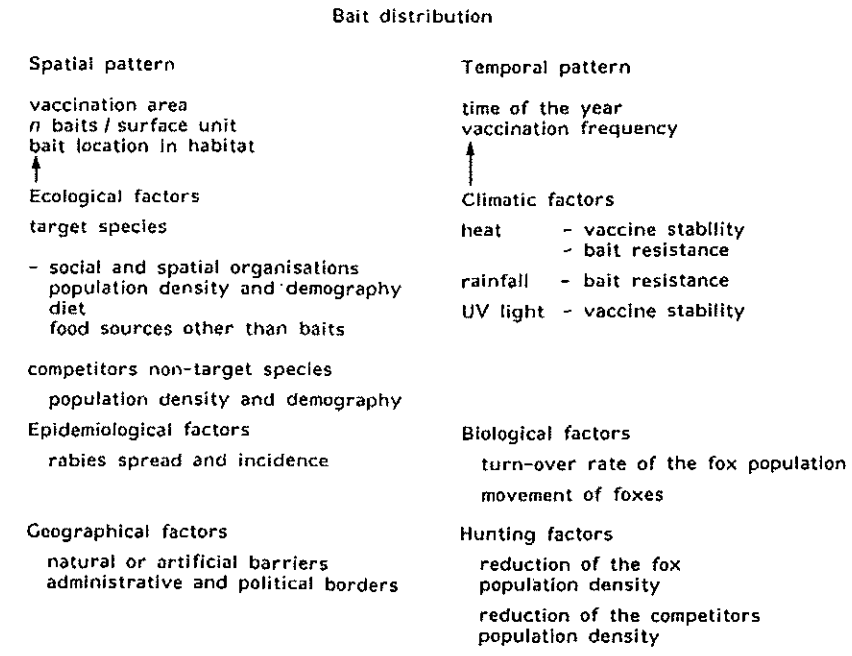


**Figure 3** Factors influencing spatial and temporal patterns of the bait distribution

currently used in the field. Furthermore, the bait used is attractive, efficient and thermostable. Nevertheless, in addition to the efficiency of a vaccine-bait system, the strategy of bait delivery is of major importance for achieving the immunization of 75% of foxes and, subsequently, the disruption of the viral infection cycle. Both spatial and temporal patterns of bait distribution must be planned according to several natural factors (*Figure 3*). In this sense, the thermostability of the VVTGgRAB bait system provides flexibility in planning a campaign.

Baits can be placed by hand or spread from airplane or helicopter. Manual dispersal requires the participation of many distributors. In Belgium, human factors were shown to play a significant role in the success or failure of vaccination. Therefore, the aerial dropping method is currently considered as more effective and should have to be used during further campaigns.

In conclusion, local eradication of fox rabies could be achieved after three or four vaccination campaigns[23] when the following criteria are met: a potent, safe and thermostable vaccine; an efficient and resistant bait system; a practical and sure method of bait dispersal; and an effective spatial and temporal pattern of bait distribution[24].

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**B. Brochier, I. Thomas, B. Bauduin, T. Leveau, P.-P. Pastoret, B. Languet*, G. Chappuis*, P. Desmettre*, J. Blancou† and M. Artois†**

*Department of Virology, Faculty of Veterinary Medicine, University of Liege, 45 rue des Vétérinaires, B-1070 Brussels, Belgium*

**Rhône Mérieux, Laboratoire Iffa, 254 rue Marcel Mérieux, F-69342 Lyon, France*

*†C.N.E.V.A., Centre National d'Etudes sur la Rage, B.P. n°9, F-54200, Malzéville, France*

## References

1 Baer, G.M. Wildlife vaccination; In: *The natural history of rabies* (Ed. Baer, G.M.) Academic Press, New York, USA, 1975, Vol. 2, pp. 261–266

2 Steck, F., Wandeler, A., Bichsel, P., Capt, S. and Schneider, L. Oral immunisation of foxes against rabies. A field study. *Zbll. Vet. Med.* 1982, **29**, 372

3 Schneider, L.G. and Cox, J. H. Ein Feldversuch zur oralen Immunisierung von Füchsen gegen die Tollwut in der Bundesrepublik Deutschland. *Tierärztl. Umsch.* 1983, **38**, 315

4 Frisch, R., Wolff, F., Krier, A., Brochier, B. and Schneider, L.G. Première campagne de vaccination antirabique du renard par voie orale menée au grand-duché de Luxembourg. Contrôles d'efficacité et d'innocuité chez le renard roux *(Vulpes vulpes,* L.). *Ann. Méd. Vét.* 1987, **131**, 449

5 Artois, M., Chillaud, T., Maillot, E., Rigal, P. and Blancou, J. Première campagne de vaccination antirabique du renard par voie orale menée en France. Contrôle d'efficacité chez le renard et d'innocuité chez les micromammifères. *Ann. Méd. Vét.* 1987, **131**, 457

6 Brochier, B., Thomas, I., Ioke, A., Ginter, A., Kalpers, J., Paquot, A. *et al.* A field trial in Belgium to control fox rabies by oral immunization. *Vet. Rec.* 1988, **123**, 618

7 Wandeler, A., Bauder, W., Prochaska, S. and Steck, F. Small mammals studied in a SAD baiting area. *Comp. Immunol. Microbiol. Infect. Dis.* 1982, **5**, 173

8 Leblois, H. and Flamand, A. Studies on pathogenicity in mice of rabies virus strains used for oral vaccination of foxes in Europe. In: *Vaccination to control rabies in foxes* (Eds Pastoret, P.-P., Brochier, B., Thomas, I. and Blancou, J.) Brussels, Belgium, Office for Official Publications of the European Communities, Brussels-Luxembourg, 1988, pp. 101–104

9 Kieny, M.P., Lathe, R., Drillien, R., Spehner, S., Skory, D., Schmitt, T. *et al.* Expression of rabies virus glycoprotein from a recombinant vaccinia virus. *Nature (London)* 1984, **312**, 163

10 Blancou, J., Kieny, M.P., Lathe, R., Lecocq, J.P., Pastoret, P.-P., Soulebot, J.P. and Desmettre, J.P. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. *Nature (London)* 1986, **322**, 373

11 Blancou, J., Artois, M., Brochier, B., Thomas, I., Pastoret, P.-P., Desmettre, P. *et al.* Innocuité et efficacité du virus recombinant vaccine-rage administré par voie orale chez le renard, le chien et le chat. *Ann. Rech. Vét.* 1989, **20**, 195

12 Brochier, B., Languet, B., Blancou, J., Kieny, M.P., Lecocq, J.P., Costy, F. *et al.* Use of a recombinant vaccinia-rabies virus for oral vaccination of fox cubs (*Vulpes vulpes,* L.) against rabies. *Vet. Microbiol.* 1988, **18**, 103

13 Tolson, N.D., Charlton, K.M., Casey, G.A., Knowles, M.K., Rupprecht, C.E., Lawson, K.F. and Campbell, J.B. Immunization of foxes against rabies with a vaccinia recombinant virus expressing the rabies glycoprotein. *Arch. Virol.* 1988, **102**, 297

14 Wiktor, T.J., Macfarlan, R.I., Reagan, K., Dietzschold, B., Curtis, P., Wunner, W.H. *et al.* Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene. *Proc. Natl. Acad. Sci. USA* 1984, **81**, 7194

15 Wiktor, T.J., Macfarlan, R., Dietzschold, B., Rupprecht, C. and Wunner, W.H. Immunogenic properties of vaccinia recombinant virus expressing the rabies glycoprotein. *Ann. Inst. Pasteur/Virol.* 1985, **136E**, 405

16 Soria Baltazar, R., Blancou, J. and Artois, M. Résultats de l'administration par voie orale au mouton de deux vaccins contenant un virus de la rage modifié (SAD B19) ou un recombinant du virus de la vaccine et de la rage (187 XP). *Ann. Méd. Vét.* 1987, **131**, 481

17 Brochier, B., Languet, B., Blancou, J., Thomas, I., Kieny, M.P., Lecocq, J.P. *et al.* Innocuité du virus recombinant vaccine-rage chez quelques espèces non-cibles. *Proc. CEC Symposium: Vaccination to control rabies in foxes,* Brussels, November 1987 (Eds Pastoret, P.-P., Brochier, B., Thomas, I. and Blancou, J.) Office for official publications of the European Communities. Brussels-Luxembourg. 1988, pp. 118–123

18 Brochier, B., Languet, B., Blancou, J., Thomas, I., Kieny, M.P., Costy, F. *et al.* Use of recombinant vaccinia-rabies virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. *J. Wildl. dis.*, 1989, in press

19 Buller, R.M.L., Smith, G.L., Cremer, K., Notkins, A.L. and Moss, B. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. *Nature (London)* 1985, **317**, 813

20 Thomas, I., Brochier, B., Languet, B., Blancou, J., Peharpre, D., Kieny, M.P. *et al.* Primary multiplication site of the vaccinia-rabies glycoprotein recombinant virus administered to foxes by the oral route. *J. Gen. Virol.*, in press

21 Brochier, B., Blancou, J., Aubert, M.F.A., Kieny, M.P., Desmettre, P. and Pastoret, P.P. Interaction between rabies infection and oral administration of vaccinia-rabies recombinant virus to foxes (*Vulpes vulpes*). *J. Gen. Virol.* 1989, **70**, 1601

22 Pastoret, P.-P., Brochier, B., Languet, B., Thomas, I., Paquot, A., Bauduin, B. *et al.* First field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus. *Vet. Rec.* 1988, **123**, 481

23 Schneider, L.G., and Cox, J.H. Eradication of rabies through oral vaccination. In: *Vaccination to control rabies in foxes* (Eds Pastoret, P.-P., Brochier, B., Thomas, I. and Blancou, J.) Office for Official Publications of the European Communities. Brussels-Luxembourg, 1988, pp. 22–38

24 Wandeler, A.I., Capt, S., Kappeler, A. and Hauser, R. Oral immunization of Wildlife against rabies: concept and first field experiments. *Rev. Infect. Dis.* 1988, **10**(4), S649