Papers and Articles

First field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus

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A field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus was carried out in Belgium on October 24, 1987. Each vaccine capsule contained a suspension of 10^8 TCID50 of the recombinant virus and was introduced into a chicken head. Each chicken head contained 150 mg of tetracycline as a marker of uptake. Two hundred and fifty heads were distributed in an area of 6 km^2 situated within a military zone. The bait uptake was monitored for 15 days after the distribution. Sixty-three per cent of the chicken heads were taken by wild animals within that period. The trial was controlled according to the rules defined by the World Health Organisation.

ATTEMPTS to control rabies by vaccinating wild carnivores with attenuated rabies virus seem very promising (Steck and others 1982, Pastoret and others 1987, Brochier and others 1988a). However, the use of attenuated rabies virus remains controversial as far as its innocuity and stability are concerned.

The available attenuated rabies viruses are still pathogenic for some small rodents (Leblois and Flamand 1988). In order to improve both the safety and the stability of the vaccine used, a recombinant vaccinia virus which expresses the immunising glycoprotein of rabies virus has been developed.

The vaccine consists of a live-modified vaccinia (Copenhagen strain) – rabies glycoprotein (ERA strain) recombinant virus (VVTGg RAB-26D3 strain) grown on VERO cells to a titre of 10^8 TCID50/ml. To construct the recombinant virus, the cDNA corresponding to the recombinant virus has been aligned with a vaccinia promoter and inserted in vitro, into a cloned segment of vaccinia DNA.

The flanking VV sequences permit homologous recombination in vivo with the viral genome, and double reciprocal recombination transfers the DNA insert from the plasmid to the VV genome, where it is propagated and expressed (Kieny and others 1984). The insertion of the glycoprotein gene into the thymidine kinase (TK) gene of the vaccinia DNA gives the phenotype TK$^-$ to the recombinant and Buller and others (1985) have demonstrated that (TK$^-$) vaccinia virus recombinants, constructed by inserting coding sequences into the vaccinia virus TK gene, are even less pathogenic than the parental vaccinia virus. Moreover, the parental vaccinia virus has been used extensively for more than 150 years to control and finally eradicate smallpox in man.

The potential of this VVTGg RAB to protect foxes (*Vulpes vulpes*) (adult and young) (Blancou and others 1986, 1988, Brochier and others 1988b), raccoons (*Procyon lotor*) (Rupprecht and others 1986) and striped skunks (*Mephitis mephitis*) (Tolson and others 1987) against rabies has already been demonstrated. The oral, intradermal or subcutaneous administration of VVTGg RAB to those target species elicits high levels of rabies virus neutralising antibodies and long term protection against rabies.

When given by the oral route the recombinant virus multiplies in the tonsils for no more than 48 hours (I. Thomas, B. Brochier, B. Languet, Ph. Desmettre, J. Blancou and P. P. Pastoret, unpublished results).

The safety of VVTGg RAB for non-target species has been tested in several domestic (Soria-Balthazar and others 1987, Blancou and others 1988), laboratory (Wiktor and others 1984) and wild species (Brochier and others 1988c). The oral administration of VVTGg RAB has been shown to be safe for fox, raccoon, skunk, wild boar (*Sus scrofa*), badger (*Meles meles*), carrion crow (*Corvus corone*), magpie (*Pica pica*), jay (*Garrulus glandarius*), common buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), water vole (*Arvicola terrestris*), bank vole (*Clethrionomys glareolus*), ferret (*Mustela furo*), laboratory mouse, rabbit, cattle, cat, dog and sheep. Furthermore, the absence of horizontal transmission was confirmed in foxes, badgers, wild boar, cattle, cats, dogs and ferrets and the oral administration of the recombinant virus to adult foxes during the incubation period of the disease did not produce asymptomatic carriers of the wild rabies virus (B. Brochier, unpublished results).

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 field trial of fox vaccination against rabies using this vaccinia-rabies recombinant virus was carried out in Belgium on October 24, 1987; it was controlled according to the rules defined by the World Health Organisation.

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Materials and methods

Experimental area and date of release

The experiment was carried out in a military zone at Marche, in Belgium. The total area of the zone is 2700 hectares and the vaccination area within it covered 600 hectares. The release of vaccine took place on October 24, 1987. Foxes

and other non-target species are numerous in the area and wildlife within it was controlled during the three months after the release.

Vaccine and baits

The vaccine was contained in capsules which were introduced into the neck region of a chicken head. Each capsule contained 1·5 ml of a suspension of 10^8 TCID50 of the recombinant virus. Each chicken head contained 150 mg of tetracycline as a marker of uptake.

Distribution of the baits

Two hundred and fifty baits were distributed at a density of 40 to 50/km² and were hidden under leaves. They were located on a map (1/25,000) and identified by a number stick.

Measurement of bait uptake

The uptake of the 250 baits was assessed on days 4, 9 and 15 after distribution, and on day 15 the remaining baits were retrieved.

Tetracycline detection

Sections (300 to 400 μm) were made of jaw or other bones from dead animals taken from the vaccination area and tetracycline incorporation was detected by ultraviolet fluorescence microscopy (Capt 1981).

Serological and biological analysis

Blood samples were collected from dead animals. Rabies virus neutralising antibodies were determined by a technique of fluorescence inhibition (Smith and others 1973). Titres were expressed in international units (iu)/ml and compared with a standard serum with a titre of 4·4 iu/ml. Titres higher than 0·5 iu/ml were considered as positive.

Control of safety for non-target species

During the three months after the distribution of the baits 145 small mammals were trapped in the vaccination area. They were of the following species: *Apodemus sylvaticus* (29), *Apodemus flavicollis* (22), *Apodemus* species (11), *Clethrionomys glareolus* (61), *Microtus agrestis* (12), *Sorex araneus* (6), *Neomys fodiens* (2), *Mustela nivalis* (1) and *Talpa europaea* (1). Each animal was tested for the presence of tetracycline and for the presence of orthopox lesions.

Results

Safety

No mortality or apparent modifications in wildlife populations were recorded during the three months of observation following the distribution of the baits.

Measurement of bait uptake

As shown in Table 1, what happened to the baits could be described in four ways; the bait and capsule had disappeared; or the bait had disappeared but a perforated capsule remained; or the bait had disappeared but an intact capsule re-

TABLE 1: Results of the assessment of bait uptake, expressed as the percentages of the baits which had disappeared, or had been interfered with or remained intact

Observations	Days after distribution of baits		
	4	9	15
Bait and capsule had disappeared	18·3%	34·2%	56·4%
Bait had disappeared, but perforated capsule remained	1·6%	3·6%	7·2%
Bait had disappeared, but intact capsule remained	1·6%	4·4%	6·4%
Bait remained intact	78·5%	57·8%	30·0%

TABLE 2: Detection of tetracycline in small mammals

Species	Tetracycline negative	Tetracycline positive	Total
Apodemus sylvaticus	29	0	29
Apodemus flavicollis	21	1	22
Apodemus species	11	0	11
Clethrionomys glareolus	61	0	61
Microtus agrestis	9	3	12
Sorex araneus	6	0	6
Neomys fodiens	2	0	2
Mustela nivalis	1	0	1
Talpa europaea	1	0	1
	141	4	145

mained, or the bait remained intact. After 15 days, 64 per cent of the baits had disappeared or their capsules had been perforated. The tracks of the animals showed that the baits had been taken by foxes, wild boar, mustelidae and small mammals.

Tetracycline in small mammals

Table 2 shows the results of tetracycline detection in 145 small mammals trapped in the vaccination area. Four animals were tetracycline positive but none of them showed lesions typical of an orthopox infection.

Serological analysis

No antirabies neutralising antibodies were detected in the sera of three boars killed in the vaccination area. No fox was taken in the area during the three first months after the distribution of the baits.

Discussion

The results obtained from this first field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus are preliminary, but they have confirmed the safety of the vaccine.

The results showed that the uptake of baits had been very good, but that of 145 small mammals trapped in the baited area only four had eaten baits.

No lesions were observed in the trapped animals and no other adverse phenomena were observed in wildlife during the three months after the distribution of the baits. A further experiment on a larger scale should therefore be done in order to confirm the field efficacy of this vaccine.

During the past 25 years, most of the research on the control of fox rabies has been devoted to the development of methods of fox vaccination against rabies by the oral route. The first campaigns carried out in western Europe with attenuated strains of rabies virus were successful. However, the use of conventional vaccines remains controversial because these vaccine strains are still pathogenic for some non-target species especially rodents which may eat the baits (Kalpers and others 1987). To solve this problem new vaccines are needed, such as

the recombinant vaccinia virus which expresses the immunising rabies glycoprotein. The oral administration of this vaccine was shown to be safe for several target species and a large number of non-target species. Moreover, this first field trial in Belgium has confirmed the safety of the vaccine.

Experimental field trials have thus demonstrated that it is technically feasible to control rabies in western Europe by the oral vaccination of foxes. Large scale vaccination campaigns must therefore be organised and coordinated between the countries concerned. The recombinant vaccinia-rabies virus seems to be the best candidate for such large scale campaigns because of its efficacy, which has been demonstrated experimentally in foxes, and its innocuity for non-target species.

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Strategic use of anthelmintics for parasitic nematodes in cattle and sheep

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Treatment regimes for the control of parasitic nematodes in cattle and sheep are reviewed. The advantages and disadvantages of each strategy are outlined and the veterinarian's judgement of the management and economic constraints involved when recommending a particular system to a client is emphasised.

STRATEGIES for the use of anthelmintics have been developed to maximise the production benefit gained by their use and to overcome the necessity for frequent drug administration and animal supervision. Strategies have also been developed to minimise the risk of anthelmintic resistance developing, thereby extending the effective life span of available drugs.

In the United Kingdom and other European countries unique systems have been devised for the convenient and effective administration of anthelmintics to cattle, thus saving labour and drug costs. These systems rely heavily on a detailed knowledge of the epidemiology of the parasite and the characteristics of the drug and in most cases depend upon reducing the number of infective larvae on the pasture during the latter half of the grazing season, when the greatest production losses due to parasitism generally occur. In the major sheep rearing areas of the southern hemisphere new strategies have become a necessity because the frequent and often indiscriminate use of anthelmintics has led to the development of parasite resistance to all the major classes of drug available.

Strategies are often complicated by pasture management requirements, and some may incorporate drug therapy with procedures designed to produce 'clean grazing' or at least minimal pasture larval contamination.

This paper outlines some of the strategies available and the rationale for their use for the protection of cattle and sheep.

Cattle

Traditionally anthelmintics were used to treat parasitic disease when the clinical signs became obvious (Fig 1). Using this method several treatments are required to suppress disease over a climatically typical season, and a production loss is

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