

Sue Kidd for extracting the VIDA data. This study will be presented in part fulfilment of the degree of DVM&S at Edinburgh University.

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A field trial in Belgium to control fox rabies by oral immunisation

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Campaigns of fox vaccination against rabies were carried out in Belgium in September 1986 and June and September 1987. The SAD B19 attenuated strain of rabies virus was inserted into baits which were distributed over an area of 2100 km² at a density of 11 baits/km². As recommended by the World Health Organisation, the efficacy and the innocuity of the method were controlled in the field and in the laboratory. Samples of blood and brain and jaw were taken from foxes which were shot or found dead in the vaccination area, for the diagnosis of rabies, the titration of antirabies antibody and the detection of tetracycline marker. In rabid animals, the virus strain was characterised by immunofluorescence using monoclonal antibodies. In September 1987, the uptake of the baits had reached 72 per cent by 14 days after distribution. Several wild species competed with foxes in taking the baits. After the last campaign, tetracycline was found in 65 per cent of the healthy foxes collected and rabies virus neutralising antibodies were detected in 77 per cent of them. In 1987, the incidence of rabies decreased markedly in the vaccination area compared with the untreated areas. No vaccine virus was isolated either from rabid animals or from 228 small mammals trapped in the vaccination area.

SYLVATIC rabies invaded Belgium 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 characterised by a succession of peaks

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which, until 1982, had a mean periodicity of four years. However, after the severe outbreak in 1982, the expected lull did not occur, and in the last five years although the annual numbers of rabies cases have steadily decreased they have remained abnormally high. It may therefore be assumed that rabies persists enzootically behind the leading edge of the invasion.

Since 1967, several methods for reducing the fox population have been used unsuccessfully. As in most other European countries, these control measures were only temporarily effective and did not stop the spread of the disease. Other methods such as the oral immunisation of foxes against rabies needed to be assessed (Baer 1975). Oral administration by the

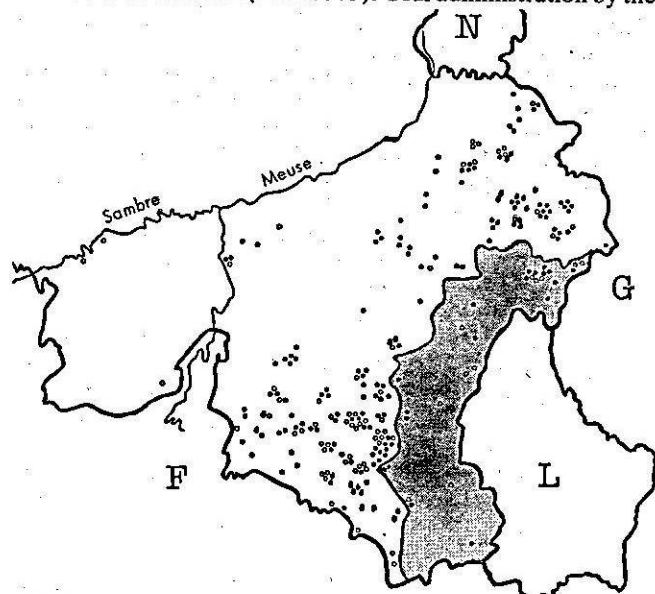


FIG 1: Geographical distribution of rabies in Belgium in 1987. ● Rabid wild animals, ○ rabid domestic animals. Dark zone indicates vaccination area

distribution of vaccine baits is the only route appropriate for the vaccination of a sufficient number of wild foxes. Since 1978, several European countries have initiated large scale field trials of fox vaccination using the SAD standard or B19 modified, live attenuated rabies virus (Steck and others 1982, Schneider and Cox 1983, Schneider and others 1985). The encouraging results obtained from these vaccination campaigns have provided evidence of the efficacy, the innocuity and the stability of the vaccine used.

The three campaigns of fox vaccination carried out in 1986 and 1987 in the south of Belgium were part of an international project involving four European countries (Pastoret and others 1987). The experimental protocol and the strategy were established, according to the rules of the World Health Organisation, by political and scientific authorities of the Grand Duchy of Luxembourg, West Germany, France and Belgium. The aims of the campaigns were first to create an immune belt around the eastern border of the Grand Duchy of Luxembourg, whose entire territory was being covered by vaccination and, secondly, to assess the efficacy and safety of oral vaccination under Belgian conditions.

Materials and methods

VACCINATION CAMPAIGNS

Area and period of vaccination.— The vaccination zone covered an area of 2100 km² in a heavily infected region of south Belgium. This area forms a 20 km wide barrier which runs along the eastern border of the Grand Duchy of Luxembourg from Germany to France (Fig 1). Vaccine impregnated baits were distributed in September 1986 and in June and September 1987.

Vaccine.— The vaccine used was the SAD B19 attenuated strain of rabies virus, adapted to and grown on baby hamster kidney cloned cells (Schneider and Cox 1983). It was produced by the Rabies Centre of the Federal Research Institute in Tübingen, West Germany. One dose consisted of 1.8 ml of virus suspension contained in a hermetically sealed capsule. The vaccine stock was stored at -20°C until distribution in the field.

Vaccine bait.— The baits consist of a machine-made mixture of fat, bone and fish meal enclosing a vaccine capsule. When a fox chews the bait, the vaccine capsule is perforated and the virus suspension spreads over the oral mucosa. Each bait also contained 150 mg tetracycline used as a marker of bait uptake; tetracycline can be detected in animal bones by ultraviolet microscopy.

Distribution of baits.— The baits were distributed in the field at a mean density of 11/km², with a range from about 5 to 15/km² according to the terrain. The bait distributors were forestry rangers and volunteer hunters who laid out the baits with gloved hands; they were placed so that they were invisible to human beings and animals and protected from sunlight by a covering of leaves or grass. Each campaign of bait distribution lasted about four days.

SURVEILLANCE

Bait uptake.— The uptake of baits by target and non-target species was monitored directly in several predetermined areas covering 200 to 400 hectares and situated in distinct administrative regions. Each vaccine bait was laid out, located with a coloured wooden stick, and then observed on days 4, 8 and 14 after distribution. One hundred and eighty-five, 312 and 292 baits were examined after the first, second and third campaigns, respectively. The ingestion of the appetising coating and, or, the vaccine liquid by non-target animals was identified by using some species identification criteria such as footprints or teeth marks on the baits.

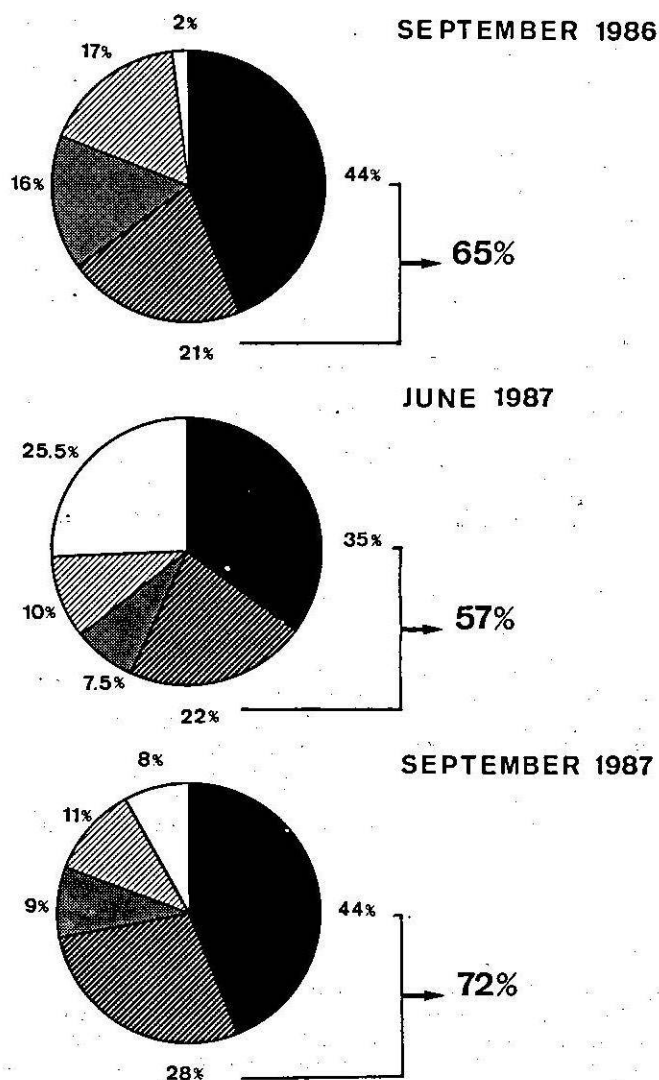


FIG 2: Uptake of baits recorded on the 14th day after distribution. These data were collected after the campaigns carried out in September 1986 (185 baits), June 1987 (312 baits) and September 1987 (292 baits). ■ Baits had disappeared, ▨ coating ingested and capsule found perforated and empty, ▩ coating ingested and capsule found intact, ▧ whole bait found intact, □ physical or biological alteration to the bait

Collection of field animals.— After each campaign, wildlife specimens were collected within the vaccination area for examination. Foxes and mustelids were shot or found dead. During the month following the first campaign, 216 small mammals (rodents and insectivores) and 11 hedgehogs were trapped in three control areas each covering about 3 km². The species, collection period and numbers of animals are detailed in Table 1.

Rabies diagnosis.— The presence of rabies virus in the brains of dead foxes was investigated by the analytical techniques recommended by the World Health Organisation (WHO), immunofluorescence and intracerebral inoculation of mice (Kaplan and Koprowski 1973).

Characterisation of virus strains.— Strains of rabies virus were characterised by using monoclonal antibodies prepared and supplied by the Tübingen WHO Centre (Schneider and others 1985).

Tetracycline marking of foxes and other wildlife.— The jaws were taken from dead animals and stored at -20°C until tested. Thin transverse sections (300 μm) were cut with a diamond saw (Isomet-Bueler) and examined directly. Tetracycline incorporation in the bones was detected by ultraviolet fluorescence microscopy (Capt 1981).

TABLE 1: Numbers of wildlife specimens collected for rabies diagnosis in the vaccination area

Vaccination campaign	Species examined	Total collected	Collection period (months)	Rabies positive n (%)
September 1986	Rodents and Insectivores	216	1	0
	Hedgehogs	11	1	0
	Foxes	68	5	16 23.5
	Foxes	32	3	8 25
June 1987	Stone martens	4	3	1 25
	Other mustelids	3	3	1 33
	Foxes	62	3	2 3

Serological analysis.— Blood samples were collected from the thoracic cavity of dead foxes. Rabies virus neutralising antibodies were determined by a technique of inhibition of fluorescence (Smith and others 1973, Frost 1978). Titres were expressed in international units (iu)/ml determined by comparison with a standard serum with a titre of 4.4 iu/ml. Titres higher than 0.5 iu/ml were considered as positive. Eleven healthy foxes collected in an unvaccinated area were used as controls.

Virological examination.— The brain of each trapped small mammal was suspended in phosphate buffered saline containing streptomycin and penicillin. After centrifugation, the supernatant was injected intracerebrally into five mice aged three to four weeks, and the inoculated animals were observed for 30 days. Mice showing signs of rabies were killed and the rabies virus was sought in the brain by direct immunofluorescence.

Results

The baits were affected in five different ways (Fig 2); they either disappeared, or their coating had been ingested and the vaccine capsule was found perforated and empty, or the capsule was found intact, or the whole bait was found intact, or there had been physical or biological alteration of the bait. In September 1986, and June and September 1987, respectively, 31 per cent, 28 per cent and 29 per cent of the baits were partially taken by small mammals. It was probable from the tracks observed that the vaccine had been ingested by rodents, mustelids and dogs.

RABIES DIAGNOSIS AND CHARACTERISATION OF THE VIRUS ISOLATES

The percentages of rabid animals are given in Table 1. The identification of the strain of rabies virus with monoclonal antibodies indicated that each rabid animal was infected with a field strain.

No case of vaccine rabies was observed in the vaccination area.

TETRACYCLINE MARKING

Table 2 gives the percentages of tetracycline positive foxes among the rabid and healthy specimens collected after each

TABLE 2: Presence of rabies virus neutralising antibodies and, or, incorporated tetracycline in rabid and healthy foxes collected in the vaccination area after each campaign of bait distribution, expressed as the number positive of the number of animals tested

	September 1986		June 1987		September 1987	
	Healthy	Rabid	Healthy	Rabid	Healthy	Rabid
Tetracycline	25/52	7/16	14/24	2/8	39/60	0/2
Rabies antibodies	—	—	13/17	7/7	31/40	1/1
Tetracycline and rabies antibodies	—	—	7/17	2/7	24/40	0/1

TABLE 3: Demonstration of tetracycline incorporated into wild species other than the fox, expressed as the number positive of the number of animals tested

Species	Tetracycline positive
Bank vole (<i>Clethrionomys glareolus</i>)	2/51
Woodmouse (<i>Apodemus sylvaticus</i>)	8/71
Yellow-necked mouse (<i>Apodemus flavicollis</i>)	7/18
<i>Apodemus</i> species	4/24
Other small mammals	0/16
Hedgehog (<i>Erinaceus europaeus</i>)	0/11
Stone marten (<i>Martes foina</i>)	3/6
Other mustelids	0/4

vaccination campaign. After the June 1987 campaign, 11 of 16 (69 per cent) adult and five of 16 (31 per cent) young foxes were tetracycline positive. As shown in Table 3, tetracycline was also detected in stone martens and rodents.

SEROLOGICAL ANALYSIS

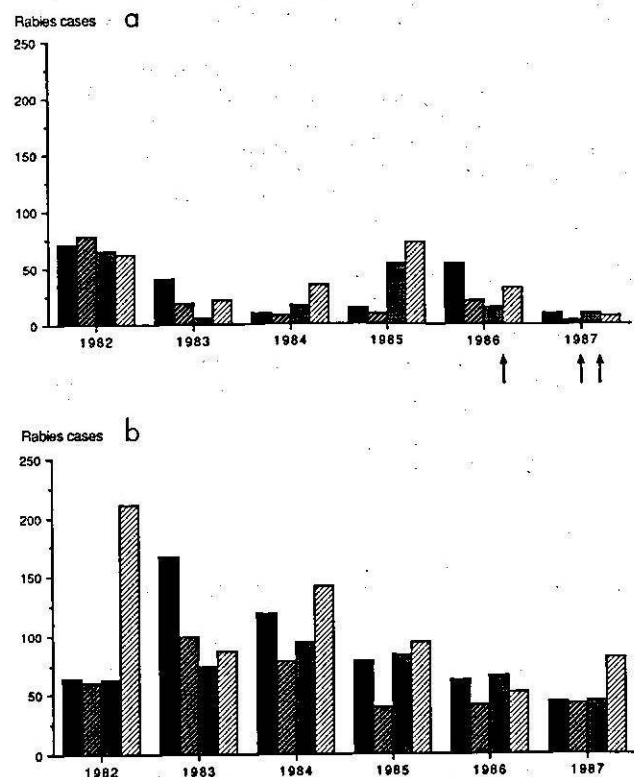
Table 2 gives the percentages of seropositive foxes among rabid and healthy specimens collected after the second and third vaccination campaigns. Three of the 11 (27 per cent) control foxes were also found to be seropositive for rabies virus.

VIROLOGICAL ANALYSIS

None of the 216 small mammals examined was infected with rabies virus.

EPIDEMIOLOGY

The annual and quarterly spread of rabies cases inside and outside the vaccination area since 1982 and after vaccination is shown in Fig 3. Figs 1 and 4 give the geographical distribution of the rabies epizootics in 1987 and 1986, respectively.

**FIG 3: Annual and quarterly spread of animal rabies in Belgium since 1982. (a) Area covered by vaccination in 1986 and 1987, (b) untreated area. Arrows show the vaccination campaigns**

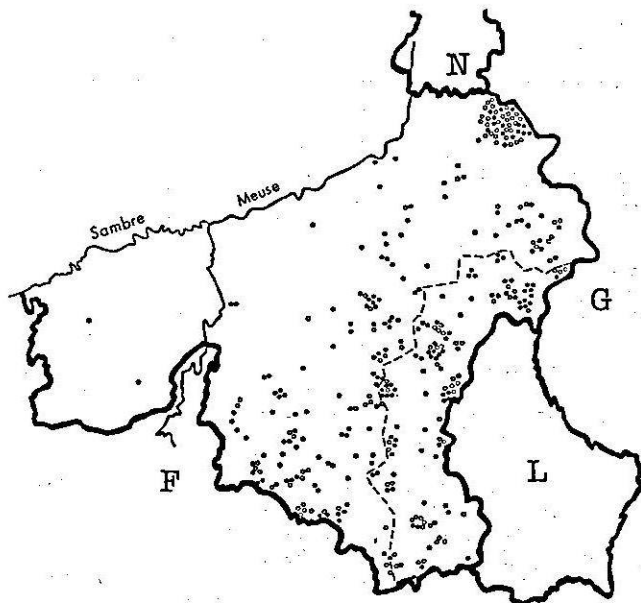


FIG 4: Geographical distribution of rabies in Belgium in 1986. ● Rabid wild animals, ○ rabid domestic animals

Only 29 rabies cases were observed in the vaccination zone in 1987 (14 foxes, nine cattle, two stone martens, two cats, one polecat and one roe deer) in contrast to the 122 cases recorded in 1986.

Discussion

The field controls of bait uptake confirm the efficiency of the 'Tübingen' baits. Most of the baits were consumed within the first week after distribution. However, during the June campaign, cool, wet weather diminished the smell of the coating and fungi developed on the bait surface. This deterioration was probably responsible for the lower bait uptake observed in June 1987.

A significant proportion of the baits was partially taken by rodents. Despite this competition, further uptake by foxes was generally not precluded. However, when baits persist in the field rodents may eat the whole coating, perforate the plastic capsule and swallow the vaccine liquid. The use of the SAD B19 attenuated rabies strain therefore remains harmful for wild rodents because mortalities have been described in mice dosed orally with the SAD B19 strain (Schneider and Cox 1983). Nevertheless, the failure to isolate rabies virus in 217 rodents trapped in the vaccination area confirms the results obtained by other groups (Wachendörfer and others 1985). Unfortunately, this safety control requires many more animals per hectare for the detection of rodents with prepatent rabies. Furthermore, the trapping methods does not help to detect specimens with patent rabies, or animals which have died.

The results of tetracycline incorporation in fox bones were inferior to those obtained in West Germany and the Grand Duchy of Luxembourg (Schneider and others 1985, Frisch and others 1987). Approximately 50 per cent of the foxes collected after the campaigns in September 1986 and June 1987 were marked with tetracycline, but after the June campaign only 30 per cent of the fox cubs were tetracycline positive. This low value suggests that it is difficult to immunise young foxes at this period; the situation might be improved by distributing bait around the breeding dens. The percentage of tetracycline positive foxes reached the expected value (>60 per cent) only after the third campaign.

The tetracycline marking showed that stone martens and rodents were strong competitors of the foxes for the uptake of the baits.

Animals positive for both tetracycline and rabies were either incubating rabies or did not perforate the capsule when

consuming the baits. The presence of vaccine induced rabies was excluded by the determination of the virus strain with monoclonal antibodies.

A poor correlation was observed between the percentages of seropositive and tetracycline-positive healthy foxes. This low correlation might have been due either to non-specific reactions inhibiting the fluorescence when blood samples of poor quality were tested, or to the low sensitivity of the tetracycline detection test. The first hypothesis is reinforced by the fact that three of 11 healthy foxes collected outside the vaccination area were also found to be seropositive.

In 1987, the incidence of rabies decreased markedly in the vaccination area because only 29 cases were recorded. Moreover, many of these rabid animals were observed in regions adjacent to the border of the vaccination area. The epidemiological map of 1987 demonstrates that the treated area became clean and was surrounded by heavily infected regions. On the other hand, in comparison with 1986, the enzootic incidence of rabies in untreated regions did not decrease in 1987. These epidemiological data confirm the efficacy of the fox vaccination method for the control of sylvatic rabies.

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Correction

Latency of equine herpesvirus 4 (equine rhinopneumonitis) (VR, November 12, p 518). The following errors in the above paper have been pointed out by the authors. In the fourth paragraph on page 519, the second and third sentences should read 'Horse 2 excreted virus from day 3 after commencement of corticosteroid treatment until day 13. Horse 3 excreted virus beginning on day 6 after commencement of corticosteroid treatment and continued to excrete virus until day 26'.

In the seventh paragraph on page 519, the first sentence should read 'There is some doubt remaining in this study as to whether reactivation was induced by corticosteroids or the trauma of nasal swabbing, or the combination of both'.