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Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

The oral vaccination of foxes against rabies

Report of the Scientific Committee

on Animal Health and Animal Welfare

Adopted on 23 October 2002

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1. MANDATE

The Commission asked the Committee to assess the reasons for failures noted in the implementation of certain rabies control protocols and identify the corrective action recommended to bring about the eradication of rabies in the Community as soon as possible.

A comparative study of the various fox vaccination protocols implemented, using the parameters listed below, may allow conclusions on the most appropriate strategy to eradicate rabies as soon as possible from the Community

1. Type of vaccines

2. Type of baits

3. Methods of release of vaccine baits

4. Density of baits and distribution patterns

5. Seasonal pattern of the releases

2. BACKGROUND

For many years the Community has contributed financially towards campaigns for the oral vaccination of foxes in a number of Member States affected by epidemics of wildlife rabies. There is particular concern at the setbacks noted in Germany, with the significant increase in cases of rabies in two regions. The situation in North Rhine Westphalia and in a common area between Hesse and Bavaria particularly demands attention, because it reduces the possibility of a final eradication of rabies from the Community. The results of a study financed by the Commission on the evaluation of effectiveness of vaccination campaigns of foxes have recently been reported (FAIR Project CT 97-3515). The combination of the results of this and other studies and the setbacks noted in Germany lead us to consider reviewing the vaccination protocols implemented.

3. RABIES AETIOLOGY

Rabies is a zoonotic viral disease, which causes an acute encephalitis in domestic and wild mammals. It is transmitted through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes). Once symptoms of the disease develop, rabies is fatal to both humans and other animals.

Rabies virus belongs to the order *Mononegavirales*, viruses with a non-segmented, negative-stranded RNA genome. Within this group, viruses with a distinct "bullet" shape are classified in the *Rhabdoviridae* family. The genus *Lyssavirus* includes rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, European bat lyssavirus 1 and 2 (EBL 1 and 2), and a newly discovered Australian bat lyssavirus (Bourhy *et al*., 1993).

The most widely used test for rabies diagnosis is the direct immunofluorescence test (FAT) on acetone-fixed smears of hippocampus, cerebellum or medulla oblongata. Virus neutralisation tests, rapid fluorescent focus inhibition tests (RFFIT) and fluorescent antibody virus neutralisation tests (FAVN), are used primarily to evaluate vaccinal antibody responses against rabies virus (OIE, 2000).

4. THE OCCURRENCE OF RABIES IN EUROPE

Since around 1939, the epizootic of fox rabies spread 1,400 km westwards from Poland, with a 20 to 60 km advance per year, resulting in the infection of several European countries. The farthest west point of spread in France was reached in 1982. Eighty-three percent of the reported cases (Aubert, 1995) were in red foxes (*Vulpes vulpes*) which is the main reservoir as well as the main vector of the virus. The racoon dog (*Nyctereutes procyonoides*), an Asian species introduced into western Russia around 1920 has also transmitted the virus in Central Europe and in Finland (10.5% of cases) (Finnegan *et al*., 2002). Nine human rabies cases have been reported in Europe in 2000 (Müller, 2000). Humans are at risk mainly through exposure to the virus from infected species of domestic animals (cattle, cats and dogs). Imported rabies cases in humans have occurred following infection from dogs in countries where canine rabies is endemic. In 2001 two human cases were recorded in the United Kingdom following infection in The Philippines and Nigeria (Fooks, 2001).

A genetically distinct virus within rabies virus type I is found in arctic foxes and this virus has been recognised in outbreaks among non-arctic animals, including the red fox and domestic animals (Nadin-Davis *et al*., 1993).

In Europe the insectivorous bat rabies cycle is independent from the epidemiological rabies cycle that involves foxes and other terrestrial mammals. Bat virus isolates of European bat lyssaviruses (EBLs) are genetically different from those found in foxes. In bats the infection seems to persist without inducing any clinical symptoms until a stress situation eventually activates the disease in some of them (Rønsholt *et al*., 1998). Later investigations have strengthened the assumption that EBL may infect most bats in a population without any noticeable clinical symptoms, possibly leaving many of them latently infected (Serra-Cobo *et al*., 2002; Wellenberg *et al*., 2002).

From 1977 to 2000, 630 bat rabies cases were recorded in free-living insectivorous as well as captive frugivorous bats in Europe, also comprising 3 human cases (Müller, 2000). Experimental infection with a Danish EBL-1a strain indicated that the cat may become infected (Fekadu *et al*., 1988). Natural infection in sheep has been observed twice in Denmark (Rønsholt, personal communication) and in 2001 a spill-over of EBL 1 in a stone marten was also reported (Müller *et al*., 2002).

4.1. Vaccination campaigns in Europe

In 1989, an increase of rabies cases occurred and produced the highest peak of rabies incidence of recent decades (Figure 1 describes the western limit of the rabies front in 1989 and in 2001). As a result of oral rabies vaccination

campaigns, the rabies situation in European countries has greatly improved since 1989 (Müller, 2000). A drastic decrease in the rabies incidence has been recorded in most western European countries (Figure 2).

In 1988, Finland experienced an outbreak of rabies in racoon dogs and foxes, close to the south-eastern border of the country. Field vaccination campaigns started in 1988 using 2 bait-layings a year, and since 1991 a single bait-laying each year in autumn. Thereafter the country has remained free of reported cases of rabies, although the disease remained endemic in Russia and Estonia. Italy carried out vaccination campaigns in the infected areas only when cases were recorded, starting from 1984. The same strategy was successfully pursued during the following years.

The Belgian programme covered the entire infected area from 1989 until 1991, with 5 campaigns in total, leading to an 80% decrease of rabies cases. Then more restricted campaigns were conducted in 1992, 1993 and 1994 only along the borders of the country. Rabies cases were recorded again from 1994 to 1996, coming from a border residual focus. In 1996, the vaccination strategy was modified and adapted to control rabies re-infection in the presence of a high density fox population. Two aerial vaccinations were carried out during the cold season (November and March: when the fox population density is at its lowest of the year). Control of aerial distribution was intensified by use of GPS (Global Positioning System) and reducing the distance between flight lines to 500 metres and finally baiting density was increased from 15 to 17 baits per km², supplemented by an additional den vaccination. Following this modification of the strategy and a close cross-border co-operation with their French counterparts, rabies was efficiently controlled (the last rabies case occurred in July 1999 in a 28 month old cow).

Switzerland proposed the vaccination strategy that has been followed by other European countries, consisting of the compartmentalisation of the infected areas using natural or artificial barriers. The last rabies case was recorded in Switzerland in 1996.

In France, with a peak of more than 4,200 rabid animals in 1989, the strategy consisted of establishing an immunological barrier from the English Channel to the Swiss border, which succeeded in stopping the westward and southward spread of the disease. During the following years the vaccinated area was shifted towards the borders resulting in a 99.7% decrease in rabies incidence from 1989 to 1996. In France the last rabies case of vulpine origin was recorded in a cat in December 1998. A part of the success of Belgium, France, Luxembourg and Switzerland in controlling fox rabies is due to the fact that they developed close co-operation for preventing cross-border contamination and improving their vaccination techniques (Aubert *et al*., 1994).

In Poland, despite vaccination campaigns, the number of recorded cases in wildlife and domestic animals (Figure 3) and the size of the infected area has been increasing since 1999. In the Czech Republic, several vaccination campaigns led to a significant decrease in rabies incidence, although two foci remain, located at the borders with Germany and Poland. To date, seven European countries are reported to be free from rabies following the use of oral rabies vaccination campaigns: Finland (since 1991), The Netherlands

(1991), Italy (1997), Switzerland (1998), France (2000), Belgium and Luxembourg (2001).

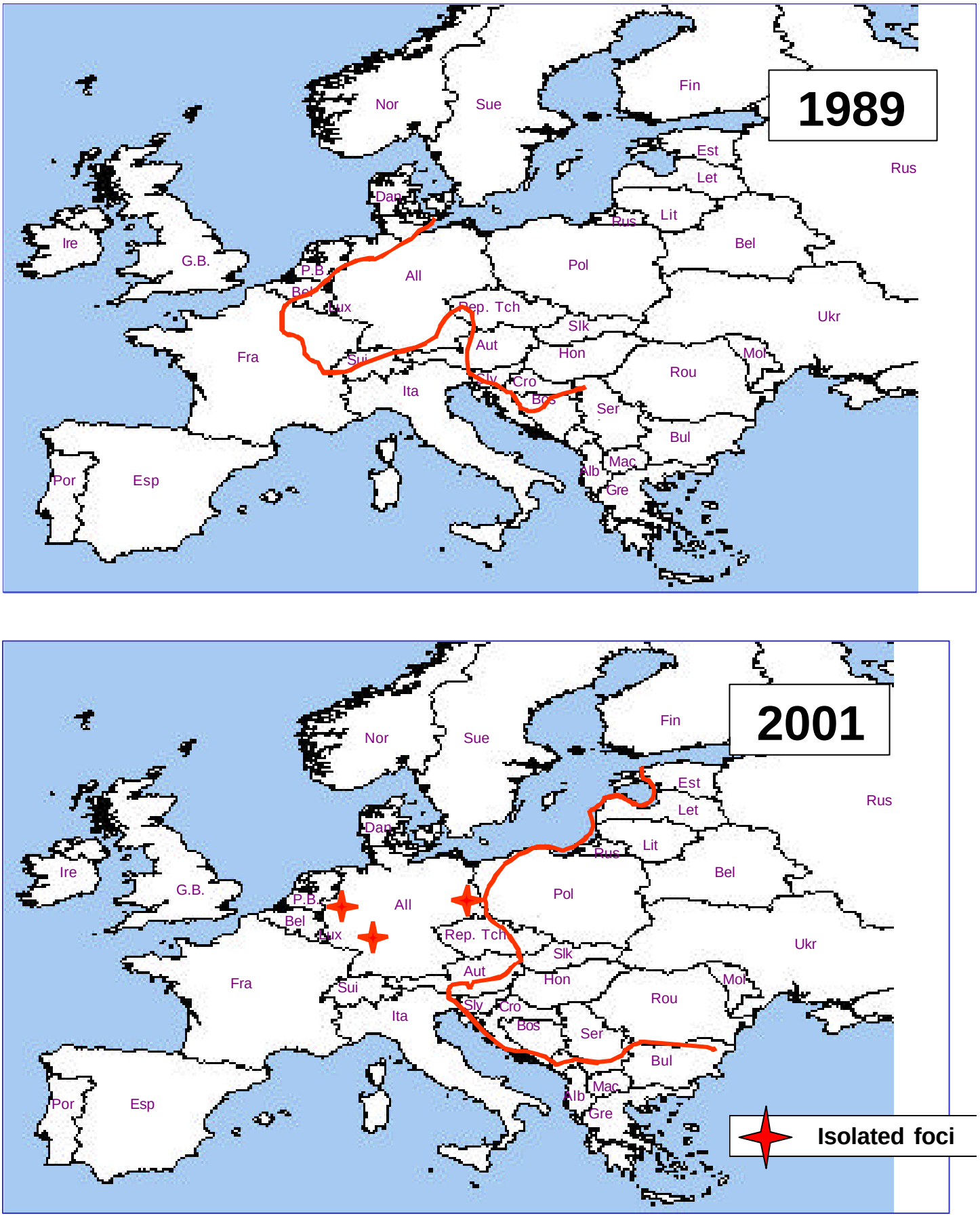
Oral rabies vaccination has, as in other European countries, drastically decreased the rabies incidence in Germany from 10,487 to 83 cases reported in 1983 and 1997 respectively. However, in contrast to the eastern parts of the country, severe setbacks occurred in some areas of the western parts. In 1998 and again in 2000 an increase in the rabies incidence was observed, with the vast majority of cases occurring in Bavaria and Hesse and in North-Rhine Westphalia. Following recent changes in vaccination strategies in Bavaria and North-Rhine Westphalia, no rabies case has been observed since March and June 2001, respectively. In Hesse since July 2000 rabies cases have occurred at a low level in a very small (65 km^2) suburban/urban area affecting two adjacent communities (Mühlheim and Offenbach). In March 2002, the disease spread into 2 further adjacent communities. In Saxony, a vaccination belt has been maintained along the border with the Czech Republic and Poland since 1997 to prevent re-infection from rabies-infected areas. Table 1 lists the total number of rabies cases in Germany from 1997 to 2002, while Figure 4 shows the distribution of rabies cases within Germany in 2000 and 2001. More detailed information on rabies control strategies in Germany is collected by the German National Reference Centre for Rabies (T. Müller, personal communication).

Table 1: Total number of rabies cases in Germany from 1997 to 2002 (excluding cases of rabies in bats)

	1997	1998	1999	2000	2001	2002*
North-Rhine Westphalia	30	55	30	35	9	0
Hesse	14	26	9	83	24	13
Rhineland Palatinate	9	2	0	0	0	0
Bavaria	2	1	8	57	3	0
Saarland	27	11	0	1	0	0
Saxony	1	9	8	6	4	0
Germany	83	104	56**	182	41***	13

* as of March 26th, ** including 1 imported rabies case from Thuringia, *** including 1 EBL 1-infection of a stone marten from Saxony-Anhalt. Source : WHO (World Health Organization) Collaborating Centre for Rabies Surveillance and Research, Wusterhausen, Germany.

Figure 1: Western limit of the wildlife rabies enzootics in Europe in 1989 and in 2001 (Compiled from the Rabies Bulletin Europe 1989-2001).



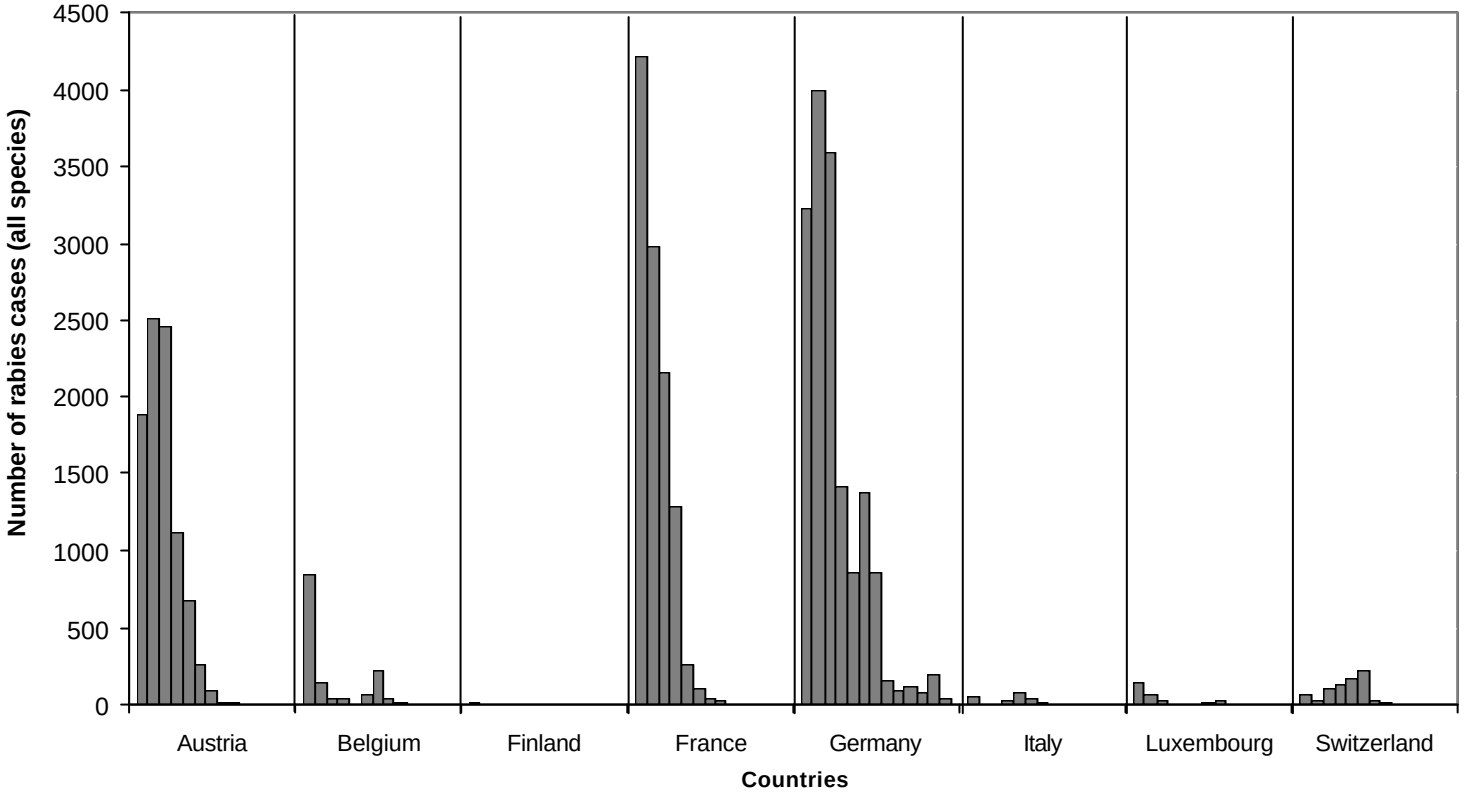


Figure 2: Number of rabies cases (all species) in the western European countries from 1989 to 2001 (Compiled from Rabies Bulletin Europe 1989-2001).

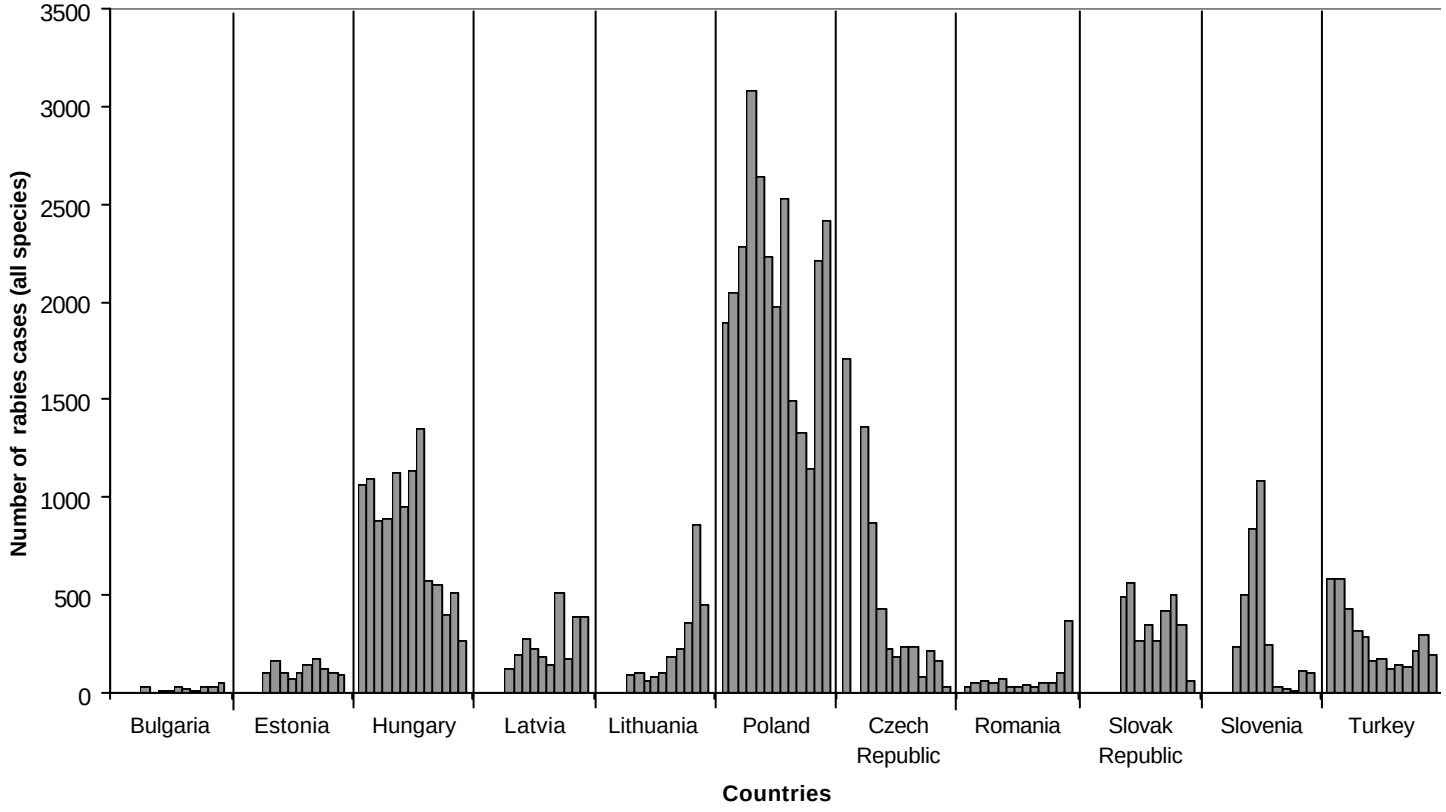


Figure 3 : Number of rabies cases (all species) in the central and eastern European countries from 1989 to 2001 (Compiled from Rabies Bulletin Europe 1989-2001).

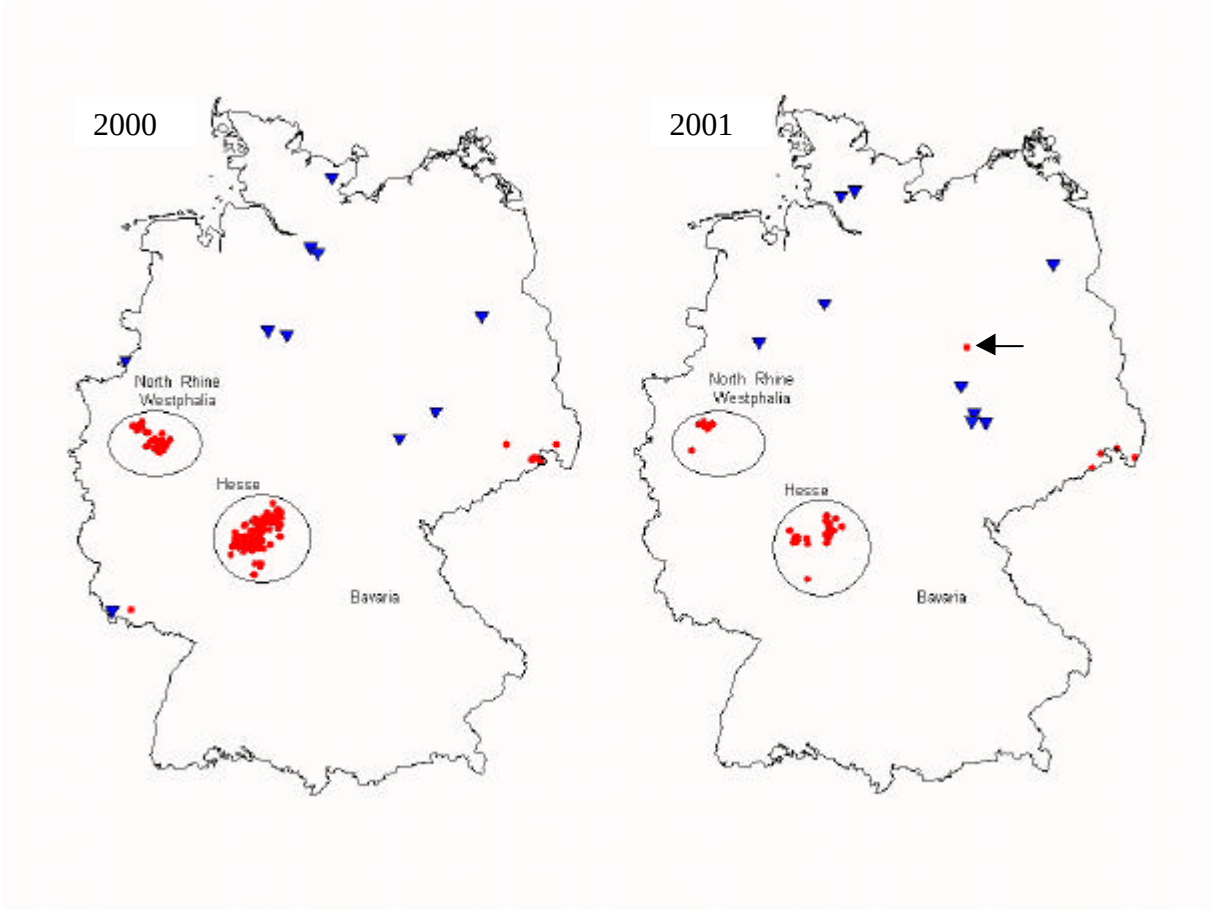


Figure 4: Rabies situation in Germany in 2000 and 2001 (Source : Institute for Epidemiology, WHO Collaborating Centre for Rabies Surveillance and Research, Wusterhausen, Germany).

The dots represent fox-mediated rabies cases in domestic and or wild animals. The triangles indicate reported bat rabies cases in the respective years. Circles mark the problematic areas in which rabies still existed at that time. Those areas affected three Federal States- Bavaria, Hesse and North-Rhine Westphalia. In 2001, a spill-over infection of EBL 1 in a stone marten was reported (Müller *et al*., 2002) as indicated by the arrow.

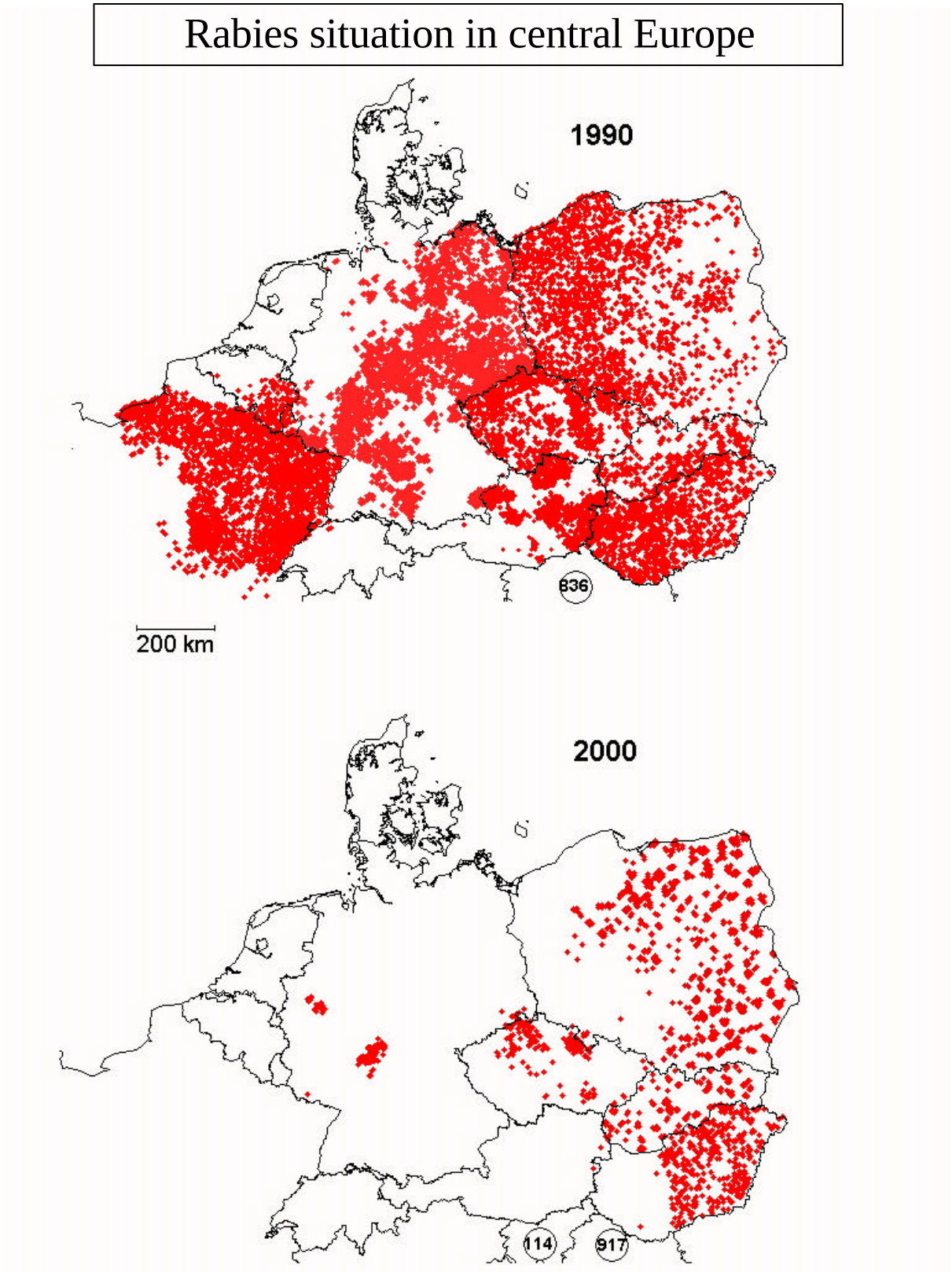


Figure 5: Geographical distribution of fox-mediated rabies in Central Europe in 1990 and 2000. Bat rabies cases are not considered. (Source: Institute for Epidemiology, WHO Collaborating Centre for Rabies Surveillance and Research Wusterhausen, Germany)

Regarding figure 5, each dot represents a single rabies case. For some countries the total number of rabies cases is indicated in circles because the spatial allocation of rabies cases for those countries is difficult due to reorganisation of administrative units and not yet updated nuts (national units of territories) format of the mapping software

5. FOXES AND RABIES

5.1. Fox biology

General information on fox biology has been published by many authors, see among others Corbet and Harris (1991).

The red fox is a medium-sized carnivore of the canid family. It is a highly adaptable species with little specific habitat requirements. It is most abundant in fragmentary and diverse habitats, including cities, which offer a wide variety of cover and food.

Foxes live in couples, in territories they may or may not share with a family group (depending on the population density). Size of territory ranges between 40 and 400 ha (Artois *et al*., 1990). This variation may be related to individual dominance and the availability of food and shelter provided by the habitat. Not all parts of the territory are used with the same frequency and adjacent territories may overlap to a certain degree.

Activity is mainly nocturnal and crepuscular (with a less active period in the middle of the night). Foraging behaviour regimens are highly adaptable. Prey species and food sources are very diverse: mammals, birds, insects, earthworms, fruits and scavenged items are important sources.

Females have a single oestrus per year and can reproduce from 10 months old (yearlings). In the northern hemisphere, mating occurs from mid-December to mid-February. Gestation lasts 53 days, with a peak of cubs being born in late March. In rural areas mainly, earths are used for litters (earths are infrequently used by foxes during other periods). Mean litter size is four to five, and cubs are born blind and deaf. By 8 weeks, their woolly coat begins to be covered by hairs with a colour and pattern very like that of the adult. They suckle until 4 weeks, and are then progressively weaned on to solids. First emergence above ground is at this age. They eat a large variety of solid items by 5-6 weeks, and become progressively more independent during summer.

Dispersal occurs principally in animals 6-11 months old (from August to March). Young males disperse earlier and further. The proportion of dispersers depends on the density of population and possibly on the level of human activity and control.

Social instability due to mating and dispersal has been cited as a cause for the peaks of rabies incidence during spring and autumn (Kauker and Zettel, 1963).

5.2. Fox population counting

Hunting statistics are an acceptable indicator for the fox population trends at a regional or national level, provided that the records have been compiled consistently over the years and the hunting pressure has not changed greatly. Although the impact of hunting on the overall fox population is not very well documented, the relationship in Switzerland between the hunting statistics and the fox population is discussed in Breitenmoser *et al*. (2000). Hunting could also affect the dispersal of animals, although available data are limited.

The effect of more intensive fox hunting on the success of a vaccination strategy has not been clearly elaborated. However, the submission of specimens by hunters aids in the monitoring and surveillance of vaccination campaigns, allowing bait uptake and seroconversion rates to be measured.

More accurate methods for measuring fox populations can be applied by trained field ecologists in smaller areas, but such data cannot be extrapolated to a larger area or an entire country. The most commonly used methods are:

- Night counting index: the number of fox sightings per 10 km is recorded driving along a defined distance at a given speed.

- Road kills: fox carcasses are collected on roads according to a standardised protocol.

- Distance sampling is a recently developed "line transect method" similar to night counting and seems to allow a direct evaluation of the population density (Buckland *et al*., 1993)

- Analysis of the population structure of foxes (age, sex) provides data on the structure, status and dynamics of the population.

5.3. Rabies in foxes

Rabies, historically mainly reported in dogs, had virtually disappeared from central Europe at the turn of the twentieth century. However, from the 1940s the disease established itself in the fox population in Eastern Europe and spread inexorably south and west, eventually to encompass almost the whole of Western Europe (Taylor, 1976; Wachendörfer and Frost, 1992). Following the high co-adaptation of the current rabies virus strain to the fox, and due to fox ecology, no other species play a significant role in maintaining the disease in the infected areas, although many domestic and wild mammals (cattle, domestic cats, dogs, badgers, roe deer, racoon dogs etc.) are affected and may transmit the disease.

Rabies in foxes is characterised by a highly variable incubation period from 11 days up to 15 months. The median duration probably does not exceed 30 days. Irrespective of the incubation duration, the morbidity phase is short (from zero to 14 days). The rabies virus multiplies in the brain and salivary glands and is transmitted through biting, either as a part of normal behaviour or provoked through a neural disorder. Up to 28 days before the onset of clinical symptoms, foxes incubating rabies have been shown to be able to transmit the disease to healthy individuals. Clinical signs are anorexia and changes in behaviour- the most visible sign is the loss of fear of people, making the foxes more visible, although aggressive behaviour towards people is rare (not more than 2% of human exposures to rabies in contaminated areas). Rabies infection in foxes is considered invariably lethal and there is no report of any fox surviving clinical rabies.

When the virus spreads into a rabies free area, either through contact of an infected fox with healthy neighbouring foxes or contacts with dispersing individuals, the disease may kill most of the resident foxes, lowering the local population density below the threshold value of rabies persistence. However,

the area will be re-populated through inward migration of foxes from neighbouring areas and the high reproductive potential of the species is also a factor. On a larger scale, a dynamic equilibrium occurs between patches of fox depopulated areas, areas without infected, or with long incubating, foxes, and areas with active foci of infection where foxes rapidly decline in numbers. Healthy or incubating foxes disperse in all directions, but stay more readily in less fox-populated areas. As a result, rabies persists in a clustered pattern, without eliminating all foxes, although it decreases their overall abundance.

These pathogenic and epidemiological features explain how the fox can be both a victim and the reservoir of the disease, and is at the same time the key to rabies control through oral vaccination.

5.4. Rabies control in foxes

The earliest attempts to control the disease in foxes focussed on radical reduction of the fox population. However, in practice it was nearly impossible to reduce the population density below a threshold value where disease transmission would cease (Aubert, 1992). More promising was the vaccination of the main host, using immune territorial foxes as a barrier to the spread of the virus. As Wandeler (1991) aptly wrote: “The wild mammal does not follow an invitation to visit a veterinarian, and there is no owner to bring it there. It has to be lured by some trick to vaccinate itself”. The combined cost-benefit balance of rabies and of fox population reduction (including the costs for culling) versus oral vaccination of foxes (including baits, bait delivery and follow-up to ensure the efficiency of the vaccination) have been compared. In France, the cumulative costs of both strategies remained comparable up to the fourth year. Thereafter, the oral vaccination strategy became more beneficial (Aubert, 1999).

Following early attempts in the early 1960s and field tests in the 1970s, the simplest, most efficient and economic method for vaccinating foxes proved to be industrially manufactured baits, made of an envelope attractive for foxes, containing a capsule or a plastic sachet filled with an attenuated anti-rabies vaccine in liquid form (Schneider *et al*., 1987). The bait envelope contains 150 mg of tetracycline to mark bait consumers. The bait has to be thoroughly chewed in order to guarantee that the sachet is punctured and the vaccine is released into the mouth of the consumer (see chapter 6.3.1). Hence, foxes and other species may have their teeth and bones marked with tetracycline deposits without necessarily being vaccinated (Kappeler, 1991).

6. ORAL VACCINATION OF FOXES AGAINST RABIES

6.1. Vaccines

It has been shown that immunisation and protection cannot be achieved when inactivated rabies vaccines are given by the oral route. For example, the preliminary results of Atanasiu *et al*. (1982) suggesting that domestic cats may be immunised using inactivated vaccines were not confirmed on the red fox, even when enteric coated tablets were used that protected the vaccine against gastric acidic pH (Aubert *et al*., 1982). Since the use of inactivated vaccines has been demonstrated not to be effective, attenuated vaccines are

therefore used. Regarding the use of attenuated vaccines and vaccines derived from the vaccinia virus, possible contact of immunosuppressed people with those vaccines is a consideration and whether infections could become established in those immunosuppressed individuals.

Three categories of oral rabies vaccine should be considered as their origin explains their difference in residual pathogenicity:

- VRG is a genetically engineered vaccine derived from vaccinia virus. It presents no rabies risk to humans and the environment, although an infection with VRG has been reported in the US in a woman who had epidermolytic hyperkeratosis and was 15 weeks pregnant (Rupprecht *et al*., 2001). She suffered swelling and erythema but healed ten days later without any anti-viral treatment and remained free of symptoms. Her pregnancy was normal and she delivered a healthy child.

- SAG1 and SAG2 were made from the SAD Bern strain following one and two successive mutations of the Arginin 333 codon, respectively. Any change in this codon leads to a considerable loss of virus pathogenicity (Lafay *et al*., 1994).

- SAD B19 and SAD P5/88 were produced from the SAD Bern strain by attenuation following several passages in cell culture. Another strain termed SAD VA1 has been used in field trials in Germany. However there is insufficient data available to include this vaccine in the present review.

Modified live virus vaccines to be used for oral vaccination of foxes fulfil all the requirements of the European Pharmacopoeia monographs, e.g. *Vaccinum rabiei per orale vivum ad vulpem*, European Pharmacopoeia, (2002), and should also take account of WHO recommendations (1989).

The main characteristics of vaccines used in the EU have been summarised in Table 2.

Table 2. Summary of main characteristics of oral rabies vaccines used in the EU (Data compiled from manufacturers and EMEA)

Vaccine	VRG	SAG2	SAD B19	SAD P5/88
Proprietary name	Raboral	Rabigen	Fuchsoral	Rabifox
Company	Merial	Virbac	IDT	IDT
Quality				
Vaccine titre,	>8 $\log_{10}$ $TCID_{50}$/dose	>8 $\log_{10}$ $TCID_{50}$/dose	7 $\log_{10}$ FFU/ml	7 $\log_{10}$ FFU/ml
Thermostability, virus titre	Stable (time and temperature details not available)	0.16 $\log_{10}$ reduction after 2 days at 25°	0.4 $\log_{10}$ reduction after 7 days at approx. 25°C	0.26 $\log_{10}$ reduction after 7 days at approx. 25°C
Melting point of bait casing	> 50°C	43°C	35°C (new bait casing under development)	35°C (new bait casing under development)
Safety				
Non-target species tested	52	approx. 30	approx 20	approx. 15
Tested Horizontal transmission	None in foxes (adults and cubs), dogs, cats, cattle, ferrets	None in foxes, may be found in salivary glands of young dogs	None in foxes, rodents, skunks and dogs	None (no information on species)
No Reversion to virulence after	7 backpassages in mice (intracerebral and footpad), 10 backpassages in vero cell cultures, 1 backpassage in fox	5 backpassages in suckling mice	5 passages in foxes and 10 passages in suckling mice	10 passages in suckling mice
Efficacy				
Lowest protective dose tested	10^{7} $TCID_{50}$/dose	$10^{8.1}$ $TCID_{50}$/dose	$10^{6.0}$ $\log_{10}$ FFU/ml	$10^{6.2}$ $\log_{10}$ FFU/ml

TCID: tissue culture infective dose, FFU: focus forming units, EMEA: European Agency for the Evaluation of Medicinal Products

6.1.1. Efficacy

Immunisation and protection may be given orally using attenuated vaccines as demonstrated by several experiments:

- in North America : Baer *et al*. (1971), Black and Lawson (1973)

- in Europe : Mayr *et al*. (1972), Dubreuil *et al.* (1979), Frost and Kiefert (1979), Steck *et al*. (1982), Artois *et al*. (1993), Neubert *et al.* (2001).

The level of protection against experimental challenge (percentage of survivals) is correlated with the dose of the vaccine (virus titre measured in mice or cell cultures, dose-effect curve). Numerous vaccination challenge studies have been performed in the laboratory and confirm this observation (for an early review see Blancou *et al*., 1986).

When delivering the vaccine to the red fox using a bait, the vaccine should be approximately 10 times more concentrated to obtain the same level of protection as the same vaccine given by direct oral instillation (Blancou *et al.,* 1986).

6.1.2. Immunity: "booster" effect and maternal immunity

The FAIR project CT 97 – 3515, "Wildlife vaccination against rabies in difficult and emergency situations and its potential impact on the environment", included a task aimed at studying several aspects of fox and fox cub immunity. The following results are summarised from the final report for this task and from other recent scientific papers.

Compared with one single oral vaccination (VRG or SAG2), two vaccinations with an interval of 35 days with the same vaccine give no advantage in terms of

(i) antibody level four months later,

(ii) cell-mediated immune response, and

(iii) protection to challenge (Lambot *et al*., 2001).

In addition, a field study has demonstrated that no significant benefit for immunisation of adult and young foxes was obtained by two delayed distributions of baits (Bruyère *et al*., 2000). On this basis, no immunological reason for performing double vaccination exists.

Four-to-five week old fox cubs are able to respond to varying extents to oral vaccination with VRG or attenuated rabies viruses such as SAG2 and SAD B19, depending on the existence of maternal immunity. When born from non-vaccinated vixens or from vixens vaccinated with VRG, cubs develop a complete protective immunity against mortality from rabies challenge (Blasco *et al*., 2001). Cubs born from vixens vaccinated with SAG2, and which are vaccinated with SAG2 produce a lower neutralising antibody response after vaccination than cubs vaccinated with VRG. In addition, they are less well protected against challenge (FAIR CT 97-3515, part 1. Immunological evaluation in captive foxes of new methods of oral vaccination, 2002).

Müller *et al*. (2001) with the SAD B19 vaccine, had shown a strong interference between passively and actively acquired immunity. With the SAD B19 vaccine, this interference affected the ability of 7 out of 10 fox cubs to resist the virus challenge.

The results indicate that VRG is better able to overcome the effects of maternal immunity than other vaccines.

6.1.3. Safety

Rabies vaccines have various levels of attenuation and there have been several meetings organised by the WHO aimed at defining the safety and efficacy requirements for the oral rabies vaccines.

Safety requirements recommended by the WHO deal with safety in target species (the red fox) and non-target species, such as wild carnivora and rodent species. Following the discovery that the original SAD Bern strain is highly pathogenic for the baboon by the oral route (Bingham *et al.*, 1992), non-human primates have been added to this list as a model for human exposure to vaccines (see also chapter 6.1). Table 3 summarises the main results on the residual pathogenicity of these vaccines.

Table 3. Summary of the main results from safety trials carried out on target and non-target species using the VRG, SAG2 and SAD B19 vaccines (When no reference is given, results have been drawn from WHO reports 1989-1998).

Vaccines	Carnivora	Rodents	Immunocompromised mice	Non human Primates
VRG	No pathogenicity	No mortality	No mortality In 40 SCID mice (10^9 $TCID_{50}$)	No pathogenicity for 11 chimpanzees (10^9PFU/ml) 24 Common squirrel monkeys (10^8PFU/ml) (Rupprecht *et al*.,1992)
SAG2	No pathogenicity	No mortality	No mortality In 10 SCID mice (10^8 $TCID_{50}$)	No pathogenicity for 10 baboons (10^9 PFU) (Bingham *et al*., 1997)
SAD B19	No pathogenicity in several species Pathogenic for Skunk at high doses (10^9 FFU) (Rupprecht *et al*., 1990, Vos *et al*., 2002)	Up to 6% mortality in several European wild species (Artois *et al*, 1992, Vos *et al*., 1999)	No mortality in 10 SCID mice ($10^{7.4}$FFU), mortality in 2/10 nude mice ($10^{7.3}$ FFU)	No pathogenicity for 12 baboons ($10^{8.3}$ FFU) (Vos *et al*., 1999)

TCID: tissue culture infective dose, FFU: focus forming units, PFU: plaque forming units, SCID: severe combined immunodeficient mice

While thermostability of oral rabies vaccine viruses is essential to guarantee vaccination success, vaccine viruses that remain stable over a prolonged period of time could also pose a potential safety risk. This is because all presently available commercial oral rabies vaccines, live-modified or recombinant-based, are self-replicating and not completely without risks (Rupprecht *et al*., 1996). Baits containing oral rabies vaccines that are thermostable over a long period of time and that are not consumed by the target species could therefore be considered as potential bio-hazardous waste (Maurer and Guber, 2001). However, generally, most vaccine baits disappear within 7 days following distribution in the field (Brochier *et al*., 1988; Hadidian *et al*., 1989).

6.2. Vaccine and Bait stability

The FAIR project CT 97 – 3515, "Wildlife vaccination against rabies in difficult and emergency situations and its potential impact on the environment", included a task studying the stability of all the vaccine baits available in the EU. The consortium gathered all the major scientific teams involved in oral vaccination in the EU (Belgium, France, Germany, Italy) and Switzerland. During a multi-site trial conducted in each of the above mentioned countries in spring, summer and autumn 1999 and a comparative controlled trial conducted in Italy in summer 2000 single batches of a genetically engineered rabies recombinant vaccine (VRG) and three attenuated rabies virus vaccines (SAG2, SAD B19, SAD P5/88) were tested for 21 days post-delivery under varying local conditions (shade, half-shade, sunlight).

The stability of the vaccine baits in terms of virus titre and physical stability of the bait casing was recorded over a 3-week period in relation to temperature, sunlight and rainfall.

At temperatures below 30°C, as reflected in trials during spring and autumn, virus titres in attenuated vaccines were only slightly reduced during the 3-week observation period, whereas all attenuated vaccines showed a significant loss of titre when exposed to high temperatures (30°C or above). The recombinant VRG vaccine retained a protective titre at all temperatures studied.

Significant differences in bait casing stability were observed between vaccines when exposed to high ambient temperatures (30-35°C) and rainfall. Under such extreme conditions only the VRG bait casing remained stable. The casings of attenuated vaccines disintegrated more or less completely following exposure to high temperature and rain, SAG2 showing an intermediate resistance and the SAD B19 and SAD P5/88 baits being least resistant. The loss of titre in attenuated vaccines at elevated temperatures is presumably aggravated by disintegration of bait casing leading to less physical protection of the vaccine capsule.

In the experiments performed in the FAIR project some trials demonstrated contrasting results and it is therefore difficult to draw firm conclusions on the basis of the data. However some relevant stability characteristics were identified:

- The VRG bait was always delivered with a high titre and was highly stable in all trials.

- The SAG2 bait was delivered with a high titre in 6 out of 7 trials. In trials where the mean maximum temperature near the baits did not exceed 30°C, the SAG2 bait remained stable for 21 days in 5 out of those 7 trials.

- The SAD B19 bait was delivered with a high titre in 4 out of 5 trials. In trials where the mean maximum temperature near the baits did not exceed 30°C, the SAD B19 bait remained stable for 21 days in 4 situations out of 9.

- The SAD P5/88 bait was delivered with a high titre in 2 out of 4 trials. This could not be interpreted as mere instability as the titres were even initially below the threshold level and no dramatic decrease indicative of instability was observed.

- Moreover, the SAD B19 bait casing was less stable than the VRG and the SAG2 bait casing when the mean maximum temperature exceeded 30° C.

- The SAD P5/88 vaccine was the least stable of all the vaccines tested in these trials.

Based on data available from the manufacturers and results from the FAIR project, under all conditions VRG appeared to be the most stable.

6.2.1. Field observations

In practical use, all of the above-tested vaccines (VRG, SAD B19, SAG2, SAD P5/88) have been shown to effectively reduce the incidence of rabies in wildlife, thereby helping to eliminate rabies in large European areas (Aubert *et al*., 1994; Schlüter *et al*., 1997; Müller, 1997; Müller and Schlüter, 1998; Vos *et al*., 2000; Breitenmoser *et al*., 2000; Brochier *et al*., 1990, 2001).

Vaccine and bait stability is, however, an important criterion for the efficacy of oral vaccination programmes. Several field observers provided direct or indirect evidence that field efficacy also depends on the dose of the vaccine (virus titre), on the stability of the vaccine titre, and on the stability of the bait envelope:

- Balbo and Rossi (1988) described oral vaccination in Italy (1984-1987) and observed that vaccine (SAD B19) stability in field conditions proved to be correlated negatively with environmental temperature, in particular when the maximum temperature approached 30°C. They found that the observed lower seroconversion rate in foxes corresponded with the rapidly decreasing virus titres from vaccines collected on the terrain.

- Thomas *et al.* (1989) explained abnormal discrepancies between tetracycline and seroconversion rates observed in some vaccinated areas in Belgium by hypothesising a rapid decrease in vaccine titre in baits in these areas.

- Masson *et al*. (1996) obtained a better field efficacy for the SAG1 vaccine bait (decrease in rabies incidence) following progress made in ensuring the stability of both the vaccine and the bait casing at higher temperatures. Similar observations were made by the Swiss team when they used the SAG1/2 vaccine (Zanoni *et al.*, 2000).

- Aubert *et al*. (1994), then Masson *et al*. (1996) showed constant differences in the efficacy in decreasing rabies incidence by the three vaccine baits distributed in France : the decreasing efficacy order was

VRG, SAG1 and SAD B19. These results were observed repeatedly following several vaccination campaigns, which covered very large areas encompassing various milieus and epidemiological conditions: 23,000 km², 37,800 km² and 31,300 km² for the VRG, SAG1 and SAD B19 bait respectively. The same authors observed that the stability of the vaccine and the bait envelope measured in the field varied similarly. The melting point of the bait envelope was shown to be above 50°C, equal to 43°C and below 40°C for the VRG, SAG1 and SAD B19 baits respectively (Masson *et al*., 1996) (see also Table 2).

6.3. Monitoring of vaccination

The WHO expert committee on Rabies (1992a) states that most field trials with oral vaccination employ three methods of evaluation:

- testing for the occurrence of a biomarker (usually tetracycline), which is incorporated into the bait, in the target species;

- examining sera from the target species for rabies virus neutralising antibody;

- analysing the incidence of rabies in animals before, during and after the oral vaccination programme.

When using attenuated rabies virus vaccines, typing of rabies virus isolates originating from vaccination areas needs to be performed to distinguish vaccine strains from field rabies strains. Freshly collected sera are preferred for virus neutralising antibody titration.

Most Western European countries carry out these follow up investigations. In some countries, the titration of the vaccine in baits sampled during bait distribution ("out of the helicopters") is also performed as this allows the stability of the vaccines to be checked during the carrying out of vaccination in local field conditions.

Several biases may arise when studying only the evaluation of rabies incidence, because it depends on the collection of animals for diagnosis. The intensity and quality of sampling depend on the motivation of the general public, veterinarians, and the facilities of veterinary authorities of the administrative units of different countries. These conditions may vary between areas and also from year to year. Unfortunately there is no easy way to measure the quality of the sampling. The method of choice for rabies diagnosis is the fluorescent antibody test (WHO, 1996; OIE, 2000).

6.3.1. Bait uptake

Tetracycline is recommended by the WHO as a marker of bait uptake and provides a life-long marking of bones and teeth that is easily detected on post-mortem. It is inocuous for both target and non-target species and is very stable when incorporated into baits. So far,

tetracycline is considered as the best long term post-mortem tissue marker and is the most commonly used. Other biomarkers have been assessed in small-scale field trials: Sulfadimethoxine (broad-spectrum antimicrobial, short-term ante-mortem seromarker), Iophenoxic acid (relatively long-lived, 6-12 weeks, seromarker), and Rhodamine B (ante-mortem external marker). Due to either insufficient reliability, unfeasibility or cost, the latter markers were never used for assessing bait uptake rates in vaccinated fox populations. Thus, there is no currently available effective marker which could be used instead of tetracycline.

Determination of tetracycline uptake provides an easy way of monitoring bait uptake and is especially useful when identifying other causes for vaccination failure.

It should be considered, however, that monitoring tetracycline uptake alone may lead to overestimating the vaccination efficacy:

- Fluorescence in teeth may be observed without any tetracycline absorption or foxes may find other sources of tetracycline besides vaccine baits (e.g. other possible sources of tetracyclines include placental remnants from cows treated with tetracyclines for infections associated with retained placentas, or from scavenging fish from fish farms where tetracyclines may be used). Therefore, if tetracyclines are proposed to be used as a marker, it is necessary to estimate "the background level" of tetracycline in fox populations before the beginning of an oral vaccination programme.

- Foxes may only consume the attractive casing of the bait and discard the vaccine sachet/vaccine, hence leading to foxes found to be positive for tetracycline but negative for rabies antibody titration.

- Contact between the vaccine suspension and the oropharyngal mucosa may be insufficient for immunisation but sufficient for tetracycline fixation.

The minimum dose of tetracycline per bait sufficient to mark an adult fox has been estimated as equal to 10 to 15 mg tetracycline/kg of fox weight (Cliquet *et al.*, 1995). The tetracycline dose in one bait, greater than or equal to 150 mg is equivalent to 25 mg/kg of adult fox. Considering the weight of young foxes, a fox cub consuming only one fifth of the envelope of one bait (i.e. 8 g) will be shown to be "tetracycline positive". Therefore, overestimation of vaccination coverage, based on tetracycline as a marker, is probably greater with fox cubs than with adult foxes.

Tetracycline examination facilitates the epidemiological surveillance of rabies in areas freed of the disease and where vaccination is no longer practised. It allows an assessment of the level of animals which are still marked (tetracycline fixation is lifelong).

6.3.2. Fox immunity

The most efficient and commonly used method to assess the efficacy of rabies oral vaccination campaigns is to measure the antibody response after vaccination in the target species. The methods currently recommended by the WHO and the OIE are the rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralisation test (FAVN test) (Cliquet *et al*., 1998; Aubert *et al*., 2000).

However, in continental Europe, fox serum samples collected in the field by hunters, gamekeepers or by technicians are in most cases "body fluids". The RFFIT, and any other cell culture-based techniques, require a specialised laboratory with fluorescent microscopy and facilities to handle tissue culture and the virulent rabies virus. These tests are sometimes too sensitive to cytotoxicity that occurs with bad quality samples, possibly leading to false positive results (WHO, 1992a), and they have to be standardised using an appropriate standard as a control.

A simple test (ELISA test) has been developed, and has been used in France since 1992, which is rapid, safe and economical for large-scale serological and epidemiological surveys following vaccination programmes. This test can be used to accurately titrate highly contaminated body fluids obtained from animals killed in the field (Cliquet *et al*., 2000). A European interlaboratory standardisation programme using this ELISA has been carried out recently and demonstrated an almost perfect agreement between four European laboratories (FAIR project CT 97-3515, Cliquet, personal communication). This ELISA test system facilitates serological evaluation of oral vaccination campaigns within European countries.

6.3.3. Rabies incidence

WHO recommends the examination of at least 8 foxes/100 km^2 for rabies each year. Priority needs to be given to examining and testing those animals showing abnormal behaviour suggestive of rabies. Animals found dead, such as road-kills, are also useful sources for rabies diagnosis as these animals can be considered to be suspect animals.

7. VACCINATION STRATEGY

Vaccination programmes are required to be conducted and continuously monitored by a scientific team dedicated to this task. The team needs to be trained in field surveys and use validated laboratory methods for rabies diagnosis, titration of vaccines, evaluation of bait uptake by the target species, and rabies antibody titration. The whole procedure, including bait distribution in the field, needs to be carefully processed, followed and documented.

7.1. Population dynamics

7.1.1. Introduction

It is a well-known phenomenon that, after the end of a rabies epizootic in a given area, the local fox population shows a strong increase (Vos, 1995; Wachendörfer *et al*., 1996; Breitenmoser *et al*., 2000; Chautan *et al*., 2000; Aubert *et al*., 1993). This is experienced as a typical consequence of a rabies vaccination campaign.

The increasing abundance of the vector species also has a considerable impact on the success of an oral vaccination campaign, especially if the control measures have to be applied over several years. Problems of persisting rabies, experienced during the final phase of the rabies epizootic in Switzerland, Belgium and Germany, coincided with a growing fox population, showing the need to adapt the rabies control strategy to the increased fox population.

In situations of continued vaccination campaigns, it is crucial to compensate for the higher abundance of the vector species through an adjustment of the vaccine bait distribution. Although this seems to be an obvious recommendation, such an adjustment was not foreseen when rabies control programmes began. As a consequence, reliable data on the dynamics of the vector population were usually not gathered and hence not available when the problem arose. The following section summarises the underlying mechanisms, using empirical data or estimations for illustration purposes.

The course and the amplitude of a fox population increase can however vary according to local conditions, and it is therefore indispensable to monitor and analyse each local situation carefully.

7.1.2. Dimensions of the increase

Although empirical data are available on trends in fox populations during the course of vaccination campaigns (Breitenmoser *et al*., 2000) it is also possible to extrapolate models of fox population changes under various circumstances. If a closed population is infected with rabies virus, the population will decrease until the density falls below the threshold value of rabies persistence (Fig. 6). From there, the population will re-increase up to the carrying capacity of the habitat, following a sigmoidal shape. The dimensions of the population growth are not precisely known, as there is a lack of reliable census data for fox populations. Usually, the population dynamic is estimated from mortality data, such as the hunting bag or road kills. These data sets indicate that the increase continues for 5–10 years after a population reaches a minimum, and that the amplitude of the increase can be from 4–5 up to 10 fold compared to the minimum. The maximum population density depends on the carrying capacity of the habitat and differs from area to area. The threshold density of rabies persistence (the minimum population density at which the disease can persist) is also influenced by the landscape and topography, but is probably a relatively constant value.

In a real situation (i.e. in a non-isolated fox population) and in the absence of rabies control measures, a local increasing population will probably face a re-infection before it reaches the carrying-capacity density again, and will hence fluctuate in the longer term around the threshold value of rabies persistence (Breitenmoser, Personal communication) (Fig. 6).

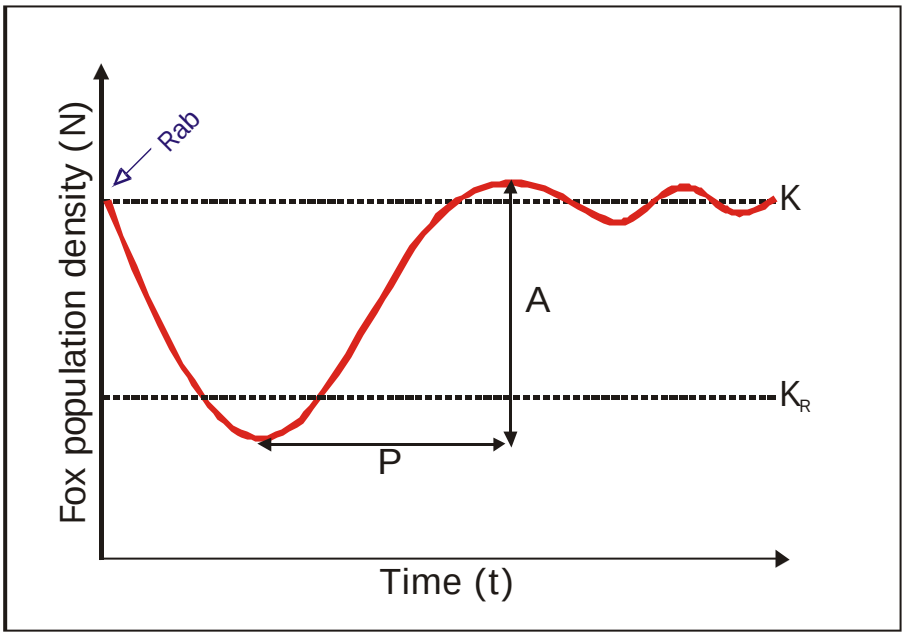


Figure 6: Course of a fox population increase after a rabies infection (Rab). The disease disappears when the population density (N) falls below the threshold value of rabies persistence (K_R). The population increases in an S-shaped curve until it reaches the carrying capacity of the habitat (K). The population growth is characterised through the amplitude (A) and the duration (P) of the increase. K_R is a conjunct of fox population density and contact rate

7.1.3. Influence of herd immunity and population size on the success of the vaccination campaign

The herd immunity, used as a standard immunological term, is a relative measure of the immunity of a population (fraction of individuals protected against infection), and it does not indicate the absolute numbers of immune or susceptible foxes in the field.

The oral immunisation of foxes against rabies has two goals: (i) to defeat the infection in a given area, and (ii) to prevent the local population from becoming re-infected. The first goal requires the rapid increase in the herd immunity – experience has proven that three vaccination campaigns might be enough to eradicate rabies from a certain region (Masson *et al*., 1996), whereas the second goal is the maintenance of a sufficient herd immunity as long as the infection persists in neighbouring areas.

It is obvious that the second goal needs to take into account the increase in the fox population. Assuming that the oral vaccination of

foxes starts when the population is at its lowest (Fig. 7), the herd immunity will increase along with the number of vaccination campaigns but will never cover the entire population. Typical values for the herd immunity, evaluated from tetracycline analyses, ranged from below 50% up to 90% in adult foxes (when antibody titration is used these percentages might be 30 to 80% respectively). When the population increases after the start of the vaccination campaigns, the number of susceptible foxes may also increase, as indicated in Fig. 7. This is not a problem as long as the density of susceptible individuals remains below the threshold density of rabies persistence. However, if this threshold value is exceeded, the population remains susceptible to the disease.

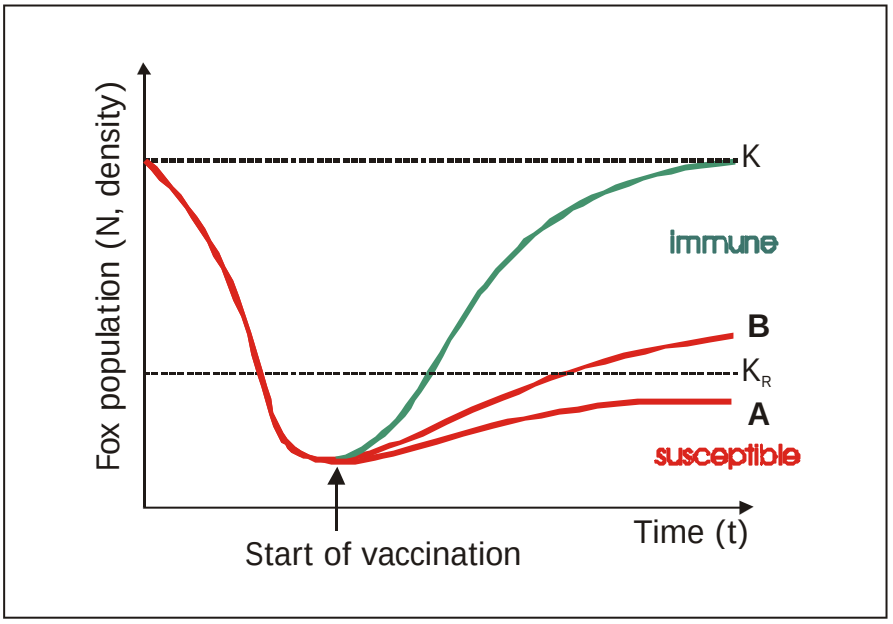


Figure 7: Population growth with oral vaccination of the vector population.

In Figure 7, the population increase follows the sigmoidal curve up to the carrying capacity K. Due to the vaccination campaigns, most foxes are immune against rabies. However, the herd immunity will never be 100 %; a certain proportion of the population will always be susceptible to the disease. If the herd immunity is below 100%, the probability of transmission of infection depends on contact rate in the region and transmissibility. As long as the density of the susceptible individuals remains below the threshold density of rabies persistence K_R (situation A), the oral vaccination campaign will still be successful. If, however, the density of the susceptible foxes exceeds K_R (situation B), the disease will persist even if the herd immunity increases.

A high level of herd immunity may give a false feeling of security when the absolute number of non-immunised foxes is high. In other words, the herd immunity required to eliminate rabies or protect a population from re-infection is required to increase along with the population. Once an absence of rabies cases is reached at a certain herd immunity level, that level of herd immunity will need to

increase with the increasing fox population in order to prevent a reoccurrence of cases. If, in a given moment, a herd immunity was empirically found to be enough to defeat rabies, a higher herd immunity may be needed to prevent a re-infection of the same population some years later, due to the increase in the population in the intervening period.

7.1.4. Modification of vaccination strategies to account for the fox population increase in prolonged vaccination campaigns

To allow for an adaptation of the rabies control strategy to the increasing fox density, the fox population should be monitored. It is not enough to sample a constant number of foxes in order to determine the herd immunity, an indicator for the dynamics of the population is needed. Such indicators can be the hunting bag, road kills, night counting, and line transects (see chapter 5.2) etc.. Even if such parameters do not really indicate the absolute number of foxes, they will be satisfactory for the population trend to be followed.

An additional complication is that an increasing population density may also influence the social structure and behaviour and the land tenure system of the fox. Social group ("family") composition, dispersal patterns and individual home range size may change. Analysis of the rabid foxes and of an independent control sample in regard to age structure and sex ratio would allow identification of the problem categories and permit adequate measures to be taken.

Problems with re-infections typically occur along administrative borders. This is the result of the immediate proximity of vaccinated, increasing fox populations to areas where rabies is endemic. Sometimes, administrative borders are also barriers to the fox movement (as for example the river Rhine between France and Germany), but very often, they are not. In the latter case, the following points need to be observed in order to avoid continued re-infections:

(i) To set up large-scale vaccination zones and

(ii) To strictly synchronise all control measures within the zone and across political or administrative borders.

(iii) A vaccination zone to ideally extend up to the next geographical or artificial physical barrier and include the entire infected area.

7.2. Temporal patterns

The annual frequency of vaccination campaigns is required to be considered with reference to the months of baiting for a variety of campaign strategies. Based on experience in previous oral rabies vaccination campaigns, it is considered important that vaccination campaigns continue for a period of at least two years after the last reported case of fox-related rabies.

7.2.1. Regular vaccination campaigns

The classical pattern of two "single" vaccination campaigns per year, carried out in spring and autumn, has been found to be successful whatever the fox population density. This biannual distribution frequency has been used in all European programmes of oral vaccination that resulted in the elimination of rabies (Zanoni *et al*., 2000; Breitenmoser *et al*., 2000; Bruyère and Janot, 2000; Brochier *et al*., 2001; Besch, 2001).

Spring distributions are preferably carried out in May or June in order to increase the efficient access of fox cubs to baits. However, early spring campaigns carried out in March-April (targeting exclusively the adult fox population at its annual lowest density) were also shown to be beneficial in Belgium, Luxembourg, and several German Bundesländer (Brochier *et al*., 1996, 2001). Where snow is abundant, its melting may degrade the vaccine baits, and in such areas vaccination is preferably performed before the snow starts to melt. Autumn distribution is generally organised in September or October.

In both autumn and spring campaigns, short delayed baiting at intervals ranging from a few days to 3-4 weeks (so-called "double" vaccination strategy), aiming either at inducing an immune booster effect or at increasing the bait uptake rate, is not advisable. However, when vaccination campaigns are initially launched repeated distribution of baits within such a short time interval can be performed. Any effect of such double distribution is probably mediated through increased bait-uptake rate in the fox population by redistributing baits along other flight lines (for targeting foxes that would not have been reached during the first distribution).

7.2.2. Additional vaccination of fox cubs at den entrances

In spring, an additional distribution of vaccine baits at den entrances (targeting fox cubs) may be carried out in focal areas from mid-May to mid-June (Vuillaume *et al*., 1997). When using rabies modified vaccines, the distribution needs to preferably take place in early-June, because of a potential interference between passive and acquired immunity in fox cubs (Müller *et al*., 2001; Blasco *et al*., 2001; Barrat *et al*., 2001) but only if external maximum temperatures do not exceed 30°C. It should be noted that when directly exposed to the sun, the temperature of baits may be 10-20°C higher than temperatures measured under shelter.

Such distributions can usefully complement the regular spring campaign (Vuillaume *et al*., 1998; Brochier *et al*., 2001; Besch, 2001; Breitenmoser, 1995) but due to their organisational burden and associated cost they can only be applied in limited areas in problem situations (residual rabies foci with high fox population density) and in particular habitats (suburban areas).

7.2.3. Emergency vaccination

In cases of re-emergence of rabies in a focus where rabies had been previously eliminated, vaccination needs to be implemented immediately, whatever the period of the year. Such an emergency vaccination might thus be carried out in summer or in winter under unfavourable weather conditions that require the use of a highly heat-stable vaccine-bait system such as the VRG (Masson *et al*., 1999; Pastoret *et al*., 1996).

In general, vaccination is not advised to be carried out at temperatures below 0°C, because:

(i) frozen vaccines do not induce a sufficient immune response and

(ii) the virus titre may decrease caused by freezing-thawing cycles, except for VRG which has been shown to remain stable in such conditions (Pastoret *et al*., 1996).

Vaccination using attenuated rabies virus vaccines is not recommended during hot weather conditions. At temperatures above 30°C, melting of the bait casing occurs and vaccine titre decreases.

7.2.4. Synchronisation of vaccination campaigns in neighbouring administrative or political entities

Examples of cross-border re-infections are numerous (Schaarschmidt *et al*., 2002). They are the result of the immediate juxtaposition of vaccinated areas (where fox populations are increasing) and areas where rabies is endemic. These re-infections can be prevented by synchronising control measures on both sides of political or administrative borders (as outlined in chapter 4.1) and when this is not possible, by the maintenance of an immune belt at the border (see also “spatial aspects” below).

7.3. Spatial aspects and patterns

7.3.1. Size of a vaccination area - “buffer”zones

The size of the vaccination zone needs to ideally include the entire infected area or be as large as possible (5,000 km² at least) and extend up to natural or artificial barriers such as a motorway, canal, river, stream, lake, or mountains (e.g. in Alpine regions a vaccination zone should include a whole valley).

Occasionally, administrative borders may constitute barriers to the movement of foxes, but in most situations vaccination zones need to be defined and vaccination campaigns synchronised across administrative borders.

When considering a "punctal occurrence" of rabies, that is an isolated residual or localised re-infection focus, the size of a vaccination area needs to range between 2,000 and 8,000 km^2 (radius of 25 to 50 km, respectively, around the site) depending on the landscape and the availability of natural or artificial barriers (Thulke *et al.*, 1999). In the absence of any barrier, the larger radius (50 km) is advisable as the rule. Consequently, to protect a rabies-free area from a neighbouring infected area, the immunological barrier (a buffer zone) along the border with the infected area should be 50 km deep. This distance appears to be the minimum allowing for a sufficient reaction time to expand the zone from one campaign to the next if rabies entered in the border area.

Similarly, the minimum buffer zone (depth of a vaccinated strip in km) ahead of the front wave of the spreading epizootic should be 50 km.

If the endemic area is limited by a natural physical barrier (e.g. a river, lake, etc.), the depth of the buffer zone beyond this barrier depends on the supposed effectiveness of this barrier, the landscape and the expected fox density on both sides of the barrier. Beyond a barrier that may be crossed by foxes (e.g. a river), the minimum distance advisable is 20 km.

If vector species other than the red fox are involved (e.g. racoon dogs), the buffer zone needs to be enlarged in respect of the maximum movement distance of this species.

7.3.2. Bait density - distribution pattern

All fox home ranges, whatever their size and shape, need to ideally receive several baits. Therefore, the general principle for the distribution of vaccine baits is as follows:

- all habitats should be treated except heavily urbanised areas and large stretches of water, taking the pattern of fox habitat into consideration;

- baits should be distributed in a regular pattern within a given area.

Concentrations of baits in clusters or along distant lines cannot be relied upon and needs to be avoided. Distances between baits cannot be neither too large nor too short, as otherwise individuals may go unvaccinated or there may be over-baiting. If a regular distribution is applied, a raster model is better than a parallel line model (this at least is valid for double vaccination).

Baits distributed along landscape interruptions such as forest edges, hedgerows, creeks, etc. will more likely be found by a fox than those in the middle of forest or farmland. Furthermore, the increased use of anthropogenic food resources by foxes is required to be considered. Foxes often visit edges of settlements or parks and the role of other species such as cats and dogs competing for baits needs also to be

considered, as well as public awareness and safety issues when distributing baits in urban and suburban areas.

7.3.2.1. Regular vaccination campaigns

According to radio-tracking studies in Western Europe (Artois *et al*., 1990), the smallest fox home range was 77 ha. In this situation, if vaccines are dropped at regular time intervals, along parallel lines separated by 400 metres, the minimal number of vaccine baits dropped in this fox home range will be 10. If the lines were 1,000 metres apart, this fox home range may receive no bait at all. The relationship between flight-line distances and the spatial arrangement of fox home-ranges is a key factor when considering bait distribution strategy (Thulke *et al*., 2001).

The distance between flight lines appears more crucial considering that:

(i) Foxes usually explore only 1/3 to 1/2 of their territory every day (Artois *et al*., 1990) which gives more opportunity for non-target species to pick up baits before the fox,

(ii) Several foxes may share the same home range when fox density increases, and

(iii) In suburban areas, the size of a fox family home range may not exceed 25 ha (Brochier, unpublished data).

When distributing baits manually, baits need to be uniformly distributed according to a raster model. The map is required to be divided into equal plots and every plot should receive at least 1 bait. For a bait density equal to 20 per km², plots will be 223 metres x 223 metres. Inside every plot the place to choose to locate baits will be a forest edge, a bunch of trees in the middle of a meadow, a village boundary, etc.. By using this method, baits can be deposited throughout the landscape giving preference to "fox-lines" (forest edges, hedges, creeks, village boundaries etc.) and fox habitat.

When using the aerial method of bait distribution, flight sectors need to be defined in advance using natural or artificial landscape features. To ensure that most of the fox territories are given at least 1 bait, baits are distributed along parallel flight lines. Based on flight lines 500 metres apart, this entails two flight lines per km and approximately 110 metres distance between baits when the bait density is equal to 18 per km^2. When increasing the bait density to compensate for an increase in fox population, the bait distribution pattern needs to be re-considered: the distance between flight lines is advised to be reduced from 500 to

300 metres (i.e. a change from two flight lines per km to three; with 100 metres between baits for a bait density of 30 per km^2).

Based on field experience gained of vaccination campaigns in various countries and use of computer simulation models, densities of 18-20 and 20-30 baits per km^2 are advisable for low and high fox population densities, respectively. Although low and high fox population densities are difficult to precisely define, relative measures of the population dynamics can be used in combination with parameters such as bait uptake rate to modify bait densities appropriately.

7.3.2.2. Optional vaccination campaigns

If considering spring vaccination of fox cubs at dens, in early spring, fox dens need to be located and recorded on detailed maps by appointed and trained people (forest rangers, hunters, gamekeepers). The knowledge on the precise number of active fox dens within a given area is essential.

At the end of May to early June, the dens recorded previously are re-visited by the same persons (wearing gloves) and at least 10 baits are deposited at the den entrances.

Emergency vaccination is required to follow the protocol used in the context of a regular vaccination campaign adapted to a high fox population density situation: 20-30 baits/km^2 – 3 flight lines/km^2.

7.4. Distribution methods and systems

Vaccine baits need to be deposited throughout the fox habitats (i.e. almost everywhere). Unfortunately, not all baits are consumed by foxes. Baits may remain undiscovered or be taken up by other wild or domestic species, or even be picked up by humans. However, vaccination campaigns carried out during spring and autumn for several years led to the durable elimination of the disease in most of Western Europe (Müller, 1997, Breitenmoser *et al*., 2000, Bruyère and Janot, 2000, Brochier, 2001).

All of the distribution systems used so far have been found to be efficient, provided bait dispersal is properly designed. Each of them has its advantages and disadvantages.

Manual distribution allows a very precise and uniform spreading of baits (according to a raster model) and may be used to encourage public involvement and awareness. Furthermore, baits can be hidden (covered with grass, leaves, etc.) to avoid human contact, ingestion by birds and exposure to direct sunlight. However, it requires a thorough organisation and important

human resources, qualitatively (competency, motivation) as well as quantitatively, and it is slow. Consequently, one can never be totally assured that baits are distributed everywhere. Any forgotten place (lack of motivation of a single team) can constitute a future rabies focus.

Distribution by hand is the preferred system in suburban areas, in combination with an aerial distribution (helicopter) whenever possible.

Aerial distribution may be performed either by helicopter or by fixed-wing aircraft flying at 100-150 m altitude and at a speed of 100-150 km/hr. Precise maps are required to be prepared before flights and followed during flights by a trained, independent person. A GPS (Global Positioning System) may be helpful for reporting the exact distribution pattern (Vos *et al*., 2001), but cannot replace the thorough work with maps (Breitenmoser and Müller, 1997).

Appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on fox habitats (hedges, village surroundings, isolated bunch of trees, etc.). An electronic metronome, connected to GPS that allows adjustment in dropping tempo to speed, may be helpful, but the dropper may increase this tempo to favour fox "places". In Germany, for aerial distribution a satellite navigated and computer supported automatic bait dropping system was developed. The exact location and time of each bait released can be recorded together with all relevant flight details, so that authorities can verify if the achieved bait distribution pattern corresponds with the previously determined baiting strategy (Vos *et al*., 2001). The delivery by helicopter is fast and allows precise dropping of baits (flexibility in both flight speed and altitude). The above-mentioned spatial pattern of bait distribution (low distances between flight lines) can be performed more easily by helicopter. In addition, the helicopter allows working in less favourable weather conditions.

Therefore the use of helicopter is advised for the treatment of all habitats (rural, agricultural, mountains, forests, suburban areas and settlements).

Delivery by fixed-wing aircraft is the most economical of all distribution systems, but does not allow for a fine distribution of baits according to the fox-habitat in the landscape. Therefore, the use of fixed-wing aircraft is only advised for the treatment of uniform, large and low density inhabited areas (e.g. large forests, mono-agricultural areas).

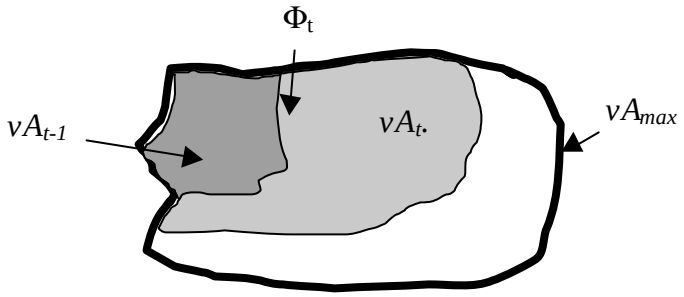
7.5. Evaluation of oral rabies vaccination programmes

In all European countries, the infected regions were initially too large to be vaccinated as a whole when oral rabies vaccination commenced. When logically planned, strategies tried to consist of vaccinating a limited part of the infected region every year, and in shifting the vaccinated area whenever possible from the areas freed from rabies to the areas still infected until the elimination of the disease in the whole region. However, in the past there have often been diverse strategies applied with varying success. These strategies mainly differed in the selection and size of the vaccination areas and the continuous treatment of these areas over the course of the vaccination programme. While in some countries vaccination areas were frequently

adapted to the concurrent rabies situation (patchwork), others used large scale or overlapping areas or utilised natural barriers (Müller *et al.*, 2001). These differing strategies seemed to result in variation in the time taken to eradicate rabies (Müller and Schlüter, 1998).

To address the reasons for this variation in eradication time, a retrospective evaluation of oral rabies vaccination programmes in European countries was conducted as part of the FAIR project CT 97-3515. Twenty-eight regions, which were involved in an oral rabies vaccination programme between the years 1978 and 2000 were selected in Belgium, Germany, Italy, and Switzerland. They were defined either by administrative units or by natural barriers and their size ranged between 313 and 66,362 km². For each region, the programme was considered from the 1st vaccination campaign to either the eradication of rabies or the end of the year 2000. Rabies was assumed to be eradicated if the disease was not recorded within a two-year surveillance period following the last confirmed case in the area.

To quantify the observed spatial and temporal differences in vaccination strategies, an Area Index (AI) was calculated. This index has been calculated for each region, using the area of the whole region concerned by the oral rabies vaccination programme (vA_{max}), the size of the areas successively vaccinated during campaigns at time t (vA_t vA_{t-1}), the number of successive vaccination campaigns (n), and the size of the overlapping of vaccinated areas successively from campaign to campaign (Φ_t).



$$AI = \frac{1}{n}\sum_{t}^{n}\frac{vA_t}{vA_{\max}}\frac{\Phi_t}{vA_{t-1}}$$

Figure 8: Concept and formula of the area index (AI)

Thus, the AI is a measurement of the proportion of areas repeatedly vaccinated within a region during the observation period, and has assigned values ranging between 0 and 1. A region in which the total area has been vaccinated since the beginning of the programme would be characterised with an AI close to or equal to 1. An AI equal to 0 would indicate that no overlapping of successive vaccinated areas has ever been done. An AI close to 0 would indicate that such overlapping was limited and/or that the proportions of the vaccinated areas over the size of the whole region were systematically small.

There was a large range of AI (from 0.18 to 1) indicating a large variety of strategies in the countries studied (Belgium: 0.56; Switzerland: 0.20-0.98; Germany: 0.13-1; Italy: 0.60-0.92). There was no significant difference in the mean AI between rabies free and regions still infected at that time. However, when rabies-free regions were divided into two groups by size (above and below 6,000 km^2), in both groups the time from the beginning of oral rabies

vaccination to eradication of rabies given with the number of campaigns is negatively correlated with the AI. In regions showing a high AI (0.8 - 1), rabies was eradicated within 3-6 campaigns for small regions (<6,000 km^2), and 12-15 campaigns for large regions (>6,000 km^2). In contrast, regions with a low AI (0.2 – 0.6) required 5-16, and 27-29 vaccination campaigns, respectively (Fig. 9).

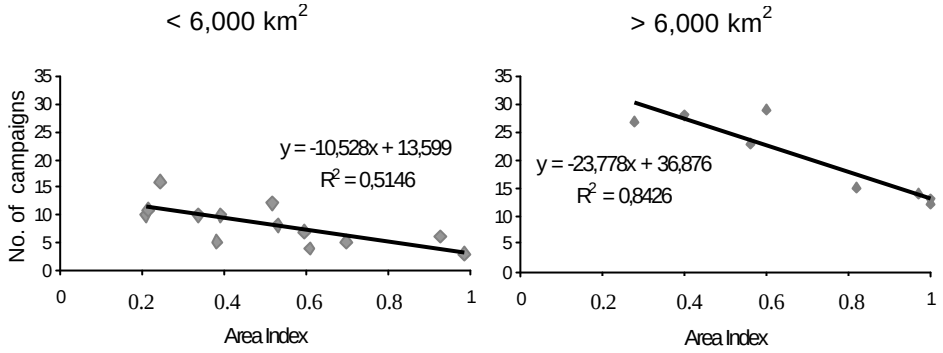


Figure 9: Linear regression of AI vs. the number of vaccination campaigns in rabies-free regions.

The validity of this approach is confirmed when regions still infected with rabies are considered. It was observed that 3 regions in Germany (all larger than 6,000 km²), which developed a strategy characterised by a low AI (lower than 0.6), were still rabies infected after 30 to 34 campaigns. In these regions, the vaccination plan did not follow a systematic approach, in contrast to the one that had been followed in the eastern part of the same country where the whole region was covered by vaccination during successive campaigns (high AI). In conclusion, these studies illustrate that an AI can explain the variation encountered in dissimilar oral rabies vaccination strategies, i.e. the differences in times taken to eradicate rabies. In order to improve the efficiency of oral rabies vaccination systems in general it is necessary to guarantee a high AI to eradicate rabies in due course. However, the AI cannot take into account the question of re-infection across the border of neighbouring regions. Logically, any correlation between the AI and the number of campaigns required for rabies elimination can only be observed as long as such re-infection can be ruled out.

8. CONCLUSIONS

General Conclusions

1. In Europe, oral immunisation by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases. However, the ultimate success of oral rabies vaccination campaigns requires a long-term strategy and cross-border co-operation.

2. Rabies in wildlife was eliminated most efficiently in those countries where the vaccination campaigns were planned on a national level and co-ordinated with neighbouring countries.

3. Thorough surveillance of a rabies epizootic and monitoring of the vaccination efficiency (using a tetracycline marker and sero-conversion rates) are important tools for the assessment and adjustment of vaccination campaigns. Standardised surveillance and monitoring methods facilitate international comparison and co-operation.

Types of vaccines and baits

4. Insufficient stability of some rabies virus vaccines is likely to have been a source of vaccination failure in specific situations (e.g. combination of climatic and meteorological conditions and areas of high fox population density). Among currently available vaccines, based on available data, the vaccinia recombinant vaccine appears to be the most stable.

5. For the den vaccination of fox cubs it is desirable that a vaccine should be able to overcome as much as possible the effects of maternal immunity. A limited number of studies indicate that the vaccinia recombinant vaccine is better able to overcome maternal immunity than other vaccines.

Methods of release of vaccine baits

6. An appropriate bait delivery system (helicopter, fixed-wing aircraft, or manual) is needed when planning vaccination strategies, in order to achieve optimal bait distribution.

Bait density and distribution patterns

7. The bait density and bait distribution pattern should take into account habitat and landscape features, species competing for baits and fox population density in order for the baits to be taken up by a sufficient number of individual foxes. Vaccination at den entrances can be used as an additional measure in situations of high fox population density.

8. During prolonged rabies-control measures, the fox population will increase and consequently the herd immunity may become insufficient to control rabies, unless compensated for in the design of the vaccination strategy and by increasing bait density applied.

Seasonal pattern of the releases

9. Selection of the months when baiting is performed is an important consideration when planning vaccination strategies, in order to ensure access of foxes and fox cubs to baits.

10. Spring distribution is best carried out in May or June in order to increase the efficient access of fox cubs to baits. However, early spring campaigns carried out in March-April (targeting exclusively the adult fox population) have also been shown to be beneficial.

9. RECOMMENDATIONS

General Recommendations

1. Dynamics of the fox population should be monitored during the vaccination campaign in order to compensate for the higher abundance of the vector species through an adaptation of the vaccination strategy. It is most important that vaccination campaigns should be designed in a way to raise herd immunity along with the fox population in order to avoid setbacks in rabies eradication. Monitoring of vaccination programmes should include a sustained, constant and intensive surveillance of (i) the rabies incidence, (ii) bait-uptake and (iii) immunity in foxes during vaccination campaigns. For the surveillance of the rabies incidence in foxes in regions where oral vaccination is carried out, an examination of all foxes suspected of having rabies, those found dead and road kills should be performed.

2. In order to ensure the success of vaccination campaigns, these campaigns should be planned and coordinated across administrative and political borders. Regular contacts and consultations between stakeholders (national veterinary authorities, local veterinary authorities, hunters and the public) are very important for the successful outcome of vaccination campaigns and should be encouraged.

3. Vaccination should be continued for at least two years after the last reported case of rabies.

4. All rabies virus isolates should be typed in areas where attenuated rabies virus vaccines are used, in order to distinguish between vaccine and field virus strains.

5. Serological methods to be used for quantification of the antibody response in foxes following vaccination should be standardised as recommended by the WHO and OIE. The Community Reference Laboratory should take a lead in standardising these methods. Standardised ELISA tests, which are now available, may replace serum-neutralisation tests.

Types of Vaccines and Baits

6. Live rabies vaccines used for oral vaccination of foxes should fulfil the requirements of the European Pharmacopoeia monographs as well as the efficacy and safety recommendations of the WHO. Vaccine titre at batch release should correspond to at least ten times the dose found to completely protect an experimental group (indicative 100% protective dose). The titre of the final vaccine in the bait should not fall below the indicative 100% protective dose following exposure to 25°C for seven days. Each vaccine batch should be tested and approved for titre and stability by an acknowledged quality control scheme according to OIE standards and WHO recommendations. Laboratories involved in the monitoring and evaluation of rabies programmes are advised to monitor the titre of all batches of rabies virus baits before and during release into the field.

7. The melting point of the bait casing should be above 40°C to ensure that the capsule of the vaccine is still covered if exposed to such temperatures in the field. Vaccine producers and National Laboratories should provide detailed information to the Community Reference Laboratory on the stability of baits to be used in the field. The Community Reference Laboratory should perform additional tests or trials if required.

8. The use of tetracycline as a biomarker in the teeth and bones of foxes is recommended to evaluate bait-uptake in target species, until alternative markers without negative biological effects become available.

9. When handling baits and vaccines, storage and transportation conditions and cold-chain requirements should be strictly adhered to.

10. The use of the most stable vaccine should be preferred in situations where high stability is considered important. For the vaccination at dens of cubs born to vaccinated vixens, the vaccine that is best able to overcome the effects of maternal immunity should be used.

Methods of release of vaccine baits

11. The advantages and disadvantages of the distribution systems should be taken into account when vaccination campaigns are planned, and detailed identification and mapping of the vaccinated areas should be performed.

12. The use of helicopters is recommended for the treatment of all habitats (rural, agricultural, mountains, forests, suburban areas etc.). The use of fixed-wing aircraft is only recommended for the treatment of uniform and large areas of low density inhabitation (e.g. large forests, mono-agricultural areas). Distribution by hand is the preferred system in urban and suburban areas, in combination with the use of an aerial distribution whenever possible. Vaccination programmes should include comprehensive training of and provision of information to hunters and pilots. A proposed bait distribution methodology is given in an Annex of the present report, based on the available knowledge and experience.

Bait density and distribution pattern

13. Rabies infected regions should be vaccinated as a whole and campaigns should be repeated until rabies elimination is ascertained (and until any risk of cross-border infection is ruled out). The minimum size of a vaccination area should be 5,000 km². However, in regions too large to be vaccinated as a whole, parts of these regions should be vaccinated repeatedly until rabies elimination is ascertained. Newly vaccinated areas should overlap the previously vaccinated ones to prevent re-infection of rabies-free areas.

14. In cases of rabies-infected neighbouring regions the following points should be considered in order to avoid subsequent re-infections:

- large-scale vaccination and buffer zones should be established with the establishment of immune belts at borders between infected and non-infected regions

- control measures within the zone and across national or international borders should be strictly synchronised

- a vaccination zone should extend up to the next geographical or artificial physical barrier.

15. In case of an isolated residual or re-emerging focus of rabies a vaccination area with a radius of 25 to 50 km around the site should be applied, depending on natural barriers.

16. To protect infection spreading to a rabies-free area from a neighbouring infected area, the minimum vaccination buffer zone beyond the front of a rabies endemic zone should be 50 km. In case of an existing natural physical barrier, the minimum distance recommended is 20 km. If vector species other than the red fox are involved (e.g. racoon dogs), this buffer zone size should be adjusted to the maximum distance travelled/ranged by that species.

17. Taking topographical factors into account (e.g. urban and suburban areas), all fox home-ranges should be included in vaccination campaigns and wherever the distribution system allows flexibility (e.g. distribution by hand or helicopters), the pattern of fox habitat should be considered.

18. Homogeneous distributions of 18-20 and 20-30 baits per km^2 are recommended for low and high fox population densities, respectively. For den baiting, at least 10 baits are recommended to be deposited at the main den entrance.

19. When using the aerial method of bait distribution, flight line distance should not exceed 500 metres and when the fox population is high it should be reduced to 300 metres. When distributing baits manually, baits should be uniformly distributed according to a raster model based on prepared maps.

Seasonal pattern of the releases

20. In general, oral vaccination campaigns should be conducted on a biannual basis, in spring and autumn while taking climatic conditions into account. Autumn vaccination should generally be performed in September or October; Spring distribution should be preferably carried out in May or June in order to increase the efficient access of fox cubs to baits. Den vaccination should be considered to effectively complement the regular spring campaign.

21. In case of re-emergence of rabies in foxes in an area where rabies has been previously eliminated, vaccination should be implemented immediately, whatever the period of the year, except under extreme climatic conditions which would severely hinder bait and vaccine stability.

10. FUTURE RESEARCH

1. There is a need to develop simple methods to define fox population densities that can easily be applied by wildlife biologists and veterinary authorities. The use of "statistically recorded mortality" (hunting bag, road kills, other findings of dead foxes) along with the gathering of more precise data (animal's sex, age, place where found) could allow the application of more sophisticated models to serve as useful trend indicators.

2. Although several vaccination protocols have been found to be successful to eradicate rabies whatever the fox population density, with respect to cost-efficiency there is, nevertheless, a need for further optimisation of vaccination strategies. This can be done either by simulation modelling and/or field trials. A disadvantage is that results obtained with simulation models often are not verified by field experiments. The use of a GIS (Geographical Information System) or GPS (Global Positioning System) model approach may allow analysis of actual situations and simulation of alternatives

3. Examination of dead foxes is an essential supplementary measure in the context of vaccination campaigns. However, once foxes are immunised in a campaign, a considerable proportion of immunised foxes may be later shot. Therefore further study is needed as to whether the hunting pressure may influence the success of vaccination campaigns or not, and as a consequence the time taken to eradicate the disease. The use of demographic and epidemiological models and economic decision support systems could help to identify the optimal strategy, simulate various options (pre- and post-vaccination mortality, absence of hunting, etc.) in order to identify the strategy resulting in the highest herd immunity and the highest density of immune foxes.

4. So far, there have been only theoretical approaches to determine immunity thresholds capable of eliminating rabies or protecting a population from re-infection. Attempts should be made to verify those theoretical thresholds and analyse them under real conditions. Detailed research on other potential rabies reservoirs is required, including the possible experimental transmission of European bat lyssavirus (EBL) to the fox population and other terrestrial mammals.

5. Further comparative studies are needed on the safety and efficacy of the various vaccines available.

6. Research is required to further develop alternative markers to the use of tetracycline to monitor bait uptake.

7. Biodegradable bait casings should be developed to replace plastic forms currently used.

11. EXECUTIVE SUMMARY

This report covers a comparative study of various rabies oral vaccination protocols, with particular reference to types of vaccines and baits, methods of release, distribution patterns and seasonal patterns of vaccination campaigns, according to the mandate given. Problems in the implementation of certain vaccination protocols are indicated and conclusions and recommendations on appropriate strategies to eradicate rabies from the European Community are made.

Following the spread of fox rabies from Eastern European countries to many countries in Western Europe from 1940 onwards extensive efforts have been made to control and eradicate the disease. Since 1989, the EU has contributed financially to oral vaccination of foxes in a major effort to eradicate the disease. Setbacks in eradication campaigns have been noted in some regions, raising concerns about the final eradication of rabies from the Community.

At present, Germany is the only Member State of the Community where rabies occurs. In recent years setbacks and problems in rabies eradication have been noted in two distinct regions in Germany involving three Federal States. These required urgent measures to eliminate the disease. Several reasons for possible problems and failure of vaccination campaigns have been identified e.g. cold chain, bait dispersal, vaccine and bait stability.

Important features of the population biology of foxes and the dynamic effects of rabies on fox populations are described to enhance understanding of the mechanisms and effects of applying oral vaccination against rabies.

Several vaccines are manufactured for oral vaccination of foxes against rabies and are formulated as baits. All vaccines are live virus vaccines, one being genetically engineered by inserting a rabies virus glycoprotein gene into a vaccinia virus genome. The others are attenuated rabies virus vaccines. All vaccines in current use comply with the respective monograph of the European Pharmacopoeia and it is recommended that they should also comply with WHO recommendations for oral rabies vaccines. Bait stability has been studied in a FAIR project on wildlife vaccination against rabies. The titre of vaccine virus in attenuated rabies virus vaccines was found to be significantly reduced at elevated temperatures. The vaccinia recombinant vaccine was found to be stable over a wide temperature range. The physical stability of bait casings varied also between vaccines, some being particularly sensitive to elevated temperature and rainfall. It is recommended that each batch of vaccine should be tested for stability prior to use.

Under experimental and field conditions, ‘double-vaccination’, with an interval of a few days to 3-4 weeks, did not lead to enhanced protection and is therefore not recommended. Fox cubs are able to respond to oral vaccination from 4 weeks of age, thus allowing vaccination of cubs at dens. Maternally derived antibodies interfere with the induction of active immunity by vaccination. However, the degree of this interference appears to depend on the type of vaccine used. Vaccinia recombinant vaccine appeared to be better able to overcome this maternal immunity.

In order to estimate population immunity and progress of eradication, vaccination campaigns should be monitored with respect to fox density, rabies incidence, bait uptake (using a tetracycline marker or alternatives when they become available) and seroconversion. Successful vaccination will normally lead to an increased fox

population and this should be addressed by an increased baiting density to preserve high population immunity.

Biannual vaccination campaigns have been successful in most situations in eradicating fox rabies. Spring campaigns should preferably be carried out in May/June, with or without den vaccination. Autumn campaigns should be performed in September/October. In case of re-emergence of disease, vaccination should be implemented immediately irrespective of season, except under extreme climatic conditions which would severely hinder bait and vaccine stability.

Vaccination areas should be carefully designed, taking into account natural barriers and should generally be at least 5,000 km^2 and coordinated across administrative and international borders. Vaccinated buffer zones extending beyond the front of a rabies endemic zone should be at least 50 km in width. In the case of an existing natural physical barrier the minimum distance recommended is 20 km. Densities of 18-20 and 20-30 baits per km^2 are recommended for vaccination campaigns in areas of low and high fox population densities, respectively. Baits can be applied by helicopter, fixed-wing aircraft, or manually. Distribution by helicopter, or by hand in urban and suburban areas, can be used to ensure that baits are delivered close to natural fox habitats. Baits should be distributed in a regular pattern with no more than 500 to 300 metres between distribution lines for regions of low and high fox population densities, respectively.

12. REFERENCES

Artois, M., Aubert, M., and Stahl, P., (1990). Organisation spatiale du renard roux (*Vulpes vulpes*) en zone d'enzootie de rage en Lorraine. Rev. Ecol. (Terre et Vie), 45: 113-134.

Artois, M., Guittre, C., Thomas, I., Leblois, H., Brochier, B., and Barrat, J., (1992). Potential pathogenicity for rodents of vaccines intended for oral vaccination against rabies: a comparison. Vaccine, 10: 524-528.

Artois, M., Masson, E., Barrat, J., and Aubert, M.F.A., (1993). Efficacy of three oral rabies vaccine-baits in the red fox: a comparison. Vet. Microbiol., 38: 167-172.

Atanasiu, P., Metianu, T., and Bolanos, A., (1982). Evaluation d'une vaccination rabique expérimentale par la voie orale et intestinale avec des vaccins tués concentrés et non concentrés. Comp. Immun. Microbiol. Infect. Dis. 5: 1-3, 187-191.

Aubert, M., (1992). Epidemiology of fox rabies. K. Bögel, F.X. Meslin and M. Kaplan (Eds.). Wildlife Rabies Control, Wells Medical Ltd, Kent: 9-18.

Aubert, M., (1995). Epidémiologie et lutte contre la rage en France et en Europe. Bull. Acad. Nat. Méd., 179: 1033-1054.

Aubert, M.F.A., (1999). Costs and benefits of rabies control in wildlife in France. Rev. Sci. Tech. Off. Int. Epiz., 18: 533-543.

Aubert, M.F.A., Blancou, J., and Hofmann, M., (1982). Oral vaccination of foxes against rabies: trials with inactivated virus in enteric coated tablets. Rabies Information Exchange: 37-38.

Aubert M., Vuillaume P., and Masson E., (1993). La vaccination orale des renards est efficace même quand leurs populations augmentent. Bulletin épidémiologique de la rage animale en France, 23: 1-4.

Aubert, M.F.A., Masson, E., Artois, M., and Barrat, J., (1994). Oral wildife rabies vaccination field trials in Europe, with recent emphasis on France. "Current topics in microbiology and immunology", C.E. Rupprecht, B. Dietzschold and H. Koprowski (Eds.). Springer Verlag, Berlin, 187: 219-243.

Aubert, M.F.A., Cliquet, F., and Barrat, J., (2000). Rabies OIE Manual of Standards for Diagnosis Tests and Vaccines. 4th edition. Office International des Epizooties, Paris, p. 276.

Baer, G., Abeleth, M.K., and Debbie, J.G., (1971). Oral vaccination of foxes against rabies. Amer. J. Epidemiol., 93: 487-490.

Balbo, T., and Rossi, L., (1988). Fox vaccination in Italy, "La vaccination antirabique du renard", P.P. Pastoret, B. Brochier, I. Thomas and J. Blancou (Eds.). Office des Publications Officielles des Communautés Européennes, Luxembourg, 1988, 140 : 61-69.

Barrat, J., Blasco, E., Lambot, M., Cliquet, F., Brochier, B., Renders, C., and Pastoret, P., (2001). Is it possible to vaccinate young canids against rabies and to protect them? Sixth SEARG Meeting, Lilongwe, 18-21 June 2001.

Besch, A., (2001). Eradication de la rage au Grand-Duché de Luxembourg. In Proceedings. Journée internationale sur la Rage. Palais Abbatial de Saint Hubert, 28 mars 2001, Saint-Hubert, Belgique, C.O.8. : 1-14.

Bingham, J., Foggin, C.M., Gerber, H., Hill, F.W.G., Kappeler, A., King, A., Perry, B.D., and Wandeler, A., (1992). Pathogenicity of SAD vaccine rabies vaccine given orally in Chacma baboons (*Papio ursinus*). Vet. Rec., 131: 55-56.

Bingham, J., Schumacher, C.L., Aubert, M.F.A., Hill, F.W.G., and Aubert, A., (1997). Innocuity studies of SAG-2 oral rabies vaccine in various Zimbabwean wild non-target species. Vaccine 15: 937-943.

Black, J.C., and Lawson, K.F., (1973). Further studies of sylvatic rabies in the red fox (*Vulpes vulpes*). Vaccination by the oral route. Canad. J. Comp. Med., 34: 309-311.

Blancou, J., Schneider, L.G., Wandeler, A.I., and Pastoret, P.P., (1986). Vaccination du renard roux (*Vulpes vulpes*) contre la rage par voie orale. Bilan d'essais en station expérimentale. Rev. Ecol. (Terre Vie), 40: 249-255.

Blasco, E., Lambot, M., Barrat, J., Cliquet, F., Brochier, B., Renders, C., Krafft, N., Bailly, J., Munier, M., Pastoret, P.-P. and Aubert, M.F.A., (2001). Kinetics of humoral immune response after rabies VRG oral vaccination of captive fox cubs (*Vulpes vulpes*) with or without maternally derived antibodies against the vaccine. Vaccine, 19: 4816-4823.

Bourhy, U., Kissi, B., and Tordo, N., (1993). Molecular diversity of the Lyssavirus genus. Virology, 194: 70-81.

Breitenmoser, U., (1995). Erfahrung mit der Baubeimpfung als ergänzende Tollwut-Bekämpfungsmassnahme in der Schweiz, Tollwut-Round-Table Gespräch Wusterhausen, 6-7 November, 2pp..

Breitenmoser, U., and Müller, U., (1997). How to do the wrong thing with the highest possible precision - a reflection on the use of GPS in rabies vaccination campaigns. Rabies Bulletin Europe, 21: 11-13.

Breitenmoser, U., Müller, U., Kappeler, A., and Zanoni, R. G., (2000). Die Endphase der Tollwut in der Schweiz. Schweiz. Arch. Tierheilk., 142: 447-454.

Brochier, B., Thomas, I., Iokem, A., Ginter, A., Kalpers, J., Paquot, A., Costy, F., Pastoret, P.-P., (1988). A field trial in Belgium to control fox rabies by oral immunisation. Vet. Rec., 123: 618-621.

Brochier, B., Languet, B., Artois, M., Zanker, S., Guittré, C., Blancou, J., Chappuis, G., Desmettre, P., and Pastoret, P.P., (1990). Efficacy of a baiting system for vaccinating foxes against rabies with vaccinia-rabies recombinant virus. Vet. Rec., 127: 165-167.

Brochier, B., Aubert, M.F.A., Pastoret, P.P., Masson, E., Schon, J., Lombard, M., Chappuis, G., Languet, B., and Desmettre, P., (1996). Field use of a vaccinia-rabies

recombinant vaccine for the control of sylvatic rabies in Europe and North America. Rev. Sci. Tech. Off. Int. Epiz., 15: 947-970.

Brochier, B., Deschamps, P., Costy, F., Hallet, L., Leuris, J., Villers, M., Péharpré, D., Mosselmans, F., Beier, R., Lecomte, L., Mullier, P., Roland, H., Bauduin, B., Kervyn, T., Renders, C., Escutenaire, S., and Pastoret, P.-P., (2001). Elimination de la rage en Belgique par la vaccination du renard roux (*Vulpes vulpes*). Annales de Médecine vétérinaire, 145: 293-305.

Bruyère, V., and Janot C., (2000). La France bientôt déclarée officiellement indemne de rage. Bulletin épidémiologique mensuel de la rage animale en France, 30 : 1-2.

Bruyère, V., Vuillaume, P., Cliquet, F., and Aubert, M., (2000). Oral rabies vaccination of foxes with one or two delayed distributions of SAG2 baits during the spring. Vet. Res., 31: 339-345.

Buckland, S.T., Anderson D.R., Burnham K.P., and Laake J.L., (1993). Distance Sampling: Estimating Abundance of Biological Populations. Chapman and Hall, London, p. 446.

Chautan, M., Pontier, D., and Artois, M., (2000). Role of rabies in recent demographic changes in red fox populations in Europe. Mammalia, 64: 391-410.

Cliquet, F., Masson, E., and Aubert, M., (1995). Impact de la consommation d'appâts vaccinaux antirabiques destinés aux renards sur l'immunité des sangliers contre la rage : essai sur une population contenue dans un enclos de chasse. Ann. Méd. Vét., 139: 15-19.

Cliquet, F., Aubert, M., and Sagne, E., (1998). Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. Journal Immunological Methods, 212: 79-87.

Cliquet, F., Sagne, L., Schereffer, J.L., and Aubert, M.F.A., (2000). ELISA test for rabies antibody titration in orally vaccinated foxes sampled in the fields. Vaccine, 18: 3272-3279.

Corbet, G.B., and Harris, S., (1991). The handbook of British mammals. 3rd edition. Blackwell Scientific Publications: p. 588.

Dubreuil, M., Andral, L., Aubert, M.F.A., and Blancou, J., (1979). The oral vaccination of foxes against rabies. An experimental study. Ann. Rech. Vét., 10: 9-21.

European Pharmacopoeia, (2002). European Pharmacopoeia monograph- *Vaccinum rabiei per orale vivum ad vulpem.*

FAIR Project CT 97-3515, (2002). Wildlife vaccination against rabies in difficult and emergency situations and its potential impact on the environment, Final Report.

Fekadu, M., Shaddock, J.H., Chandler, F.W., and Sanderlin, D.W., (1988). Pathogenesis of rabies virus from a Danish bat (*Eptesicus serotinus*): neuronal changes suggestive of spongiosis. Arch. Virol., 99: 187-203.

Finnegan, C.J. *et al*., (2002). Rabies in North America and Europe. J. R. Soc. Med., 95: 9-13.

Fooks, A.R., (2001). Two imported human rabies cases in the United Kingdom, Rabies Bulletin Europe, 2: 11.

Frost, J.W., and Kiefert, C., (1979). Zur oralen immunisierung des Fuchses gegen Tollwut, Z. Säugetierkunde, 44: 52-55.

Hadidian, J., Jenkins, S.R., Johnston, D.H., Savarie, P.J., Nettles, V.F., Manski, D., and Baer, G.M., (1989). Acceptance of simulated oral rabies vaccine baits by urban raccoons. J. Wildl. Dis., 25: 1-9.

Kappeler, A., (1991). Die orale Immunisierung von Füchsen gegen Tollwut in der Schweiz, Inauguraldissertation, Bern.

Kauker, E., and Zettel, K., (1963). Zur. Epidemiologie der sylvatischen Tollwut in Mitteleuropa und den Möglischkeiten ihrer Bekämpfung. Vet. Med. Nachr., 2/3 : 181.

Lafay, F., Benejean, J., Tuffereau, C., and Coulon, P., (1994). Vaccination against rabies: construction and characterisation of SAG2, a double avirulent derivative of SAG-Bern. Vaccine, 12: 317-320.

Lambot, M., Blasco, E., Barrat, J., Cliquet, F., Brochier, B., Renders, C., Krafft, N., Bailly, J., Munier, M., Aubert, M.F.A., and Pastoret, P.-P., (2001). Humoral and cell-mediated immune responses of foxes (*Vulpes vulpes*) after experimental primary and secondary oral vaccination using SAG2 and VRG vaccines. Vaccine, 19: 1827-1835.

Masson, E., Aubert, M.F.A., Barrat, J., and Vuillaume, P., (1996). Comparison of the efficacy of the antirabies vaccines used for foxes in France. Vet. Res., 27: 255-266.

Masson, E., Bruyère-Masson, V., Vuillaume, P., Lemoyne, S., and Aubert, M., (1999). Rabies oral vaccination of foxes during summer with the VRG vaccine bait. Vet. Res., 30: 595-605.

Maurer, W., and Guber, S.E., (2001). Rabies vaccination of foxes: vaccine residues as potential biohazardous waste. Pediatr. Infect. Dis. J., 20: 1184-1185.

Mayr, A., Kraft, H., Jaeger, O., and Haacke, H., (1972). Orale Immunisierung von Füchsen gegen Tollwut. Zbl. Vet. Med., 19: 615-625.

Müller, W.W., (1997). Where do we stand with oral vaccination of foxes against rabies in Europe. Arch. Virol., 13: 83-94.

Müller, W.W., (2000). Review of rabies case data in Europe to the WHO Collaborating Centre Tübingen from 1977 to 2000. Rabies Bulletin Europe, 4: 11-19.

Müller, T., and Schlüter, H., (1998). Oral immunization of red foxes (*Vulpes vulpes*) against rabies - a review. J. Etlik Vet. Microbiol. Ankara, 9: 35-59.

Müller, T., Schuster, P., Vos, A., Selhorst, T., Wenzel, U., and Neubert, A., (2001). Effect of maternal immunity on the immune response to oral vaccination against rabies in young foxes, Am. J. Vet. Res., 62: 1154-1158.

Müller, T., Cox, J., Peter, W., Schäfer, R., Bodamer P., Wulle, U., Burow, J., and Müller, W., (2002). Infection of a Stone Marten with European Bat Lyssa Virus (EBL1). Rabies Bulletin Europe: 9-11.

Nadin-Davis, S.A., Casey, G.A., and Wandeler, A., (1993). Identification of regional variants of the rabies virus within the Canadian province of Ontario. J. Gen. Virol., 74: 829-837.

Neubert, A., Schuster, P., Müller, T., Vos, A., and Pommerening, E., (2001). Immunogenicity and efficacy of the oral rabies vaccine SAD B19 in foxes. J. Vet. Med. B, 48: 179-183.

OIE (Office International des Epizooties), (2000). Rabies: Manual of Standards for Diagnostic Tests and Vaccines, 4th edition. Office International des Epizooties, Paris.

Pastoret, P.-P., Brochier, B., Languet, B., Duret, C., Chappuis, G., and Desmettre, P.-P., (1996). Stability of Recombinant Vaccinia Rabies Vaccine in Veterinary Use. In: New Approaches to Stabilisation of Vaccines Potency. F. Brown (Ed.). Dev. Biol. Stand. Basel, Karger, 87: 245-249.

Rønsholt, L., Sørensen, K.J., Bruschke, C.J., Wellenberg, G.J., van Oirschot, J.T., Johnstone, P., Whitby, J.E., and Bourhy, H., (1998). Clinically silent rabies infection in (zoo) bats. Vet. Rec., 142: 519-520.

Rupprecht, C.E., Charlton, K.M., Artois, M., Casey, G.A., Webster, W.A., Campbell, J.B., Lawson, K.F., and Schneider, L.G., (1990). Ineffectiveness and comparative pathogenicity of attenuated rabies virus vaccines for the striped skunk (*Mephitis mephitis*). J. Wildl. Dis., 26: 99-102.

Rupprecht, C.E., Hanlon, C.A., Cummins, L.B., and Koprowski, H., (1992). Primate responses to a vaccinia-rabies glycoprotein recombinant virus vaccine. Vaccine.;10: 368-374.

Rupprecht, C.E., Hanlon, C.A., and Koprowski, H., (1996): General considerations in the use of recombinant rabies vaccines for oral immunization of wildlife. In: Laboratory techniques in rabies, 4th edition, F.X. Meslin, M.M. Kaplan, and H. Koprowski, (Eds.), World Health Organization, Geneva, pp. 341-346.

Rupprecht, C.E., Blass, L., Smith, K., Orciari, L.A., Niezgoda, M., Whitfield, S.G., Gibbons, R.V., Guerra, M., and Hanlon, C.A., (2001). Human infection due to recombinant vaccinia-rabies glycoprotein virus. New Engl. J. Med., 345: 582-586.

Schaarschmidt, U., Müller, T., Albert, G., Muluneh, A., Cox, J., Selhorst, T., and Schlüter, H., (2002). Erfahrungen mit der Begleitdiagnostik zur oralen Immunisierung der Füchse in Sachsen unter besonderer Berücksichtigung einer standardisierten Tollwutserologie. Dtsch. Tierärztl. Wschr. (in press).

Schlüter, H., Müller, T., Staubach, C., and Fröhlich, A., (1997). Rabies control in central Europe - Results after more than 10 years of oral immunization of foxes. Epidémiologie et santé animale, 31-32.

Schneider, L.G., Cox, J.H., and Müller, W.W., (1987). Der Feldversuch zur oralen Immunisierung von Füchsen gegen die Tollwut in der Bundesrepublik Deutschland (BRD)- eine Zwischenbilanz. Tierärzliche Umschau, 42: 184-198.

Serra-Cobo, J., Amengual, B., Abellán, C., and Bourhy, H., (2002). European bat lyssavirus infection in Spanish bat populations. Emerg. Infect. Dis., 8: 413-420.

Steck, F., Wandeler, A., Bischel, P., Hafliger, U., and Schneider, L., (1982). Oral immunisation of foxes against rabies. Laboratory and field studies. Comp. Immun. Microbiol. Infect. Dis., 5: 165-171.

Taylor, D., (1976). Rabies: epizootic aspects. Vet. Rec., 99: 157.

Thomas, I., Brochier, B., Levaux, T., Bauduin, B., Costy, F., Peharpre, D., Hallet, L., and Pastoret, P.P., (1989). Première campagne de vaccination antirabique du renard à l'aide de la souche atténuée SAD B19 du virus rabique réalisée en Belgique en 1988 sur la totalité du territoire contaminé par la rage. Ann. Méd. Vét., 133: 403-412.

Thulke, H.H., Tischendorf, L., Staubach, C., Selhorst, T., Jeltsch, F., Müller, T., Schlüter, H., and Wissel, C., (1999). The spatio-temporal dynamics of a post-vaccination recovery of rabies in foxes and emergency vaccination planning. J. Prev. Vet. Med., 47: 1-21.

Thulke, H.H., Selhorst, T., and Müller, T., (2001). 'The more the better, but not always for anti-rabies baiting'. Conference Presentation, Society for Veterinary Epidemiology and Preventive Medicine, Noordwijkerhout, The Netherlands, (http://www.svepm.org.uk/Posters2001index.htm).

Vos, A.C., (1995). Population dynamics of the red fox (*Vulpes vulpes*) after the disappearance of rabies in county Garmisch-Partenkirchen, Germany, 1987-1992. Ann. Zool. Fennici, 32: 93-97.

Vos, A., Neubert, A., Aylan, O., Schuster, P., Pommerening, E., Müller, T., and Chivatsi, D.C., (1999). An update on safety studies of SAD B19 rabies virus vaccine in target and non-target species. Epidemiol. Infect., 123: 165-75.

Vos, A., Müller, T., Schuster, P., Schlüter, H., and Neubert, A., (2000). Oral vaccination of foxes against rabies with SAD B19 in Europe, 1983-1998: A review. Veterinary Bulletin, 17: 1-6.

Vos, A., Muerke, H., Holzhofer, E., Gschwender, P., and Schuster, P., (2001). A satellite navigated and computer-supported fully automatic system for distributing oral vaccine-baits against rabies: SURVIS, Proceedings of the 12th Rabies in the Americas conference, November 12-16, 2001, Peterborough, Ontario, Canada, p. 66.

Vos, A., Pommerening, E., Neubert, L., Kachel, S., and Neubert, A., (2002). Safety studies on the oral rabies virus vaccine SAD B19 in striped skunks (*Mephitis mephitis*). J. Wildl. Dis., 38: 407-411.

Vuillaume, P., Aubert, M., Demerson, J.M., Cliquet, F., Barrat, J., and Breitenmoser, U., (1997). Vaccination des renards contre la rage par dépôt d'appâts vaccinaux à l'entrée des terriers. Ann. Méd. Vét., 141: 55-62.

Vuillaume, P., Bruyère, V., and Aubert, M., (1998). Comparison of the effectiveness of two protocols of antirabies bait distribution for foxes (*Vulpes vulpes*). Vet. Res., 29: 537-546.

Wachendörfer, G., and Frost, J.W, (1992). Epidemiology of fox rabies: a review. In: Wildlife Rabies Control, K. Bögel, F.X. Meslin, M. Kaplan (Eds.), Wells Medical Ltd., Kent; pp. 19-31.

Wachendörfer, G., Frost, J.W., Gutmann, B., Scharf-Zentek, G., von Schöppenthau, C., Gongal, G.N., Dingeldein, W., Wagner, K., Hofmann, J., and Gerbig, H., (1996). Orale Immunisierung von Füchsen gegen Tollwut - Feldergebnisse und Erfahrungen im Zeitraum 1983 bis 1995 in Hessen. Amtstierärztlicher Dienst und Lebensmittelkontrolle, 3: 343-352.

Wandeler, A.I., (1991). Oral immunization of wildlife. In: The Natural History of Rabies, G.M. Baer, (Ed.). 2nd edition, CRC Press, Boca Raton; pp. 485-503.

Wellenberg, G.J., Audry, L., Rønsholt, L., van der Poel, W.H., Bruschke, C.J., and Bourhy, H., (2002). Presence of European bat lyssavirus RNA in apparently healthy *Rousettus aegyptiacus* bats. Arch. Virol., 147: 349-361.

WHO, (1989). Report of WHO Consultation on requirements and criteria for field trials on oral rabies vaccination of dogs and wild carnivores, Geneva, 1-2 March 1989, Doc. WHO/Rab.Res./89.32.

WHO, (1992a). 8th Report of WHO Expert Committee on rabies, Geneva.

WHO, (1992b). Report of the 3rd WHO consultation on oral immunization of dogs against rabies, Geneva, 21-22 July 1992, Doc. WHO/Rab.Res./92.38.

WHO, (1993). Report of the 4th WHO Consultation on oral immunization of dogs against rabies, Geneva, 14-15 June 1993, Doc. WHO/Rab.Res./92.42

WHO, (1994). Report of the 5th WHO Consultation on oral immunization of dogs against rabies, Geneva, 20-22 June 1994, Doc. WHO/Rab.Res./94.45

WHO, (1995). Oral immunization of dogs against rabies : report of the 6th WHO consultation, Geneva, 24-25 July 1995, Doc WHO/EMC/ZDI/98.13, p. 28.

WHO, (1996). Laboratory techniques in rabies., 4th edition, Geneva. F.X. Meslin, M.M. Kaplan, and H. Koprowski, (Eds.).

WHO, (1998a). Field application of oral rabies vaccines for dogs. WHO-OIE consultation, Geneva, 20-22 July 1998, Doc. WHO/EMC/ZD/98.15, p. 24.

WHO, (1998b). WHO Conference on rabies control and oral immunization of foxes, Zagreb, 22-23 October 1998.

WHO/OIE, (1993). 4th WHO/OIE Concertation meeting on rabies control in Europe, Piestany, 5-7 October 1993.

Zanoni, R.G., Kappeler, A., Müller, U.M., Müller, C., Wandeler, A.I., and Breitenmoser, U., (2000). Tollwutfreiheit der Schweiz nach 30 Jahren Fuchstollwut / Rabies free status of Switzerland after 30 years of fox rabies. Schweizer Arch. Tierheilk., 142: 423-429.

13. ANNEX: PROPOSAL FOR A BAIT DISTRIBUTION METHODOLOGY

This methodology is based on the data reviewed, field experience, and the conclusions and recommendations of the report.

1. Aerial distribution of baits

All habitats should be treated except large stretches of water (e.g. lakes, rivers) and motorways.

The vaccination area should be divided into plots by using natural or artificial landscape features (roads, railway tracks, canals, etc.)

1.1. Low fox population density:

Bait density: 18-20 baits / km^2

Distribution pattern: linear: 2 flight lines (2 x 9 baits) / km^2

Distance between flight lines: 500 m

Distance between each bait (along the same flight line): approx. 110 m

Vehicle: helicopter

Flight altitude: 100-150 m

Flight speed: 100-150 km/h

Dropping procedure: Human: appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on the most convenient places for the fox (hedges, village surroundings, isolated bunch of trees etc.). An electronic metronome, connected to GPS (Global Positioning System), that allows adjustment of dropping tempo to speed may be a help, but the dropping tempo may be altered to favour more likely fox habitats.

Control method: detailed map or preferably GPS.

1.2. High fox population density:

Bait density: 20-30 baits / km^2

Distribution pattern: linear: 3 flight lines (e.g. 3 x 8 baits) / km^2

Distance between flight lines: 300 m

Distance between baits along the same flight line: 125 m

Flight altitude: 100-150 m

Flight speed: 100-150 km/h

Vehicle: helicopter

Dropping methodology: Human: appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on the most convenient places for the fox (hedges, village surroundings, isolated bunch of trees etc.). An electronic metronome (connected to GPS that allow to adjust dropping tempo to speed) may be a help, but the dropper has not to stick to this tempo to favour the places where foxes may live.

Control method: detailed map or preferably GPS.

Comments:

The above mentioned pattern of bait distribution (low distances between flight lines) can be performed more easily using a helicopter.

The use of a helicopter is more adapted to the treatment of low-densely inhabited zones in rural areas.

The delivery by helicopter is fast and, unlike fixed-wing aircraft, allows precise dropping of baits (flexibility in both flight speed and altitude).

The helicopter allows operation in less favourable weather conditions.

Fixed-wing aircraft may be used for the coverage of large uninhabited areas (such as large forested areas) allowing long flight lines.

2. Manual distribution of baits

The manual method of baiting allows a very precise and uniform dispersal of baits but requires a thorough organisation and important human resources, qualitatively (competency, motivation) as well as quantitatively. Therefore, it should be applied for the coverage of small size areas.

2.1. Distribution of baits at fox dens

Vaccination of fox cubs at dens can usefully complement aerial vaccination for the treatment of local residual foci and reinfected areas (especially when fox density is high). It can also be used to supplement manual uniform distribution in suburban areas (see below).

Methodology:

In early spring, fox dens should be located and recorded on detailed maps by appointed and trained people (forestry rangers, gamekeepers, hunters).

At the end of May – early June, previously located dens are visited again by the same persons (bearing gloves) and baits are deposited (generally at least 10) at the den entrances according to their status, whether inhabited or not:

-when occupation indices are present, 10-20 baits are deposited in the close surroundings of the breeding den regardless of the number of entrances (there are often only one or 2 entrances);

- in the absence of occupation indices, 6 baits are deposited in the close surroundings of the den regardless of the number of entrances.

Baits should be hidden (with grass, leaves etc.) to avoid ingestion by birds and exposure to direct sun light.

2.2. Uniform distribution of baits in suburban areas

In such a habitat, up to 4-5 fox family groups can be counted per km^2. Consequently the percentage of foxes needed to be immunised to eliminate rabies might theoretically approach 100%. In addition, the high density of competing pets population can significantly affect the rate of bait uptake in foxes. For safety and feasibility reasons, aerial distribution is replaced by manual distribution in such high-densely inhabited areas. However, whenever possible, the combination of several distribution systems (hand-helicopter) is to be recommended.

Methodology:

Hand distributors: appointed and training persons (bearing gloves).

Bait density: 50 baits / km^2

Dispersal of baits: Raster pattern: uniform but more intensive in predicted fox habitats.

Baits should be hidden to avoid human contacts, ingestion by birds and exposure to direct sunlight.

Control method: detailed map

14. ACKNOWLEDGEMENTS

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