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Control of Rabies in Slovenia

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ABSTRACT: Red foxes (*Vulpes vulpes*) are the main reservoir of rabies in Slovenia, whereas cases of rabies in other wildlife species occur sporadically. In 1995, a program of oral vaccination of wildlife in Slovenia was initiated; baits with oral vaccine were distributed by air at a density of 20 baits/km^2. During 1995, when the oral vaccination program was started, 1,089 cases of rabies (including both wild and domestic animals) were reported. Five years later (1999), only six positive animals were detected among 1,195 tested (0.5%). Despite an increase in bait density (25 baits/km^2) during the years 2000 and 2001, reported rabies cases increased to 115 and 135, respectively. In 2003, following initiation of a new bait-dropping strategy, which incorporated perpendicular rather than parallel flight lines, the number of rabies cases decreased to eight.

Key words: Rabies, Slovenia, vaccination, wildlife.

Rabies is a zoonotic disease of mammals caused by a negative-stranded RNA virus belonging to the *Lyssavirus* genus of the family Rhabdoviridae; it is transmitted most frequently via saliva following a bite from an infected animal (Bourhy et al., 1999). The genus *Lyssavirus* is divided into seven genotypes. Genotype 1 viruses are distributed worldwide and generally are found in terrestrial mammals (Bourhy et al., 1999; Finnegan et al., 2002). In central and southeastern Europe, red foxes (*Vulpes vulpes*) are the primary wildlife reservoir (Lontai, 1997; Office International des Epizooties [OIE], 2000a), and in red fox–mediated rabies epizootics, the numbers of cases vary over season and year (Müller, 2000). Oral vaccination of red fox as a method of rabies eradication started in Switzerland, and by 1984, this method was adopted by several other European countries. In central and eastern European countries, including the Czech Republic, Slovak Republic, Poland, Slovenia, and Hungary, cases of rabies were vastly reduced after 9 yr of oral vaccination (Matouch and Vitasek, 2002). Rabies cases peaked in Europe in 1989, when 24,377 cases were reported (Potzsch et al., 2002). As a result of oral vaccination programs since 1989, several European countries, based on World Health Organization principles, are rabies-free in terms of terrestrial animals (Müller, 2000); these countries include Finland (1991), The Netherlands (1991), Italy (1997), Switzerland (1998), France (2000), Belgium (2001), and Luxemburg (2001) (Potzsch et al., 2002). In other eastern and southern European counties where oral vaccination programs were not implemented, the number of reporting cases has increased.

In 1995, after preliminary experimental vaccination trials (Curk and Carpenter, 1994), a national eradication program to eliminate rabies throughout Slovenia was initiated. The objectives of this report are to describe the implementation of this rabies eradication program, to analyze the rabies situation during the vaccination period from 1995 to 2003, and to describe the use of two different airborne bait-delivery models (a parallel-line model and raster model) in these eradication efforts.

According to Slovenian legislation, all veterinary organizations are required to submit samples from animals with neurologic signs of rabies for laboratory testing. Hunter organizations also have collected samples from red fox and other wildlife species and submitted them to the local veterinary service for rabies testing. All samples were tested with a fluorescent antibody test (FAT) using impression smears of Ammon's horn and cerebellum as described in the *Manual of Standards for Diagnostic Tests and Vaccines* (OIE, 2000b). A commercial immunofluorescent

TABLE 1. Cumulative rabies and vaccination data during the oral vaccination period.[a]

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
No. of vaccine baits used per km^2	15–17	15–17	ND	16–18	16–18	18–20	23–26	23–26	24–28	24–28
No. of animals tested for rabies	3,787	2,285	1,267	1,382	1,195	1,509	2,153	1,495	993	1,612
No. of rabies cases	1,089	247	29	14	6	115	135	15	8	2
% of biomarker positive animals	ND	ND	ND	47.1	68.8	54.6	69.5	71.3	76.2	75.7

[a] ND = not done.

conjugate (Bio-Rad, Marnes la conquette, France) was used in this test. Samples with questionable FAT results were retested using virus isolation on murine neuroblastoma cells (Webster and Casey, 1996). Only laboratory-confirmed cases were included in this analysis.

A commercial vaccine containing attenuated rabies strain SAD (Steck et al., 1982; Schneider and Cox, 1983) was used. Vaccine delivery protocols were based on preliminary studies conducted from 1988 to 1992. During the first 2 yr of these studies, baits were distributed manually; during the last 2 yr, they were distributed by helicopter and plane. Based on the results from these trials, a required bait density of between 16 and 20 baits/km^2 was estimated for the vaccination area. A countrywide vaccination campaign within Slovenia started in 1995, and baits were distributed by plane at a height of between 300 and 500 m. Pilots used a global positioning system for navigation and orientation, and flight paths were 1,000 m apart. Bait discharge was monitored using the computer program FICO3J® (constructed by computer engineer Aleksander Modic S. P., Ajdovscina, Slovenia), which calculated the density of distributed baits based on route and flight time.

Vaccination efficiency was monitored by detection of the biomarker in the bones (lower jaw) of the captured red foxes. A mineral saw (Buehler Isomet, Wusterhausen, Germany) was used to prepare bone sections (thickness, 1.0 mm), and a fluorescent microscope was used to determine the presence of biomarker. Samples for biomarker detection were collected each year in winter (from November to March). Results are presented in Table 1.

Vaccination was carried out in the autumn of 1995 and in the spring and autumn of 1996. In 1997, no vaccines were distributed, but vaccination has continued annually since 1998. In 1999, an area of 18,800 km^2 was covered, but the high mountain area was not included. Later, in 2000, when rabies was eradicated in the neighboring countries of Italy and Austria, vaccination was restricted to an area of 12,500 km^2 in southern Slovenia along the border with Croatia, and bait density was increased to between 23 and 26 baits/km^2. Because the number of rabies cases increased in 2000, a new bait distribution system was implemented in 2001. Baits were dropped twice using perpendicular flight paths. The total number of vaccines distributed by year are given in Table 1.

From 1992 to 2003, a total of 22,082 samples from different animal species were tested, and 3,269 of them were positive by FAT for rabies (Table 2). Most of the positive cases (95.2%) were wildlife species, whereas domestic animals accounted for only 4.8% of positive cases. The incidence of rabies in wildlife populations (3,112 cases) was 19.7-fold higher than that in domestic animals (158 cases). Among domestic animals, cats (51.3% of rabies cases in domestic animals) were positive more often than dogs (37.3% of rabies cases in domestic animals).

From 1979 to 1995, rabies cases were common and widely distributed through

TABLE 2. Rabies cases registered in different animal species in Slovenia during the period from 1992 to 2003.[a]

Year	No. of tested animals	No. of positive animals	% of positive animals	Cats	Dogs	Cattle	Sheep	Horses	Old World rabbit	Red fox	European pine marten	Eurasian badger	Roe deer	European polecat	Red deer	Wild boar	Eurasian lynx
1992	1,365	238	17.43	4	6	1				203	10	4	6	3		1	
1993	2,019	531	26.3	13	8	1	1			476	10	14	7	1			
1994	2,632	842	32.02	11	12	4		1		753	19	21	19	1	1		1
1995	3,787	1,089	28.75	24	12	4	3	1	1	996	21	11	15	1			
1996	2,285	247	10.8	17	11	1				208	5		4		1		
1997	1,267	29	2.28	6	1					20		2					
1998	1,382	14	1.01							14							
1999	1,195	6	0.50	1						5							
2000	1,509	115	7.62	2	2					104		3	2		1	1	
2001	2,153	135	6.27	3	7					117	3	2	3				
2002	1,495	15	1.00							14		1					
2003	993	8	0.8							8							
Total	22,082	3,269	14.80	81	59	11	4	2	1	2,918	68	58	56	6	3	2	1

[a] Old World rabbit, *Oryctolagus cuniculus*; red fox, *Vulpes vulpes*; European pine marten, *Martes martes*; Eurasian badger, *Meles meles*; roe deer, *Capreolus capreolus*; European polecat, *Mustela putorius*; red deer, *Cervus elaphus*; wild boar, *Sus scrofa*; and Eurasian lynx, *Felis lynx*.

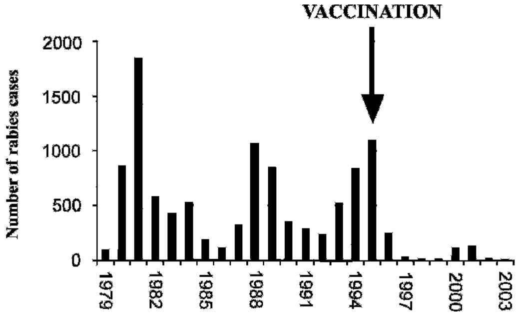

FIGURE 1. Rabies cases during the period from 1979 to 2003.

Slovenia (Fig. 1). The distribution of cases in 1995 is shown in Figure 2, and a rapid decline of positive cases was evident after 1995, when the nationwide oral vaccination program began (Fig. 1). In 1995, a total of 1,089 cases of rabies were registered, predominantly in red foxes (Table 2), and from 1995 to 1998, the highest prevalence of rabies was detected in the central part of Slovenia. In 1998, only 14 cases were detected, and 12 of these occurred in the center of the vaccinated region, within an area with a radius of 30 km around aerodrome Brnik (a restricted flight area, 30×15 km). The remaining two occurred near the Croatian border. In 1999, only six cases were detected (Table 2). In 2000 and 2001, rabies cases were restricted to the southern part of Slovenia, along the border with Croatia, and during these years, the number of positive cases increased (115 in 2000 and 135 in 2001). The number of cases declined to 15 in 2002 and then to eight in 2003. All of the 2000 and 2003 cases were detected near the Croatian border (Fig. 2).

The European epizootic of red fox rabies is thought to have originated south of Kaliningrad on the Polish-Russian border. The infection spread from this area southward and westward, and in a very short time, it was widely distributed throughout central Europe (Matouch, 1996). In Slovenia, the threat of rabies entering the county along the eastern border was recognized in 1973 (Železnik and Janc, 1977), and as a preventive measure, intensive hunting was performed in this region. Vaccination of dogs, pasture cattle, sheep, and horses at the expense of the state became compulsory, and voluntary vaccination of cats was recommended. Nevertheless, rabies was detected throughout Slovenia, and case data indicated that red foxes were most affected. During this study (1995–2003), wildlife rabies accounted for 95.2% of all cases, and most of these involved red fox. Among domestic animals, cats and dogs were most affected, but overall, cases in domestic animals represented a small proportion (4.8%) of total rabies cases in Slovenia. This low percentage demonstrates the effectiveness of the domestic animal vaccination programs.

During the past 25 years, the success of oral mass vaccination in European wildlife has been demonstrated repeatedly (Brochier et al., 1988; Masson et al., 1996; Vos et al., 2000). The management aim of an oral vaccination program is to provide accessible vaccine baits to every fox within the treatment area. Consequently, bait density has been designed empirically, according to population estimates and minimum bait number per capita (Breitenmoser and Müller, 1997; Selhorst et al., 2001). The recommended strategy of applying approximately 20 baits/km^2 (Brochier et al., 1988; Müller and Schlüter, 1998; Thulke et al., 2004) was adopted in Slovenia when the wildlife vaccination program started in 1995, and in that year, rabies cases were distributed throughout Slovenia (Fig. 2). After 3 yr of vaccination, a marked decrease in rabies cases was observed; only 14 rabies cases were recorded in 1998. The persistence of rabies within a 30-km radius area in the center of the vaccinated area (12 cases) is believed to be related to the failure to place baits in this area as a result of restricted airspace. The two additional cases were registered near the border of Croatia, which was not vaccinating wildlife

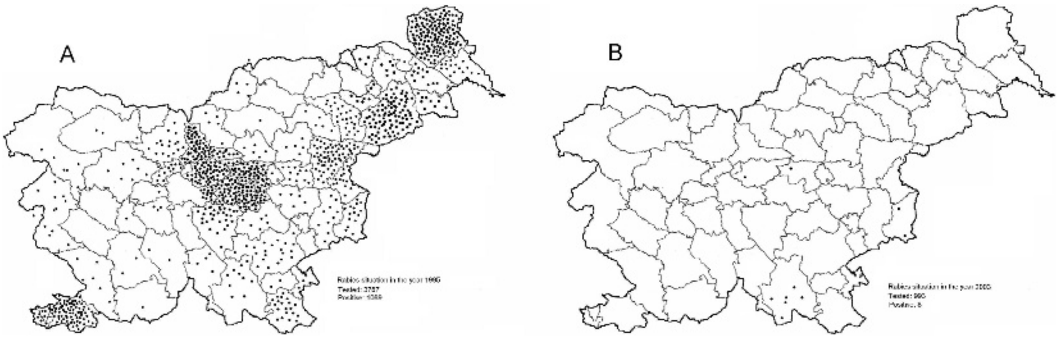

FIGURE 2. Distribution of rabies cases in Slovenia: (A) 1995 (before vaccination), and (B) 2003 (after vaccination).

at that time. Rabies persisted in this region for three additional years. In 1999, all six positive cases were found in this same area, and despite an increase in bait density (from 20 to 25 baits/km^2), the number of positive cases dramatically increased in the years 2000 and 2001. With single flight lines separated by 1,000 m, it was suspected that the area had not been covered with vaccine baits effectively, so the parallel-line model was replaced with the raster model (double-baiting system using perpendicular flight lines). During the next 2 yr, the number of rabies cases decreased; only 15 and eight cases were registered in 2002 and 2003, respectively. Historically, rabies cases in Slovenia (Fig. 1) have peaked in approximately 7-yr intervals, but after oral vaccination started in 1995, the disease appears close to being eradicated. These activities have greatly aided in preventing the spread of rabies to rabies-free parts of central Europe.

Red foxes are solitary, and the home range of adults can vary in size, depending on the quality of the habitat. Home ranges may cover between 5 km^2 and 12 km^2 in quality habitats to between 20 km^2 and 50 km^2 in poorer habitats. An individual red fox can move up to 8 km per night (Corbet and Harris, 1991). It is possible that in some areas, perpendicular flight paths would not transect an individual's home range, and we believe that this is the basis for the improved results when the raster model was used (Fig. 3). This strategy seemed to be especially effective when the landscape was configured with high hills and deep valleys, which may have influenced the shape and dimensions of home ranges. The configuration of dispensed baits also may have affected the results, because the baits have a very intense smell that is easily detected by red foxes from a distance of 100 m (Corbet and Harris, 1991). The single-flight strategy with the density of 25 baits/km^2 (Fig. 3) resulted in baits being dispensed 40 m apart, which may have increased the chance of multiple baits being eaten by the same fox following the transect line. When baits were distributed by double-baiting using perpendicular flight lines, a more uniform delivery was achieved, which may have resulted in more baits being accessible to more animals.

Consistent with results from Slovenia, a dramatic decrease in rabies incidence associated with oral vaccination of red fox

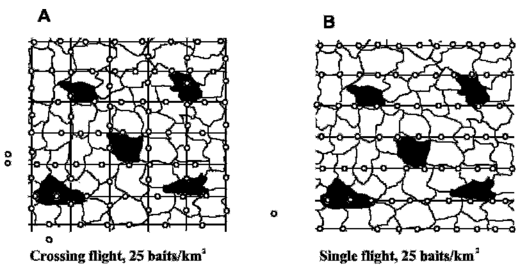

FIGURE 3. Simulation of baits distribution using (A) crossing flights and (B) single flights.

has been recorded in most western European countries. Setbacks and unexpected outbreaks, however, as occurred in Slovenia, also have been reported in other countries (Müller, 1997; Müller, 2000; Matouch and Vitasek, 2002). These problems often were resolved by increasing the number of vaccine baits (>30 baits/km^2), changing the timing of vaccination, or improving and optimizing flight-line distances (Thulke et al., 1997). Our results suggest that perpendicular-flight bait distribution also may improve vaccination results by providing improved bait dispersion.

Slovenia is densely covered with forests and has rich biotypes favorable to potential wildlife vectors. Although the incidence of rabies has been greatly reduced because of the success of the vaccination program, a continuation of surveillance and oral vaccination of wildlife each year as well as the coordination of vaccination campaigns between countries are highly important.

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