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An Epidemic of Sylvatic Rabies in Finland

- Descriptive Epidemiology and Results of Oral Vaccination

By *Matti Nyberg, Katariina Kulonen, Erkki Neuvonen, Christine Ek-Kommonen, Marja Nuorgam* and *Bengt Westerling*

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Nyberg, M., K. Kulonen, E. Neuvonen, C. Ek-Kommonen, M. Nuorgam and B. Westerling: An epidemic of sylvatic rabies in Finland - descriptive epidemiology and results of oral vaccination. Acta vet. scand. 1992, 33, 43-57. – When rabies reappeared in Finland in April 1988, the country had been rabies free since 1959. Soon a picture of sylvatic rabies become evident, its main vector and victim being the raccoon dog *(Nyctereutes procyonoides)*. Between 8 April 1988 and 16 February 1989, 66 virologically verified cases were recorded (48 raccoon dogs, 12 red foxes, 2 badgers, 2 cats,1 dog and 1 dairy bull) in an area estimated at 1700 km^2 in south-eastern Finland. The greatest distance between recorded cases was 67 km. A positive reaction with monoclonal antibody p-41 indicated that the virus was an arctic-type strain.
A field trial on oral immunization of small predators was initiated in September 1988 using Tübingen fox baits according to the Bavarian model of bait distribution. Each bait contained $5*10^7$ $TCID_{50}$/ml modified live rabies virus (SAD-B19). The 6 months' surveillance indicate a seroconversion rate of 72% (N=126) in the raccoon dog population, 67% (N=56) in the red foxes and 13% (N=16) in the badgers, when titers ≥ 1.0 IU/ml are considered seropositive. In the whole follow-up period, no statistically significant difference could be detected between the raccoon dogs and red foxes in the rate of seroconversion or in the uptake of tetracycline from the baits. Notably high antibody levels were recorded in both raccoon dogs and red foxes within 4-5 months after vaccination. Of the seropositive animals, the proportion of animals with titers 3.0 IU/ml or greater was higher in raccoon dogs (73%) than in red foxes (51%) (x^2= 5.29, $p< 0.05$). The trial shows that raccoon dogs can be immunized against rabies in the field with vaccine baits originally developed for controlling sylvatic rabies in foxes.

oral immunization; raccoon dog; red fox; Finland.

Introduction

Finland was first declared free from rabies in 1936. Since then local epidemics have occured in the years 1940-1942, 1952-1954 and 1956-1959. During the years 1940 to 1959, 57 dogs, 2 cats, 3 cows and 1 red fox were found to be rabid. All these epidemics started in areas close to the south-eastern national border and were controlled by restricting the movement of dogs and cats, shooting stray animals and vaccinating the dog population (*Holmberg* 1954). In wild animals, rabies had been recorded during this century in 1 wolf and 2 foxes only, which indicated the absence of an established reservoir of sylvatic rabies. In the 1950s the raccoon dog population in the country was still negligible.

On 8 April 1988 rabies was diagnosed in 1 dog and 1 red fox in the province of Kymi. It soon become evident that this time rabies occurred in its sylvatic form. In contrast to the situation in Central Europe, the major species involved was the raccoon dog *(Nyctereutes procyonoides)* and not the red fox *(Vulpes vulpes)*. The first raccoon dogs were observed in Finland in the 1930s, but a significant population was established much later, during the 1970s and 1980s (*Helle & Kauhala* 1987).

In the 1988 outbreak area, vaccination of dogs at the expense of the state become compulsory and voluntary vaccination of cats, cattle and horses was recommended. In the country as a whole, vaccination of dogs used for hunting and those put on show become compulsory at the owner's expense and voluntary vaccination of pet dogs and cats was strongly recommended.

The promising results obtained from field trials on oral immunization of foxes agains rabies in Central Europe (*Steck et al.* 1982a, 1982b, *Wachendörfer et al.* 1985, *Schneider et al.* 1988) prompted a vaccination trial undertaken in callaboration with the European Centre for Rabies Surveillance and Research, WHO, Tübingen, FRG. The objectives were:

1) to study the feasibility of the Bavarian model (*Schneider et al.* 1983, *Wilhelm & Schneider* 1990) of bait distribution in a boreal biotope with the raccoon dog as the primary target species,
2) to study the efficiency of the live attenuated SAD-B19 virus in Tübingen Fox Baits for immunizing free-living raccoon dogs,
3) to try to inhibit further spread of rabies and if possible to eradicate it.

In this article we report the main descriptive data for the local rabies epidemic 1988-1989 and the results from the first campaign of a field trial on oral vaccination in Finland.

Material and methods

Collection of field specimens

The general public and hunters all over the country were asked to send carcasses of wild predators found dead or behaving abnormally to the National Veterinary Institute, Helsinki. Carcasses were also requested of all animals which had bitten people or other animals, and had been judged by the owners or authorities to be suspects of rabies. All the predators killed by hunters in and near the observed rabies area were also requested. The efficiency of hunting was increased, but the use of poisons or gassing of dens was not allowed. According to the law the red fox, raccoon dog, mink, polecat and badger can be hunted throughout the year, except for female animals with litters from 1 May to 31 July. Bounties were not paid for the animals killed or for specimens sent for examination.

Virus detection and isolation

From the animals to be examined for rabies, tissue samples were taken at autopsy from the cerebral cortex, Ammon's horn and the cerebellum. From each location, 2 impressions were made for a direct immunofluorescence test (*Wiktor et al.* 1980). The impressions were stained, respectively, with the anti-rabies conjugates: Centocor FITC anti-rabies monoclonal globulin (Centocor Inc, Malvern, PA, USA) and rabies antinucleocapsid conjugate (Institut Pasteur Production, Marnes-la-Coqette, France). Positive cases were typed further and differentiated from the SAD B19 vaccine strain with the monoclonal antibodies W-239, P-41 and W-187.5, provided by Drs. Schneider and Cox, WHO Reference Centre, Tübingen, FRG.

In cases with a known history of contact with people or domestic animals, or when the suspected animal was a ruminant, the direct immunofluorescence method was complemented with a virus isolation test. From April to October 1988 inoculation on mice (*Koprowski et al.* 1973) was used. Briefly, a 10% brain suspension in Hank's medium (Orion Diagnostica, Helsinki, Finland) was injected intracerebrally into 10 suckling, 3-4 days old mice (NMRI outbred stock). The mice were inspected daily for 28 days and animals dying or showing symptoms of disease were tested for rabies by direct immunofluorescence on brain tissue. From November 1988 on, the tests were made by using murine neuroblastoma cells instead of suckling mice. The sample preparation and infection of cells followed a previously described method of *Kulonen et al.* (1991).

Bait

Tübingen Fox Baits consisting of fat and fish meal, each containing 1.8 ml live attenuated SAD-B19 rabies virus in a plastic-tinfoil capsule were used (*Schneider et al.* 1989). The baits contained tetracycline as a biomarker. In view of the results of a laboratory experiment on the immunizability of the raccoon dog with the SAD-B19 vaccine (*Tanskanen et al.,* unpublished), $5*10^7$ $TCID_{50}$/ml was chosen as the concentration of vaccine virus in the baits.

Distribution of baits

The organization for the bait distribution has been described previously (*Westerling* 1989). The Bavarian model (*Schneider et al.* 1983, *Wilhelm & Schneider* 1990), adapted to Finnish conditions, was used. On the 24 September 1988, 36000 baits were distributed (15/km^2) by some 800 volunteer hunters on an area of 2400 km^2. A week later a rabid raccoon dog was recovered inside the vaccination area only 7 km from its north-western border. Because of this 4500 more baits were distributed by air (20/km^2) over an area of 225 km^2 on 11 October (Fig. 2 c-d).

Bait uptake

Uptake of baits was observed on days 4, 8 and 14 after distribution in 4 different sites by the respective hunter teams. Each team had marked 60 baits by placing numbered wooden sticks directly next to the baits. A search was made for vaccine capsules around the original bait sites to examine the proportion of punctured versus intact capsules and to determine, if possible, the animal species in question on the basis of the tooth marks.

To determine the proportion of bait uptake from cadavers sent to the laboratory, a mandibular bone was removed and cut through at the level of the third premolar tooth with an ordinary plaster saw (Aesculap). The cut surface was smoothed on a grinder, whereafter a 2-3 mm thick slice, including a section of the tooth, was cut out. For tetracycline detection a Leitz-Dialux 22 microscope equipped with a l-Lambda Ploemopak epi-illuminator and filterblock D was used.

Serology

Collection of samples was started 1 month after bait distribution and was continued for 6 months, i.e. until a 2nd bait distribution campaign was carried out on 21-22 April 1989. Samples to be tested for antibodies were taken from either blood-containing liquid in the thoracic cavity or blood in the heart cavities from carcasses of raccoon dogs, red foxes and badgers sent to the laboratory from the vaccination area. Control samples were also collected from areas further than 100 km of the vaccination area. The samples were centrifuged to clarify the serum and

Table 1. Numbers of animals examined and rabies cases recorded (I) during 1.1.1988 - 31.12.1989 in the whole country and (II) during the epidemic (1.4.1988 - 28.2.1989) in the municipalities affected.

Species	Examined		Positive	%Pos.	
	I	II		I	II
Wild animals:					
Raccoon dog	1215	229	48	4.0	21.0
Red fox	591	89	12	2.0	13.5
Badger	112	15	2	1.8	13.3
Other mustelids 1)	96	12			
Large predators 2)	19	1			
Artiodactyls 3)	8	2			
Lagomorpha 4)	42	11			
Rodents 5)	82	12			
Bats	18	2			
Others 6)	12	3			
Domestic animals:					
Cat	518	67	2	0.4	3.0
Dog	199	16	1	0.5	6.3
Cattle	41	3	1	2.4	33.3
Fur animals 7)	46	7			
Others 8)	16	2			
Totals	3015	471	66		

1) Mink, pine marten, pole cat, ermine, weasel, otter.
2) Bear, wolf, lynx.
3) Wild boar, moose, fallow deer.
4) European brown hare, mountain hare.
5) Muskrat, squirrel, flying squirrel, beaver, brown rat, mice, voles.
6) Hedgehog.
7) Red fox, blue fox, silver fox, raccoon dog.
8) Horse, sheep.

stored at –20 C for further use. The antibody level was determined by a microscale modification (WHO Reference Centre, Tübingen) of the RFFIT-test (*Smith et al.* 1973). The samples were titrated with international reference serum 0.5 IU/ml (WHO, Tübingen) in 3-fold dilutions after predilution 1:10. In the test used the titer 0.5 IU/ml corresponded to dilution 1:30. If the result was inconclusive, the titer was estimated to be in the middle of the 2 dilutions. The corresponding serological test as applied at the WHO Reference Centre, Tübingen, FRG, was relied on as a reference test when determing agreement between the teste (e.g. *Martin et al.* 1987). The result of the comparison supported the recording of the the dilution 1:30 as negative in this field study, although designated positive in Tübingen. The kappa-value of agreement between the serological tests, calculated from 92 field samples studied in both Tübingen and Finland, was 0.53. The

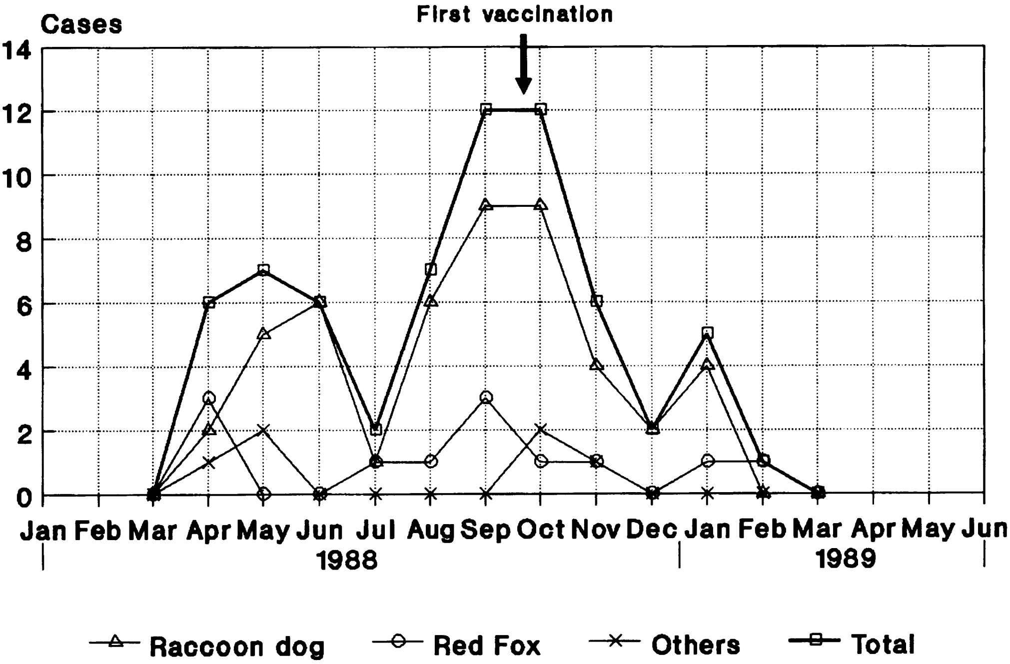


Figure 1. Rabies cases recorded monthly from April 1988 to February 1989.

= total = raccoon dog = red fox = others

(2 badgers, 2 cats, 1 dog, 1 bull calf).

geometric mean titre and 95% confidence limits were calculated from $\log_{10}$ transformed data, which were then retransformed into international units. Parametric statistical tests (t-test and ANOVA) were applied on $\log_{10}$ transformed IU/ml values.

Results

Between the beginning of 1988 and the end of 1989, 2195 wild animals and 820 domestic animals from the whole country were examined for rabies. Forty-eight raccoon dogs, 12 red foxes, 2 badgers, 2 cats, 1 dog and 1 dairy bull were found positive (Table 1). All the virus isolates reacted positively with the monoclonal antibodies W-239, W-187.5 and P-41, thus indicating that they contained a nucleocapsid typical of arctic strains of rabies virus. The 1st case was diagnosed on 8 April 1988 and the last on 16 February 1989. The peak of the cases was observed in September and October 1988 (Fig. 1).

In April, May and June 1988 all the cases, except the 2nd (a dog), were concentrated in an area near the town of Kuusankoski. The greatest distance between cases was 37 km (excluding the dog, 27km) (Fig. 2 and 2a). In

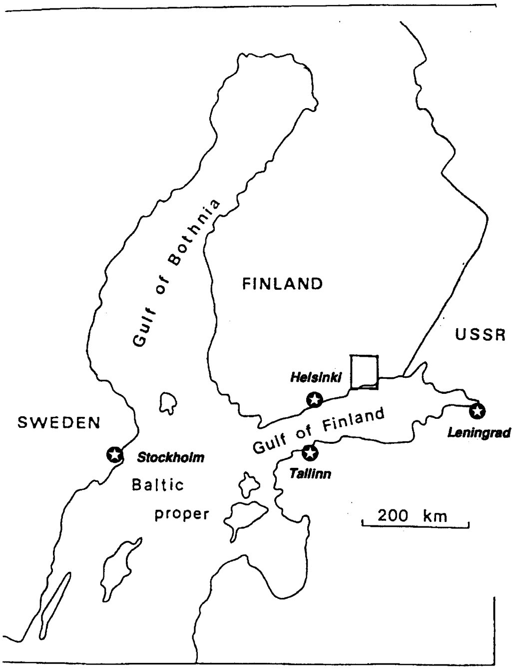


Figure 2. Location of the epidemic area □.

July and August no expansion was observed but in September 1988, a westward spread was noted into areas south of the population centre of Nastola. One case was also recorded on the eastern side of the Kymi River. The greatest distance between the new cases was 42 km (Fig. 2b). In October, November and December 1988, cases still occurred in the primary area on the western side of the Kymi River and in areas south of Nastola. In the beginning of the epidemic, the expansion southwards along the western river bank was slow, but it gained speed from October on, when 1 case was recorded on the southern bank of the western river arm. In December 1 case occurred on the eastern side of the eastern river arm. The greatest distance between cases during this period was 62 km (Fig. 2c). During the 2 last months of the epidemic (January and February 1989) there was only 1 case near Kuusankoski and other cases occurred between the eastern and western arms of the Kymi River, near the Gulf of Finland. The greatest distance between cases was 53 km. There were also 1 case on an island at the southern rim of the coastal ice (Fig. 2d). The greatest distance between all the cases recorded during this epidemic was 67 km.

Uptake of baits was observed on days 4, 8 and 14 after vaccination in 4 different distribution sites and the mean cumulative percentage of baits taken by predators were 12, 31 and 51, respectively. The total uptake of baits in 14 days varied between 38 and 72%. The highest uptake was recorded in vicinities of brooks and lakes, along small roads and field and forest edges. Baits placed in deep forests and open fields mostly remained untouched. The difference probably reflects habitat preferences of the raccoon dog. On an average, 42% (range 13-54%) of the capsules of the eaten baits (N=123) were found. The capsules (N=51) were sent to the laboratory from 3 distribution sites only and (N=28) 79% of these were punctured (range 67-85%). The puncture marks were subjectively examined to try to determine the species in question, and the major bait consuming species was judged to be the raccoon dog. Of the animals examined during the whole follow-up period, 72% of the raccoon dogs (N=126), 66% of the red foxes (N=56) and 13% of the badgers (N=16) were judged to be seropositive, with titers 1.0 IU/ml or greater (Table 2 and Fig.3). The difference between raccoon dogs and red foxes in the relative numbers of seropositive animals is not statistically significant (X^2 = 0.70, p= 0.40). Of the seropositive animals, the proportion of animals with titers ≥ 3.0 IU/ml was higher in raccoon dogs (73%) than in red

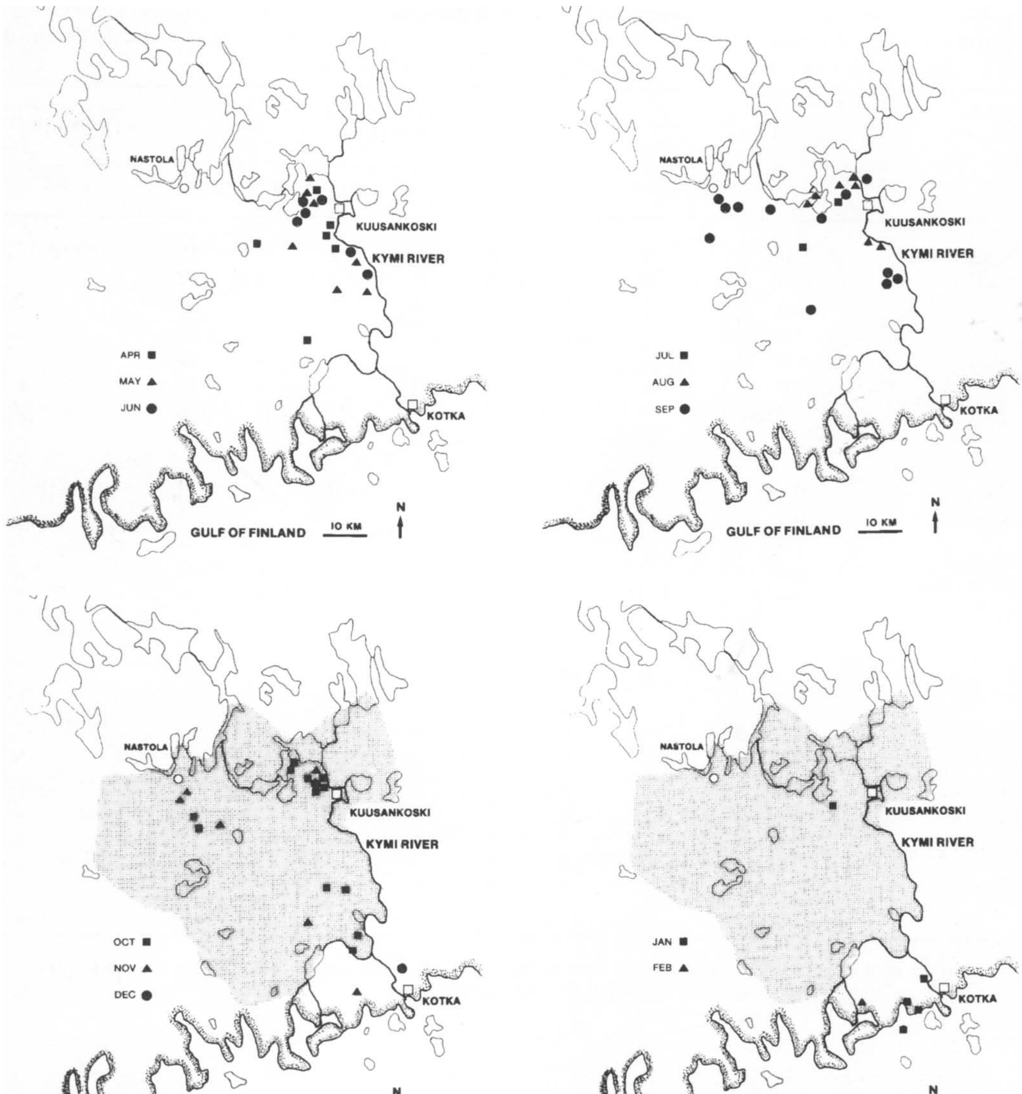
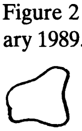


Figure 2 a-d. Temporal and spatial distribution of rabies cases observed between 8 April 1988 and 16 February 1989.

 = lakes  = rivers

□ = the towns of Kuusankoski and Kotka

O = the population center of Nastola.

The area vaccinated (2625 km^2) is shaded. The terrestrial bait distibution was performed by hunters on 24 September 1988 in an area of 2400km^2 (15 baits/km^2). In the westernmost area of 225 km^2 the baits were distributed by air (20 baits/km^2) on 11 October 1988.

Table 2. Antibody titers and tetracycline positivity of specimens examined from vaccination area. Collection of specimens was started 1 month after the vaccination and continued for 6 months. Blood samples found to be toxic or without a corresponding bone sample were excluded from the analysis.

Species	Titer IU/ml	No. with indicated Ab-titer on month after vaccination							No. of titers ≥ 1.0 IU/ml (%)
		[2)]1-2	2-3	3-4	4-5	5-6	6-7	sum	
Raccoon dog	0.08[1)] 0.17	0	0	0	0	9	9	18	91 (72.2)
N=126	0.33 0.5	6	3	3	0	3	2	17	
	1.0 1.5	11	2	6	1	2	3	25	
	3.0 4.5	9	2	4	1	3	1	20	
	9.0 13.5	17	0	5	8	0	0	30	
	27.0 40.5	6	0	3	2	3	2	16	
	Sum	49	7	21	12	20	17	126	
	% Tetra+	88	86	76	58	65	88		
Red fox	0.08 0.17	0	1	1	0	0	0	2	37 (66.1)
N=56	0.3, 0.5	5	6	3	0	2	1	17	
	1.0 1.5	2	10	1	2	2	1	18	
	3.0 4.5	1	5	1	2	3	0	12	
	9.0 13.5	2	0	0	3	1	0	6	
	27.0 40.5	1	0	0	0	0	0	1	
	Sum	11	22	6	7	8	2	56	
	% Tetra+	91	82	83	86	100	100		
Badger	0.08 0.17	1	0	0	0	6	3	10	2 (12.5)
N=15	0.33 0.5	1	0	0	0	1	2	4	
	1.0 1.5	0	0	0	0	1	0	1	
	3.0 4.5	0	0	0	0	1	0	1	
	9.0 13.5	0	0	0	0	0	0	0	
	27.0 40.5	0	0	0	0	0	0	0	
	Sum	2	0	0	0	9	5	16	
	% Tetra+	50				33	60		

1) Samples with less than 50% reduction in fluorescing cells in the 1st dilution are recorded as 0.08 IU/ml.
2) 1-2 indicates ≥ 1 and < 2, with the accuracy of 1 day, etc.

foxes (51%) (X^2 = 5.29, p< 0.05). When the results are grouped into 3 time periods using the upper limits 3, 5 and 7 months after vaccination (with the accuracy of 1 day), the proportion of seropositive raccoon dogs and red foxes in the 1st period are 92% (N=56) and 64% (N=33), in the 2nd period 91% (N=33) and 69% (N=13) and in the last period 38% (N=37) and 70% (N=10), respectively. The difference is statistically significant only in the 1st time period (X^2 = 4.74, P< 0.05).

The means and 95% confidence intervals of the $\log_{10}$-transformed titers of raccoon dogs and red foxes, grouped in the 2 month periods, are shown in fig. 4. In the whole follow-up period raccoon dogs had significantly higher titers than red foxes, both when nega-

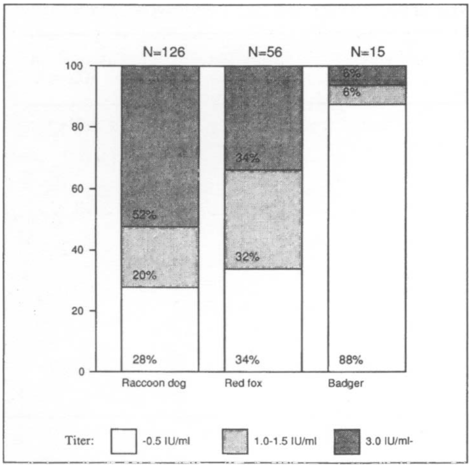


Figure 3. Percentage distribution of classified titers in the raccoon dog, red fox and badger.

tive titers are included (t= –1.979, p= 0.05, df= 180) and excluded from (t= –3.309, p= 0.001, df= 127) the analysis. When all the samples are grouped into the 3 periods, raccoon dogs had significantly higher titers than red foxes during the 1st period (t= –3.871, p< o.oo1, df= 86) and during the 2nd period (t= –2.378, p< 0.05, df= 44). During the last period raccoon dogs had lower titers than red foxes, but the difference was not statistically significant (t= 1.316, p= 0.09, df= 45).
The highest antibody levels of both raccoon dogs and red foxes were recorded in the 5th month after vaccination and a drop in the titers of raccoon dogs took place in the 6th month after vaccination. The titers of raccoon dogs in the last period differed significantly from the titers in both the 1st and the

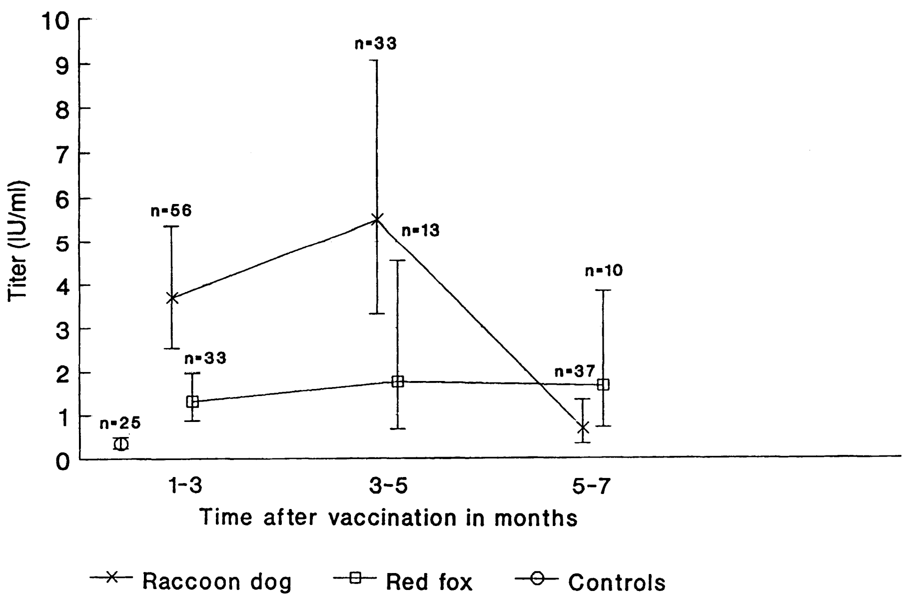


Figure 4. The means and 95% confidence intervals of titers (≤0.05 IU/ml included) in raccoon dogs and red foxes, grouped in 2 month periods. The bar at left represents the 95% confidence interval of 25 control samples (21 raccoon dogs, 3 red foxes and 1 cat) obtained more than 100km from the vaccination area. The follow-up period lasted from 24 October 1988 to 21 April 1989.

Table 3. Agreement of seroconversion and mandibular tetracycline in raccoon dogs, red foxes and badgers.

Species	Ab IU/ml	Tetra+	Tetra-	% Tetra+	Agreement Kappa
Raccoon dog	Ab+	75	16	78.6	0.12
N=126	Ab-	25	10		
Red fox	Ab+	30	7	87.5	–0.22
N=56	Ab-	19	0		
Badger	Ab+	1	1	46.7	0.02
N=15	Ab-	6	7		
All	Ab+	107	25	78.7	0.07
N=198	Ab-	49	17		

+ = ≥ 1.0 IU/ml.

2nd period (F=17.40, P< 0.001, df= 125, ANOVA, one-way and Tukey-Kramer method). No significant difference in the titers of raccoon dogs was observed between the 1st and the 2nd periods. No significant difference in the titers of red foxes was observed between the 3 periods (F= 0.32, df= 55, ANOVA, one-way and Tukey-Kramer method). When the titers of the control animals are included in the analysis, the titers of raccoon dogs differ statistically from the titers of the control animals during the 2 first periods (p< 0.001) but not in the last period (p< 0.20, ANOVA, one-way and Tukey-Kramer method). The titers of red foxes differ statistically from the titers of the control animals during all the periods (p< 0.001 in the 1st and 2nd period, p< 0.01 in the last period, ANOVA, one-way and Tukey-Kramer method)

There was no significant difference between raccoon dogs and red foxes in the proportions of tetracycline-positive animals in either the whole follow-up period X^2= 1.73, p= 0.19) or any of the 3 time periods. The agreement between positive titers and tetracycline-positive bone samples was low; the kappa-values for raccoon dogs and red foxes were 0.12 and –0.22, respectively (Table 3).

Discussion

The spread of rabies to Finland over the eastern and south-eastern national border had been feared for a long time, because sylvatic rabies was known to occur on the Kola Peninsula and in Estonia (*Cherkasskiy* 1980, 1988). As a precautionary measure, all dogs used for hunting in the south-eastern border districts of Finland have been vaccinated since 1981. Little was known in Finland about the rabies situation in the Karelian Autonomous Soviet Socialist Republic or the Leningrad area before 12 March 1988, when rabies was reported (Leningradskaja Pravda) to occur in the wildlife in the Leningrad area. After that the surveillance for rabies was intensified in Finland.

When spreadind into Finland in 1988, rabies appears to have by-passed the dog vaccination belt, most likely along the coastal ice, being carried by wolves incubating rabies. Spread of rabies by land through an animal to animal chain from behind the south-eastern border seems unlikely, because no rabies cases have been observed within 60 km of the national border in spite of intensive sampling. Only 2 cases have occurred on the eastern side of the Kymi River, both less than 5 km from the river and obviously connected with the epidemic west of the river. According to a 3rd and perhaps the least plausible hypothesis, rabies could have entered Finland with raccoon dogs hiding in goods wagons from the USSR, since the animals have occasionally been observed to behave in this way.

Rabies virus strains considered to be arctic owing to the positive reaction with MAb P-41 have previously been found in terrestrial animals in the Soviet Union, on Spitzbergen, Norway, in Greenland and Alaska (*Schneider et al.* 1985), in the North Western Territories of Canada, in Quebec and Ontario (*Charlton et al.* 1982, *Webster et al.* 1986), and in parts of the north-eastern United States (Smith et al. 1984). In the Soviet Union over 60 virus isolations from the Arctic regions and over 20 virus isolations from Estonia, and the Pskov and Leningrad regions have been positive to the monoclonal antibody P-41 (*Selimov et al.* 1990), hence the occurrence of such a strain in Finland is understandable.

In Finland the raccoon dog population increased greatly from the end of the 1960s, and reached its present level around the mid-1980s. In the rabies area, however, population growth started even earlier, in the mid-1960s, and had nearly attained its present level as early as the mid-1970s. The population densities and turnover of raccoon dogs have been evaluated in Finland by *Helle & Kauhala* (1987, 1989) on the basis of the game harvest and studies on the reproductive capacity, age structure and relative abundance in different regions. According to these calculations, the population density of the raccoon dog in the outbreak area is approximately 0.3 animal/km^2 in the spring and 1.0 animal/km^2 in the autumn. The population density of the red fox is about 0.15 and 0.3 animal/km^2, respectively (*Helle & Kauhala,* pers.com.). Much greater population densities of red foxes have been estimated from hunting statistics in Central Europe, for instance 0.8-6.0 fox/km^2 in flat and hilly terrain in the absence of rabies or strict control measures (*Steck & Wandeler* 1980). In Hessen (*Wachendörfer et al.* 1985) hunting has yielded 1.0-5.4 foxes/km^2 depending on the year.

As a treshold level for rabies to maintain itself in a fox population 0.25 fox/km^2 (*Toma & Andral* 1977) and 0.3-0.4 hunted fox/km^2 (*Steck & Wandeler* 1980, *Steck et al.* 1982 b) have been proposed. The population density of the red fox in Finland is estimated to be lower. No estimations of the threshold level for the raccoon dog have been reported and the threshold levels for mixed populations of raccoon dogs and red foxes are unknown. The above estimates by *Helle & Kauhala* allow the preliminary conclusion that for a mixed raccoon dog- red fox population in Finnish conditions the density level of 0.45-1.3 animal/km^2 is above the treshold. The population densities of the component species need not, however, be directly additive in determination of the threshold level for a composite population.

The raccoon dog hibernates for a variable time during winter, depending on the snow depth and the length of winter. This phenomenon may be of importance in the epidemiology of rabies, because the contact rate may fall to the level at which the disease disappears. The animals incubating rabies may become ill and die while hibernating or leave the den without meeting a new victim. On the other hand, the same den usually contains more than 1 raccoon dog and some-times also badgers, so that an animal becoming ill during winter can infect its fellow hibernaters. Unverified theories (*Bacon & MacDonald* 1980) of longer incubation periods in hibernating raccoon dogs have been presented, which suggest that infected animals could survive the winter and transmit the disease to new victims in the spring.

According to Swiss observations (*Kappeler et al.* 1988), the most important natural or artificial obstacles to the spread of rabies are, in descending order: lakes, high mountain ranges, large and rapidly flowing rivers, major cities and freeways lined with game-proof fences. In Finland also, the importance of lakes and the Kymi River in preventing and directing the expansion of the epidemic was obvious. The raccoon dog is known to favour lowlands with deciduous or mixed forests often bordering rivers, while coniferous forests, swamps or open fields are less favoured. Consequently, the population densities can be expected to be high along rivers, and the rivers will direct migration. The findings on bait uptake in different habitats seem to support this reasoning.

For organizing a field trial under Finnish conditions, the Bavarian model was found both practical and effective. The baits could be distributed cheaply, rapidly and evenly by experienced hunters at predetermined sites, where they were most likely to be found by the target animals. The involvement of hunters from the very beginning of the field trial obviously also increased the number of cadavers obtained for follow-up examinations. The planning and final terrestrial distribution of baits were not regarded as being too laborious, by either the responsible authorities or the voluntary helpers from the local hunting clubs. The area vaccinated by air was too small for comparisons between the 2 methods of bait distribution.

In a previous laboratory experiment raccoon dogs were shown to develop neutralizing antibodies and immunity to rabies, when vaccinated with SAD-B19 virus (*Tanskanen et al.,* unpublished). In view of the results of this experiment, $5*10^7$ $TCID_{50}$/ml was chosen as the concentration of vaccine virus used in the field trial. The results of this 1st campaign of the field trial show that the seroconversion rate of raccoon dogs is comparable to that of red foxes in Europe and agrees with the previous seroconversion rate of 3 out of 4 obtained from field trials in the Federal Republic of Germany (*Schneider et al.* 1988).

Interesting phenomena are the long occurrence of antibodies in both raccoon dogs and red foxes as compared to the results of previous laboratory experiments with SAD-B19 attenuated rabies vaccine virus administered by oral route to different animal species, and the higher average titers among raccoon dogs as compared to red foxes during the 1st 5 month after vaccination. In a laboratory experiment with red foxes (*Schneider & Cox* 1983) the antibody titers dropped below the selected level of positivity in 2 of 5 foxes within 6 months, when the vaccine concentration was $2*10^7$ $TCID_{50}$/ml, and a drop in titers was already evident after 2 months. None of 10 raccoons *(Procyon lotor)* vaccinated with baits containing 1.8 ml ($1*10^7$ $TCID_{50}$/ml) of vaccine had detectable antibodies left 4 months after vaccination, though their titers had shown an initial rise (*Rupprecht et al.* 1989), and no antibodies were detected in striped skunks (*Mephitis mephitis*) vaccinated with baits containing even 4 ml ($1*10^{9.5}$ $TCID_{50}$/ml) of vaccine (*Rupprecht et al.* 1990). Our field trial results showed remarkably high titers in both raccoon dogs and red foxes as long as 4-5 months after vaccination. The tetracycline results do not support the conclusion that the drop in average titer among raccoon dogs in March was due to animals dispersing from outside the vaccination area at the beginning of the mating season.

Ingestion of the baits was estimated in the present field trial by tetracycline-positive bone and tooth samples. However, the agreement of the tetracycline and antibody results was unexpectedly low. One explanation of the discrepancy can be that some animals ate the baits after the vaccine virus concentration had dropped below the immunizing level, or that some animals had a poor response to the antigen. Also toxic and nonspecific factors in blood samples which at times were rather spoiled could affect the results. The possibility of failure to detect tetracycline in old animals, or the existence of other sources of tetracycline cannot be excluded, either. In addition, some animals may simply have eaten the bait mass without puncturing the capsule or punctured the capsule without eating the bait mass.

It remains unclear, whether the diminution of the animal numbers by increased hunting and rabies itself and the relatively long hibernation period of raccoon dogs would have sufficed to cut the infection chain to the effect that rabies had disappeared. The observed rabies cases ceased also from the limited region outside the vaccination area about 2 months before the 2nd vaccination campaign was conducted in that area. However, it is not known if the disease still existed unnoticed in that area, and if the spring vaccination had a final effect to the ending of the epidemic. If the above estimate of the spring density of 0.45 animal /km^2 for the combined population is accepted, the immunization results of the present vaccination trial, 66-72%, would have decreased the density of sensitive animals well below the threshold level proposed for the red fox population in Central Europe. Also, a strong argument for the decicive role of the oral vaccination is the endemic status of wildlife rabies in Estonia, on the other side of the Gulf of Finland, where oral vaccination has not been implemented.

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Sammanfattning

En epidemi av sylvatisk rabies i Finland - deskriptiv epidemiologi samt resultat av oral vaccinering.

Då rabies i april 1988 återuppträdde i Finland hade landet varit rabiesfritt sedan 1959. Bilden av sylvatisk rabies stod snart uppenbar. Dess främsta bärare och offer var mårdhunden *(Nycrereutes procyonoides)*. Mellan 8.April 1988 och 16.Februari 1989 registrerades 66 virologiskt verifierade fall (48 mårdhundar, 12 rödrävar, 2 grävlingar, 2 katter, 1 hund och 1 ungtjur). Det till 17oo km^2 uppskattade smittområdet ligger i sydöstra Finland. Det längsta avståndet mellan registrerade fall var 67 km. Med monoklonal antikroppsteknik visades virusstammen reagera positivt med Mab P-41 och således vara av arktisk typ.

I september 1988 inleddes ett fältförsök avseende oral immunicering av markrovjur mot rabies med s.k. Tübingen vaccinebeten för räv och distribution av betena enligt s.k. bayersk modell. Varje bete innehöll modifierat, levande rabiesvirus (SAD B19) i en dos av 5 x 10^7 $TCID_{50}$/ml. Under en 6 månaders uppföljningsperiod erhållna resultat visade serokonvation hos 72% av mårdhundarna (N=126), 67% av rödrävarna (N=56) och 13% av grävlingarna (N=16), då titer ≥1.0 IU/ml bedömes som seropositiva. Under hela uppföljningsperioden kunde ingen statistiskt signifikant skillnad påvisas mellan mårdhund och räv angående vare sig frekvens av serokonvation eller upptagning av tetracyklin från betena. Hos både mårdhundar och rävar påvisades anmärkningsvärt höga antikropps-titer under 4-5 månader efter vaccineringen. Proportionen av seropositiva individer med titer ≥3.0 IU/ml var högre bland mårdhundarna (73%) än bland rödrävarna (51%), x_2=5.29, $p< 0.05$). Försöket visar, att mårdhunden kan immuniceras mot rabies i fält med vaccin och beten som ursprungligen utvecklats för bekämping av sylvatisk rabies hos rödräv.

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