

EXPERIENCES WITH RABIES ERADICATION PROGRAMS*
ISKUSTVA SA ISKORENJIVANJEM BESNILA U SLOVENIJI**P. Hostnik, Darja Barlič-Maganja, J. Grom, T. Malovrh, A. Bidovec****

Oral vaccination as a method of rabies eradication in the field was first started in Switzerland in 1978 and after 1984 several other EU countries followed this practice. Due to oral vaccination some European countries are now rabies-free in terrestrial animals.

In Slovenia, after the first experimental oral vaccination and study of vaccination models from 1988–1992, the spring-autumn campaigns have been carried out since 1995. The model of oral vaccination of wildlife requires 16–20 baits per km² in the vaccination area. The baits were distributed by plane. They were dispersed from a height of 300–500 m. The aeroplanes' paths were 1000 metres apart. In the vaccination campaigns two vaccines were used. Lysvulpen®, produced by the Bioveta company at the Czech Republic, was laid down in the southwestern part of the country, and Fuchsoral®, produced by the German company Impfstoffwerk Dessau-Tornau, was placed in the eastern part of Slovenia. A rapid decline of rabies was evidenced from 1995 to 1999, when the oral vaccination program in the whole territory using the aircraft baits distributing system was practiced. In 1999, only 6 rabies cases were laboratory-confirmed, whereas in 1995, 1089 rabies cases were documented. Of the 14 rabies cases detected in 1998, 12 were found as an island in a circle with a radius of 30 km in the centre of the vaccinated area. In 2000 and 2001, rabies incidence increased again, so it was decided to change the baits distribution system in the year 2001. The vaccination by crossing flights in certain areas was introduced. In the next year (2002), after changing the vaccination strategy, positive cases rapidly dropped and only 15 cases in 2002, and 8 cases were found in 2003, near the non-vaccinated border with Croatia.

Key words: rabies, eradication, Slovenia

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Introduction / Uvod

Rabies is a zoonotic disease of mammals, caused by infection with a negative-strained RNA virus belonging to the *Lyssavirus* genus of the *Rhabdoviridae* family, mainly transmitted via saliva following a bite from an infected animal [1]. In central and southeast Europe, the red fox (*Vulpes vulpes*) from the wild reservoir species is the main source of infection [2, 3]. In Slovenia, only the urbane rabies was present at the beginning of the 20th century. The sylvatic rabies was introduced in 1973, and the whole country had become infected by the end of 1980. The infection of domestic animals was always connected to the epizootics in wildlife. Immunization of dogs is obligatory and advisable for cats. In the period from 1997 to 2003, when the trend of epizootics was high, cattle, sheep and horses in pasture were also vaccinated against rabies.

Oral vaccination as a method of rabies eradication in the field was first started in Switzerland in 1978, and after 1984, several other EU countries followed this practice. Due to oral vaccination, some European countries are now rabies-free in terrestrial animals; Finland (1991), Netherlands (1991), Italy (1997), Switzerland (1998), France (2000), Belgium (2001) and Luxembourg (2001) [4]. In other eastern and southern European countries, the number of reporting rabies cases has increased [4].

Rabies surveillance in Slovenia / Posmatranje besnila u Sloveniji

A rabid animal was first registered in Slovenia in 1973. It was found in the Prekmurje region. The disease spread in the southwest direction. The nature of this breakout was that it only spread to the river Mura area and didn't go any further [5]. The disease reached its peak in Slovenia in 1979, when it crossed the borders in the upper Sava valley region and in the Carinthian region. In 1980, the first cases were reported on the outskirts of the capital Ljubljana and a year later it spread to the southern parts of the country. Ever since, we have had rabies occurrences among wild animals, some cases even in non-vaccinated domestic animals in the period before oral vaccination was carried out. The Institute for Microbiology and Parasitology at the Veterinary Faculty in Ljubljana annually examines 2,000 – 4,000 samples for the rabies virus. The percentage of positive cases has varied from 20 to 45%. Cyclic oscillations in the numbers of positive cases have been a common feature for Slovenia as well as the rest of Europe. This cycle is repeated every 3 to 5 years and starts with a high occurrence followed with a fall in the number of positive cases and then again the number rises. This is closely associated to the size of the fox population at a given time.

The rabies surveillance has been in effect since the beginning of the 1970's. According to the Slovene legislation the veterinary organisations are obliged to send the animal carcasses with neurological clinical symptoms for laboratory examination. In the territory of 21.000 km² from 1195 to 3787 samples were

collected yearly and tested to rabies antigen. The fluorescent antibody test (FAT) was used as the laboratory technique for rabies diagnosis. The diagnostic technique for rabies is standardised by the Manual of Standards for Diagnostic Tests and Vaccines. Immuno-fluorescent conjugate made by the Bio-Rad Company from France was used.

First period of oral vaccination in Slovenia / Prvi period oralne vakcinacije u Sloveniji

In Slovenia, after the first experimental oral vaccination and study of vaccination models from 1988–1992, the spring-autumn campaigns have been carried out since 1995. A Fuchsoral (Tubingen Institute, Germany) vaccine containing the attenuated SAD-B19 virus (titre 10^{-7} TCID₅₀/ml) raised on BHK21 cell culture was used. Each foil-wrapped ampoule contained 1 ml of vaccine and was covered with meat and fish flour mixed with fat. A biomarker in the form of tetracycline was also added to each bait.

The first oral vaccination in Slovenia was performed in 1988 (Gorenjska region). After carefully considering the geographical layout of Slovenia and examining the results acquired from Austria, West Germany, France and Italy, where this kind of vaccination started in the early eighties, we decided upon the Tubingen model of vaccination. This model requires laying 16–20 baits per km² in the vaccination area. Some areas were laid using the „common sense” method where the baits were hand-laid in areas where foxes were registered. The other areas were laid systematically using a pattern of squares (each square being 250 m wide and 250 m long) in which 16 baits were to be laid (16 baits per km²). The latter proved to be more efficient. The job of laying the baits by hand was given to the game wardens that also registered how many baits were actually taken from the spot where they had been laid (4, 8 and 12 days after the bait placement). The wardens also shot foxes, which were examined to the tetracycline biomarker. The laying of baits was completed in one day. The purpose of the control was to determine the vaccine intake and the vaccination level. Based on positive results from the Gorenjska region, we extended that method of bait laying to all Slovenia. Due to financial reasons, we baited the country gradually, starting at the west and ending in the east. The vaccination was performed in six-month intervals autumn/winter. The vaccination in the springs of 1990 and 1991 was cancelled due to financial difficulties. Each vaccination saw an enlargement of the area by 5 to 10 thousand km² taking into consideration the fox biology and ecological closures. Up to 1992, the entire country was covered in this way.

Oral antirabies vaccination in Slovenia / *Oralna vakcinacija protiv besnila u Sloveniji*

In the following section, we present brief data about oral antirabies vaccination of free living common foxes in Slovenia in the period from 1995 to 2004. The vaccination was performed first in autumn 1995, and then in spring and autumn 1996, in the surface of the whole country. In 1997, the vaccination was not done. Two campaigns per year have been carried out since 1998, the first two years in the whole country, later the vaccination was spread to 18.800 km², but in the year 2000, it was reduced to 12.500 km² while the density of baits increased to up to 23-26 baits/km². The Northwestern part of the country has not been vaccinated since 1999, because no rabies positive cases were found. In the vaccination campaigns two vaccines have been used. Lysvulpen® produced by the Bioveta company from the Czech Republic was laid down in the southwestern part of the country, and Fuchsoral® produced by the German company Impfstoffwerk Dessau-Tornau, was placed in the eastern part of Slovenia. The baits were distributed by plane. They were dispersed from a height of 300 – 500 m. The aeroplanes' paths were 1000 metres apart in a parallel line. The pilots used the GPS navigation system for orientation and the discharge was monitored by a computer. A special computer program named FICO3J® (constructed by computer engineer Aleksander Modic) monitored the route and time of flying and calculated the density of distributed baits. Due to the increased incidence of rabies in 2000, the vaccination strategy in the year 2001 was changed. When a crossing flight instead of a single flight model was introduced, rabies cases dropped again in the year 2002, and positive cases were still found only in the non-vaccinated region near the border of Croatia.

Thermostability of vaccine in the field / *Termostabilnost vaccine na terenu*

In this field study the vaccine was used in Slovenia (Lysvulpen® and Fuchsoral®) both for oral vaccination, of foxes containing the live-modified rabies virus SAD – strain, and the thermo-stability of baits and the virus were observed. The vaccine baits were placed in three different locations: (a) in the open cut-grassing places, where direct sun was possible, (b) in the high grass, and (c) under trees and bushes where direct sunlight was not possible. This study was done during the summer period (from July 6, to August 14, 2003), when maximum daily temperatures were higher than 30°C. Ten baits A and ten baits B in the open land (location a), thirty baits A and thirty baits B in the high grass (place b), and five baits A and B in the shadow (location c) were placed. Baits were dropped directly on the ground and the distances between baits were at least 2 cm.

Results / Rezultati

The Rabies investigation showed that during the last 10 years, rabies prevailed among red foxes (2918 cases, 89,26%), rare rabies positive cases were found in other wildlife animals (194 cases, 8,01%; 68 martens, 58 badgers, 56 roe, 6 polecat, 3 deer, 2 wild boar, 1 lynx), and in domestic animals (158 cases, 4,85%; 81 cats, 59 dogs, 11 cattle, 4 sheep, 2 horses, 1 rabbit) (Table 1). In the period from 1992 to 1996, the territory of Slovenia was heavily affected by rabies. A rapid decline of rabies is evidenced from 1995 to 1999, when an oral vaccination program in the whole territory using the aircraft baits distributing system was practiced. In 1999, only 6 rabies cases were laboratory-confirmed, whereas in 1995, 1089 rabies cases were documented (181-fold decrease) (Table 1). Of the 14 rabies cases detected in 1998, 12 were found as an island in a circle with a radius of 30 km in the centre of the vaccinated area. In 2000 and 2001, rabies incidence increased again. In the next year (2002), after changing the vaccination strategy, positive cases rapidly dropped and only 15 cases were found near the non-vaccinated border with Croatia (Fig. 2). The number of positive cases then declined and 8 positive cases were found in 2003.

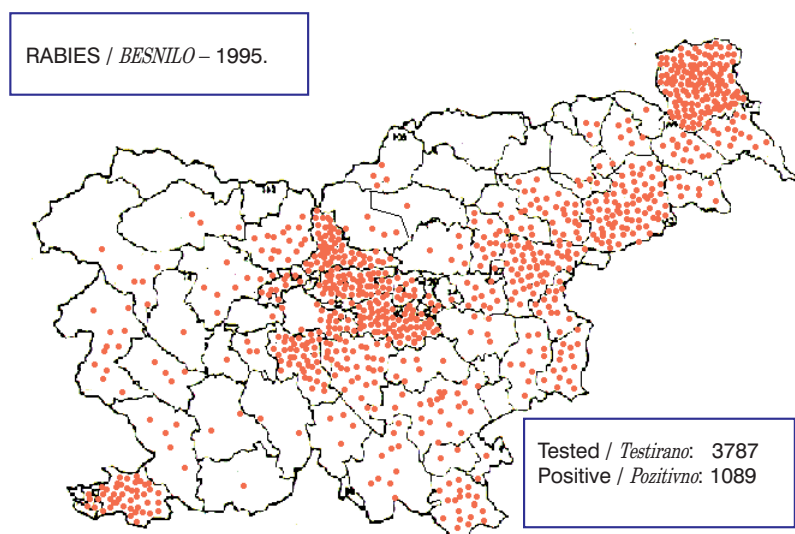


Figure 1. Rabies situation before vaccination
Slika 1. Situacija sa besnilom pre vakcinacije

The stability of the baits matrix and virus titre was recorded over an 8-week summer period (maximum daily temperatures were over 30°C) in relation to temperature, sunlight and rainfall. The SAD Bern vaccine was less stable than the

Table 1. Number of rabies cases from 1992-2002 in Slovenia /
 Tabela 1. Broj slučajeva besnila u Sloveniji od 1992. do 2002.

Year / Godina	No. of tested animals / Broj ispitivanih životinja	Positive animals / Pozitivne životinje	Cats / Mačka	Dogs / Pas	Cattle / Stoka	Sheep / Oveca	Horses / Konj	Rabbit / Zec	Foxes / Lisica	Martens / Lasica	Badgers / jazavac	Roe / Košuta	Polecat / Tvor	Deer / jelen	Wild boar/ Divlja svinja	Lynx / Ris
1992	1365	238	4	6	1				203	10	4	6	3		1	
1993	2019	531	13	8	1	1			476	10	14	7	1			
1994	2632	842	11	12	4		1		753	19	21	19	1	1		1
1995	3787	1089	24	12	4	3	1	1	996	21	11	15	1			
1996	2285	247	17	11	1				208	5		4		1		
1997	1267	29	6	1					20		2					
1998	1382	14							14							
1999	1195	6	1						5							
2000	1509	115	2	2					104		3	2		1	1	
2001	2153	135	3	7					117	3	2	3				
2002	1495	15							14		1					
2003	993	8							8							
Total / Ukupno	22082	3269	81	59	11	4	2	1	2918	68	58	56	6	3	2	1

SAD B19. The loss of virus titre was highest when baits were placed in the sunlight, but when they placed in the shadow, the virus was detected still on the 53rd day of observation. After 24 hours, the bait matrix of Lysvulpen placed in the sunlight showed total destruction and the vaccine container was not covered with fat, paraffin-fish meal matrix. In the case of the vaccine Fuchsoral, the bait matrix was partially destroyed and the vaccine container was visible, too. After 72 hours, the titre of the rabies virus in the vaccine Fuchsoral and after 48 hours the virus titer in the vaccine Lysvulpen were not detectible in the virus isolation test using BHK 21 cell culture, when baits were placed in the sunlight and the maximum daily temperature was 32°C.

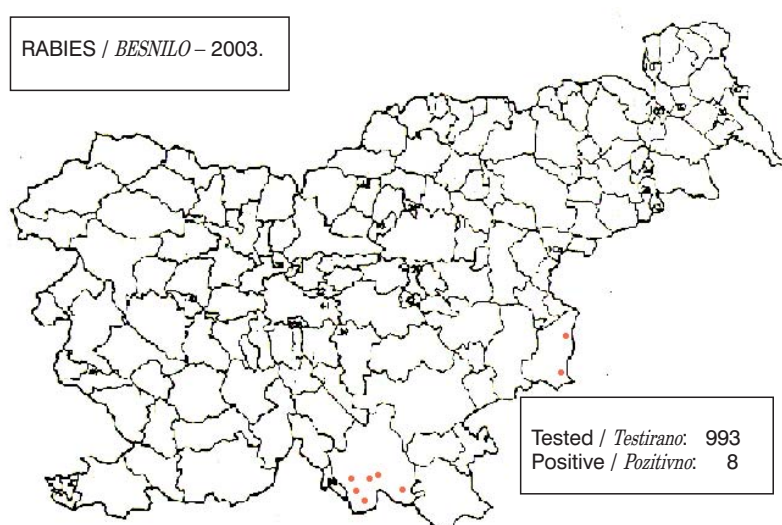


Figure 2. Rabies situation 8 years later
Slika 2. Situacija sa besnilom posle 8 godina

Discussion / *Diskusija*

The evaluation of animal rabies cases in Slovenia during the last decade indicates that foxes represent the main source of infections among wild animals, and cats and dogs among domestic animals. Before the vaccination program of wildlife, rabies was detected all over Slovenia. After the vaccination of wildlife, the number of laboratory-confirmed rabies cases in animals in Slovenia decrease rapidly. A table 1 show that foxes are most affected. Domestic animals infected with rabies represent only a small proportion (4,85%) of all rabid animals

in Slovenia. This low percentage of rabies cases in domestic animals shows that the vaccination program in domestic animal is useful.

During the years 1996 and 1997, rabies was dispersed practically in the whole territory of Slovenia, while in 1998, only 14 positive cases were found. Twelve of them were concentrated within a radius of 30 km in the centre of the vaccinated area. Only 2 cases occurred near the national border connected with the epidemic east. The persistence of rabies cases in the centre of the vaccinated area could be explained by the fact that during all three years of the vaccinating program, aircrafts were not allowed to fly in this area, and the baits were not distributed in this territory. All 6 rabies cases detected during the year 2000 have been found along the national border with Croatia. A significant increase of positive cases in 2000 and in 2001 in the southern part of the country was evidenced. The reason (lower funds) for that increase could be the fact that in the year 1999, lower baits were distributed (13 – 15 baits/km²) in this territory than in the years before (15 – 17 baits/km²). This territory is densely covered with forests and rich biotypes favourable to natural rabies vectors/reservoirs. After the increase of baits density per km² (23 - 26 baits/km²) the number of rabies positive animals decreased. In the year 2002, 15 rabies and in 2003, 8 rabies cases were confirmed. All were located near the Croatian border, except one badger, positive in the IF test, which was shot in the unvaccinated area. Later, this positive result in the IF test was not confirmed by the RT-PCR test.

We could notice that when 25 baits per km² were laid by single flight in parallel lines, the fox had less chances to find the bait. When the crossing flight distribution was used, the baits were more dispersed and more accessible for foxes. This strategy using the raster model for baits distribution was found suitable especially when a geographical configuration with high hills and deep valleys was present. After the introduction of this distribution model the number of rabies cases declined in 2002 and 2003.

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References / *Literatura*

1. Bourhy H., Kissi B., Audry L., Smreczak M., Sadkowska-Todys M., Kulonen K., Tordo N., Zmudzinski J. F., Holmes E. C.: Ecology and evolution of rabies virus in Europe. *J Gen Virol*, 80, 2545-2557, 1999. - 2. Lontai I.: The current state of rabies prevention in Europe. *Vaccine*, 15 (suppl.), 16-9, 1997. - 3. OIE (Office International des Epizooties): Multi annual Animal disease status. Europe/Rabies, 1-12, 2000. - 4. Potzsch C. J., Müller T., Kramer M.: Summarizing the Rabies situation in Europe 1990 – 2002 from the Rabies Bulletin Europe. *Rab Bull Eu*, 4, 11-17, 2002. - 5. Bidovec A., Železnik Z., Tomašič A.: Uporaba peroralne vakcinacije za preprečevanje širjenja steline v Sloveniji. Prvi veterinarski kongres, Portorož, 465-471, 1993.

SRPSKI

ISKUSTVA SA ISKORENJIVANJEM BESNILA U SLOVENIJI

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Oralna vakcinacija je prvi put započeta kao metoda za iskorenjivanje besnila na terenu u Švajcarskoj 1978, a posle 1984. godine nekoliko zemalja Evropske unije je takođe uvelo ovu praksu. Zahvaljujući oralnoj vakcinaciji, u nekim evropskim zemljama danas nema besnila kod terestrijalnih životinja.

U Sloveniji, posle prve eksperimentalne oralne vakcinacije i ispitivanja modela vakcinisanja od 1988 do 1992, primenjuju se prolećno-jesenje kampanje od 1995. godine. Model oralne vakcinacije divljači zahteva 16-20 mamaca po kvadratnom kilometru u teritoriji na kojoj se obavlja vakcinacija. Mamci su raspoređeni iz aviona. Razbacani su sa visine od 300 do 500 m. Putanje aviona su bile na razmaku od 1000 m. Dve vakcine su korišćene u kampanjama vakcinisanja. Lysvulpen®, proizvod čehoslovačke kompanije Bioveta, postavljen je u jugozapadnom delu zemlje, a Fuchsoral®, nemačke kompanije Impfstoffwerk Dessau-Tornau, postavljen je u istočnom delu Slovenije. Uočen je brzi pad slučajeva besnila od 1995 do 1999, kada je primenjivan program oralne vakcinacije u celoj teritoriji putem raspoređivanja mamaca iz aviona. Godine 1999, laboratorijski je potvrđeno samo 6 slučajeva besnila, dok je dokumentovano 1089 slučajeva besnila 1995. godine. Od 14 slučajeva besnila otkrivenih 1998, 12 se nalazilo kao ostrvo unutar kruga u radijusu od 30 km u sredini teritorije pokriveno vakcinama. Godina 2000. i 2001, pojava besnila je ponovo bila veća, tako da je 2002. odlučeno da se promeni sistem raspoređivanja mamaca. Uveden je sistem vakcinacije putem unakrsnih letova na određenim površinama. Sledeće godine (2002), posle promene strategije vakcinacije, naglo je opao broj pozitivnih slučajeva i bilo je samo 15 slučajeva 2002, a 8 slučajeva 2003. godine i to blizu granice sa Hrvatskom, gde nije obavljena vakcinacija.

Ključne reči: besnilo, iskorenjivanje, Slovenija

РУССКИЙ

ОПЫТЫ С ИСКОРЕНЕНИЕМ БЕШЕНСТВА В СЛОВЕНИИ

P. Hostnik, Darja Barlič-Maganja, J. Grom, T. Malovrh, A. Bidovec

Оральная вакцинация впервые начата как метод для искоренения бешенства на месте в Швейцарии 1978, а после 1984 несколько стран Европейского союза также ввело эту практику. Благодаря оральной вакцинации, в некоторых европейских странах в настоящее время нет бешенства у наземных животных.

В Словении, после первой экспериментальной оральной вакцинации и испытания модели вакцинирования от 1988 до 1992, применяются весенне-осенние кампании от 1995. Модель оральной вакцинации дичи требует 16-20 приманок по квадратному километру в территории на которой совершается вакцинация. Приманки распределены из самолёта. Разброшены с высоты от 300 до 500 м. Орбиты самолёта были на расстоянии 1000 м. Две вакцины использованы в кампаниях вакцинирования. Lysvulpen®, продукт чехословацкой компании Bioveta, поставлен в

югозападной части страны, а *Fuchsoral*®, немецкой компании Impfstoffwerk Dessau-Tornau, поставлен в восточной части Словении. Замечено быстрое падение случаев бешенства от 1995 до 1999, когда применена программа оральной вакцинации в целой территории путём распределения приманок из самолёта. Года 1999, лабораторно подтверждено только 6 случаев бешенства, пока документировано 1089 случаев бешенства 1995. Из 14 случаев бешенства, открытых 1998, 12 находились как остров внутри круга в радиусе от 30 км в середине территории, покрытой вакцинами. Год 2000 и 2001, явление бешенства снова было больше, так, что 2002 решено изменить систему распределения приманок. Введена система вакцинации путём перекрёстных полётов на определённых поверхностях. Следующего года (2002), после изменения стратегии вакцинации, резко опало число положительных случаев и было только 15 случаев 2002, а 8 случаев 2003, а именно близко границы с Хорватией, где не совершена вакцинация.

Ключевые слова: бешенство, искоренение, Словения