The successful eradication of sylvatic rabies by oral vaccination of foxes in Slovenia

Danijela Černe [1], Peter Hostnik [1], Ivan Toplak [1] and Jedrt Maurer Wernig [2]

[1] Institute of Microbiology and Parasitology, Virology Unit, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia

[2] Administration of the Republic of Slovenia for food safety, veterinary sector, and plant protection, Dunajska cesta 22, 1000 Ljubljana, Slovenia

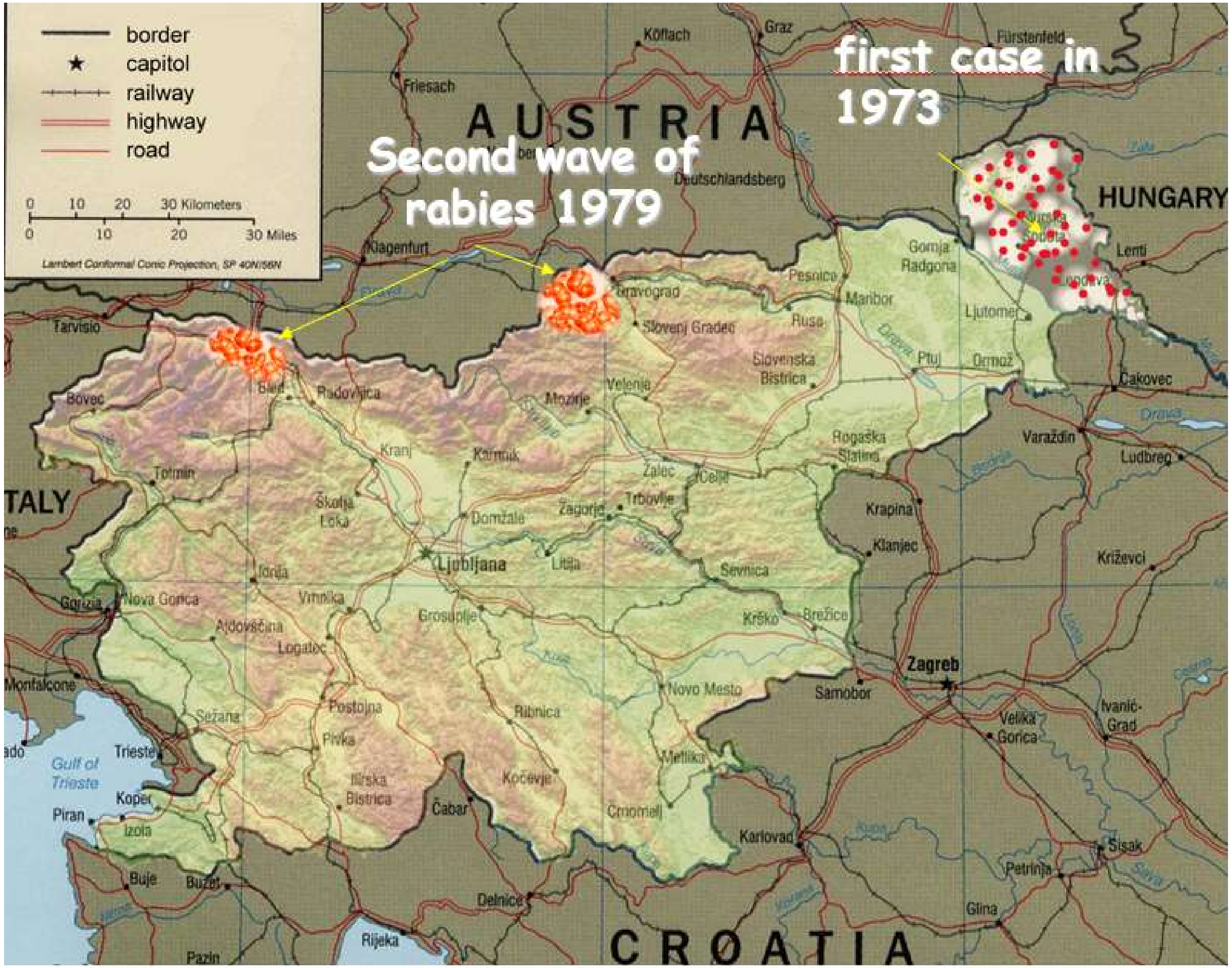
14th Workshop for Rabies, 21-22.june 2023, Ljubljana

1947 – compulsory vaccination of dogs

1950 – last case in humans

1973 – first case of rabies in fox

1988 – carrying out oral vaccination of foxes

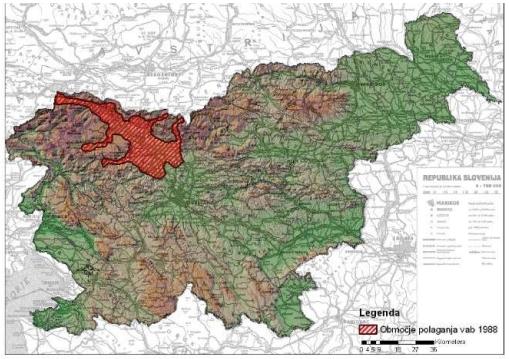

border
capitol
railway
highway
road
0 10 20 30 Kilometers
0 10 20 30 Miles
Lambert Conformal Conic Projection, SP 40N/56N
first case in 1973
Second wave of rabies 1979
AUSTRIA
HUNGARY
CROATIA
ITALY
Graz
Köflach
Fürstenfeld
Friesach
Deutschlandsberg
Klagenfurt
Tarvisio
Dravograd
Slovenj Gradec
Pesnica
Maribor
Ruše
Gornja Radgona
Ljutomer
Lenti
Ptuj
Ormož
Slovenska Bistrica
Čakovec
Bovec
Bled
Radovljica
Mozirje
Velenje
Rogaška Slatina
Varaždin
Ludbreg
Tolmin
Kranj
Kamnik
Žalec
Celje
Škofja Loka
Domžale
Zagorje
Trbovlje
Krapina
Klanjec
Križevci
Ljubljana
Litija
Sevnica
Idrija
Nova Gorica
Gorizia
Vrhnika
Ajdovščina
Logatec
Grosuplje
Krško
Brežice
Zagreb
Samobor
Novo Mesto
Monfalcone
Sežana
Postojna
Ribnica
Velika Gorica
Ivanić-Grad
Pivka
Trieste
Gulf of Trieste
Ilirska Bistrica
Kočevje
Metlika
Koper
Piran
Izola
Čabar
Črnomelj
Karlovac
Sisak
Petrinja
Buje
Buzet
Delnice
Glina
Rijeka
Pazin

anses

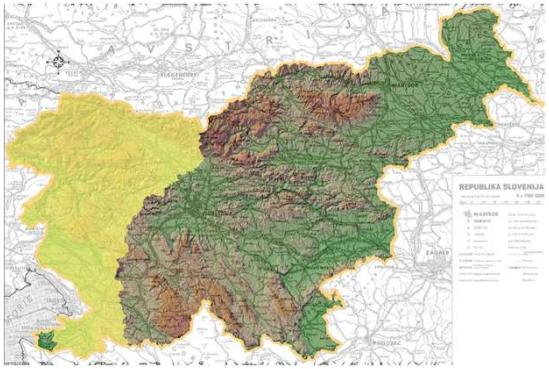
14th Workshop for Rabies, 21-22.june 2023, Ljubljana

Univerza *v Ljubljani*
Veterinarska fakulteta

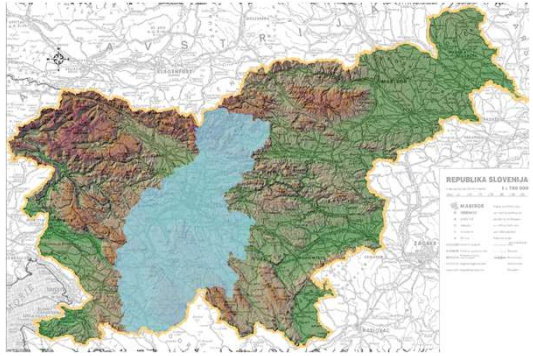
The first ORV in period 1988 - 1994



1988

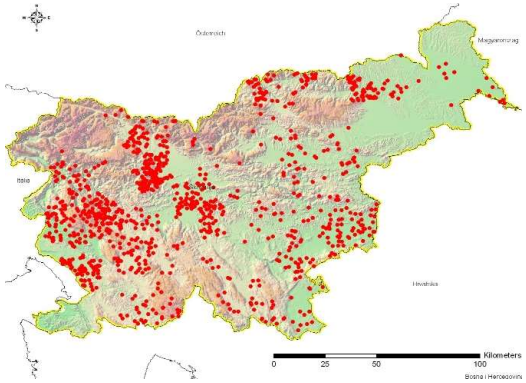


1990

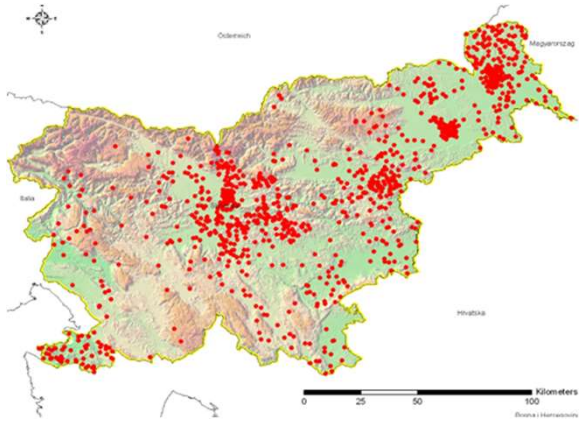


1991

The strategy of gradually pushing rabies from west to east did not work



1988; 1.067 rabies cases



1995; 1.089 rabies cases

Univerza v Ljubljani
Veterinarska fakulteta

2016 – SLOVENIA DECLARED FREE FROM RABIES

1995 – aircraft laying of baits

2000 – cross-flight technique

2013 – last case of silvatic rabies

2018 – last case of vaccine induced rabies

2020 – *Lyssavirus* in *Myotis capaccini*

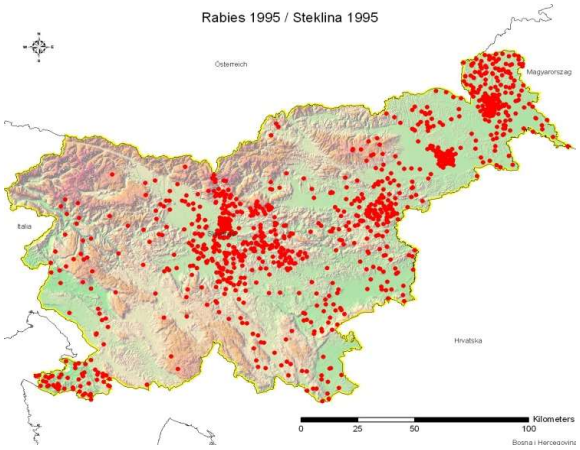
Univerza v Ljubljani
Veterinarska fakulteta

The second ORV in period 1995 - 2020

1995

Tested: 3787

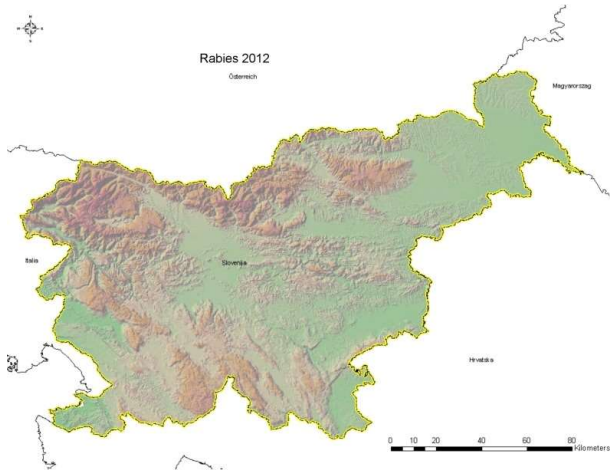
Rabies positive: 1089



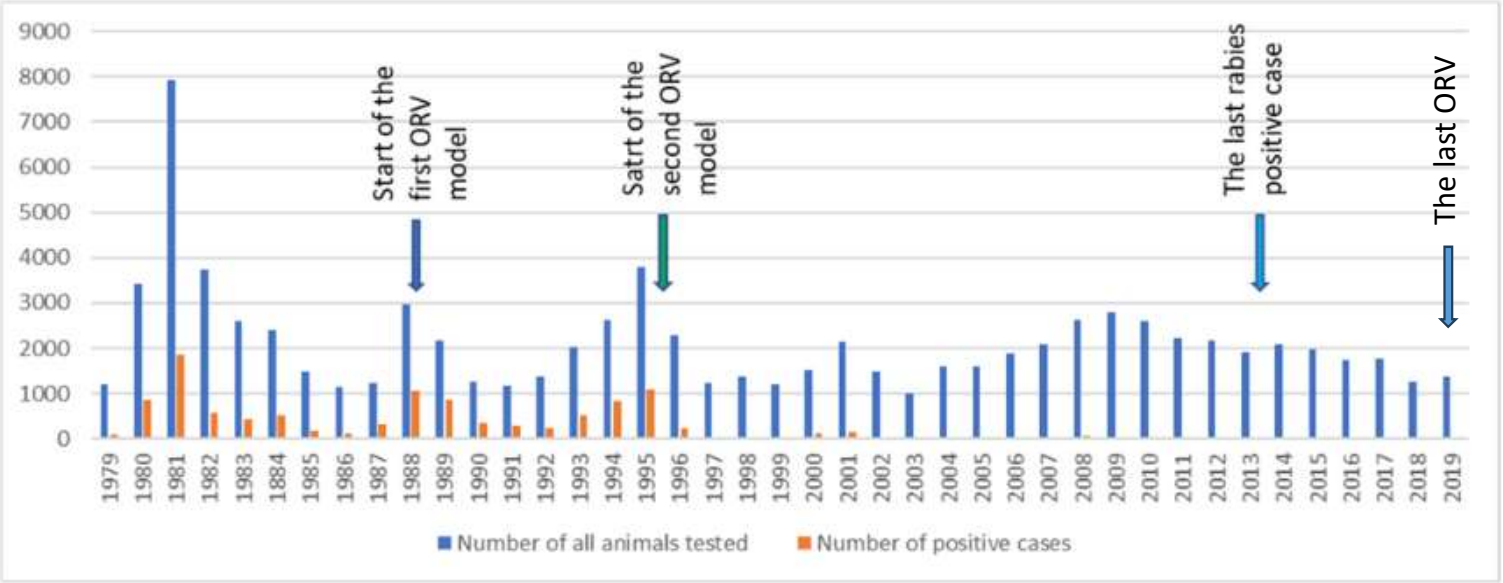
2013

Tested: 1918

Rabies positive: 0



Univerza v Ljubljani
Veterinarska fakulteta

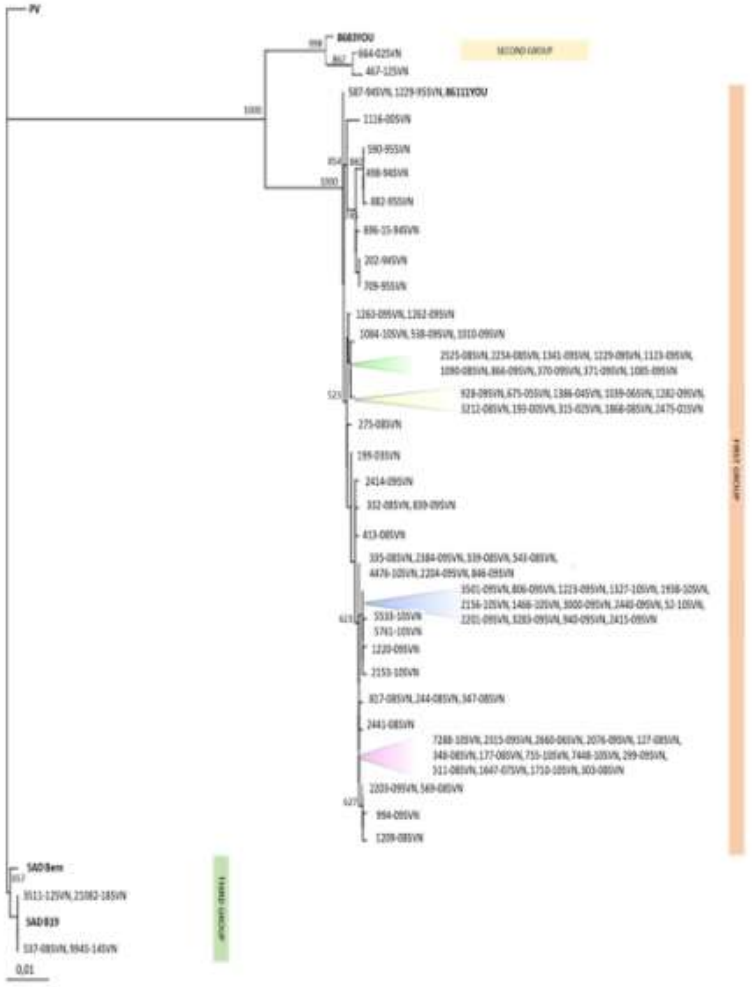


Number of tested animals in period 2010 – 2020: 10,2 animals/100 km2

Genetic Characterizatiom of Rabies virus in Slovenia

(Černe D. et all. *Viruses 2021*, 13, 405)

- 95 rabies positive samples
- strains were clustered into three geneticaly groups
  - Western Europe group
  - Eastern Europe group
  - SAD B19 vaccine-associated group
- The four vaccine-induced cases (3 foxes an 1 marten)
  - unusual behaviour
  - aggressiveness
  - uncoordinated movements
  - not afraid of dogs and people



Bats *Lyssavirus* surveilance in Slovenia

Period 2008 -2012: first active and passive surveillance program for bat lyssaviruses:

913 oropharyngeal swabs
806 blood samples
171 brain samples

Period 2020 – 2022: active and passive surveillance program for bat lyssaviruses:

256 oropharyngeal swabs
235 blood samples
74 brain samples

Retrospective study: dead bats collected for Slovenian museum of natural history in period 2012 – 2019:

225 dead bats
21 bat species

Retrospective bat lyssavirus study: Methods

- 225 dead bats were collected by biologists
- Samples were stored more years in freezer (– 20 °C)
- Identification of bat species using morphological keyes and molecular characterization
- Brain samples were collected through the foramen occipitale
- Samples were tested by pan-lyssavirus specific real-time RT-PCR method: 1.) brain tissue homogenates
  2.) automated RNA extraction
  3.) real time RT-PCR (WHO 2019)

Retrospective bat lyssavirus study: Results

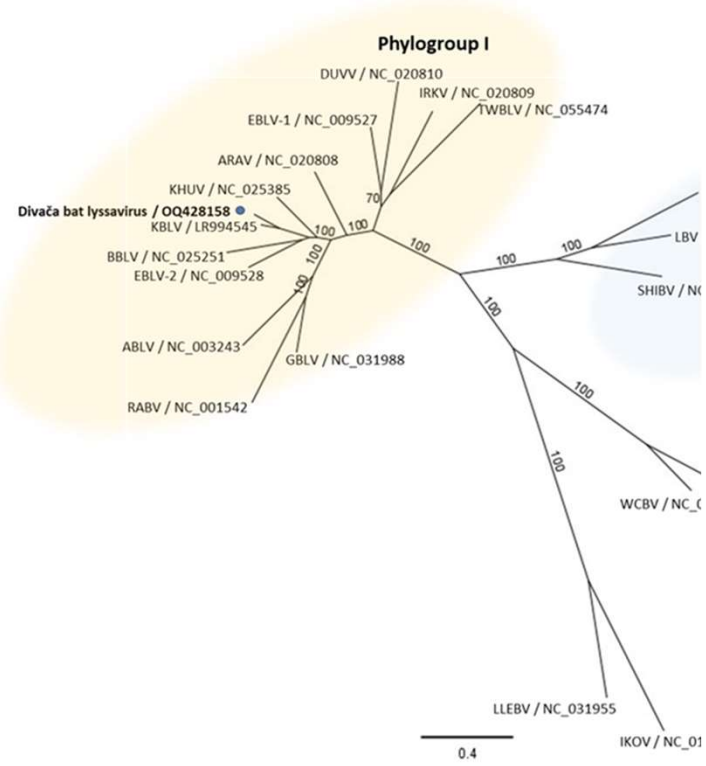
All samles collected in active and passive surveilance program were negative to *Lyssavirus*.
In retrospective surveillance study (225 dead bats), only one sample PP-0868/2014 were positive to Lyssavirus

Sample PP-0868/2014:
(Myotis capaccinii)

RTq-PCR	**positive**
FAT	**positive**
RTCIT	negative

Divača bat lyssavirus: genome determination by NGS

- The nearly complete genome of Divača bat lyssavirus from Slovenia was determined by NGS (Illumina, USA).
- Genome consists 11,871 nucleotides and reflects the characteristic gene organization known for lyssaviruses.
- Phylogenetic analysis of Divača bat lyssavirus revealed that it belongs to phylogroup I lyssaviruses and is most closely related to Kotalahti bat lyssavirus (KBLV) with 87.20% nucleotide and 99.22% amino acid identity.
- Together with KBLV, Khujand virus, European bat lyssavirus 2, Bakeloh bat lyssavirus, and Aravan virus , Divača bat lyssavirus was detected in the bat genus *Myotis*
- Lyssavirus was first time detected in *Myotis capaccinii*
- Lyssavirus was firs time detected in bat in Slovenia



THANKS FOR THE ATTENTION