



STATE VETERINARY INSTITUTE ZVOLEN

NATIONAL REFERENCE LABORATORY FOR RABIES

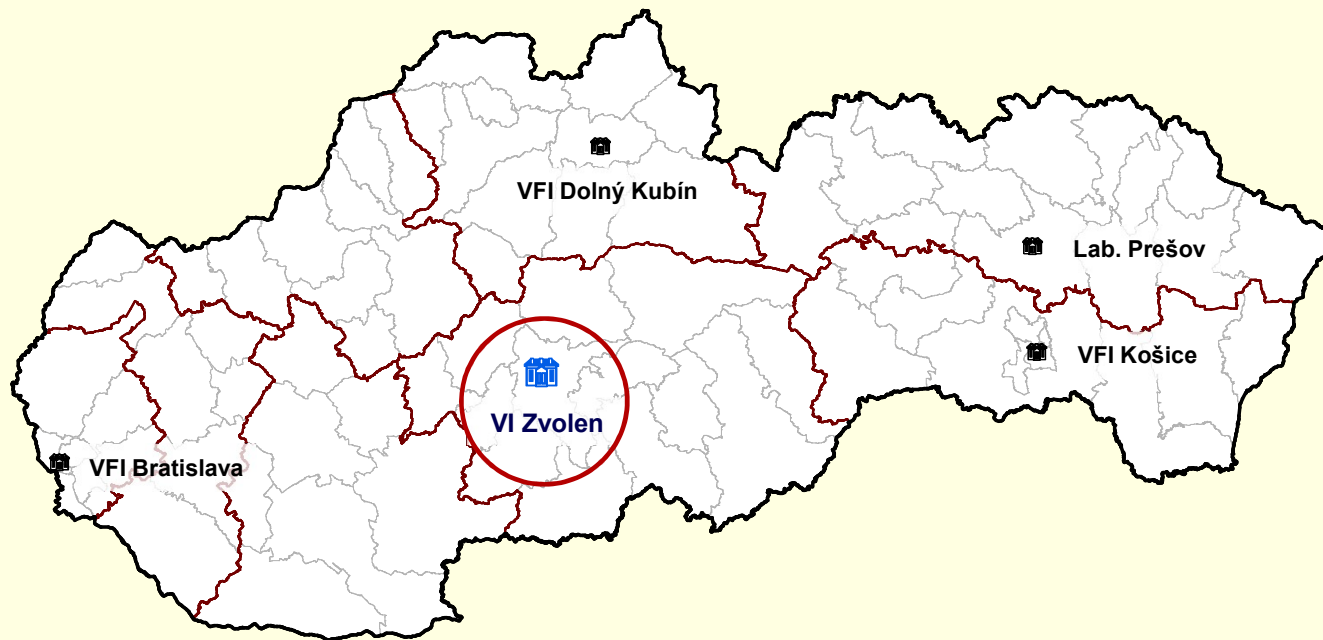
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**RABIES SITUATION IN SLOVAKIA
FROM VIEW LAST REINTRODUCTIONS OF VIRUS**

**14th Workshop for Rabies
Ljubljana, Slovenia 21st – 22nd June 2023**

Introduction

NRL for Rabies in VI Zvolen is only laboratory involved in laboratory diagnoses of Rabies in Slovakia

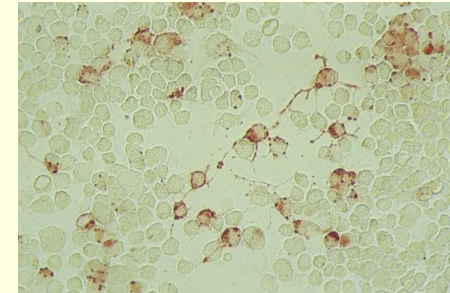


Introduction

Laboratory methods that are performed in National Reference Laboratory for Rabies

Detection of virus

- FAT – antigen detection on impressions or smears with FITC conjugated antibodies
(WHO, 1996 OIE 2000)
- Virus cultivation on Neuro-2a cell cultures in microtitration plate. Visualisation of antigen is performed with indirect immunoperoxidase technique using sheep polyclonal anti-nucleoprotein serum
(WHO, 1996 OIE 2000)



Detection of viral RNA

- RRT-PCR method (multiplex) genotypes 1, 5, 6
(Wakeley et al., 2005)
- RRT-PCR method (SYBR Green) all genotypes
(Heaton et al., 1997, Coertse et al., 2010)

Introduction

Laboratory methods that are performed in National Reference Laboratory for Rabies

Typing of virus

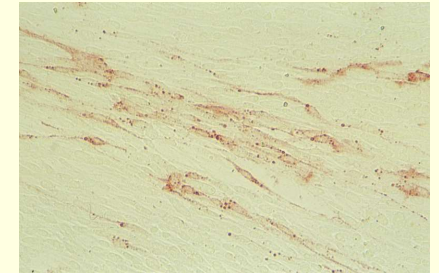
- Indirect immunoperoxidase technique using monoclonal antibodies:
W 239.17, W 187.5, W 187.11, MW 187.6, MSA 6.3, LBV 7.3.6, DUV 6.15,
S 62 1.2, P 41, Z 144.88
(purchased from Tübingen)
- Sanger full genome sequencing and phylogenetic analyses
(Dirbakova, SVI Zvolen)

Introduction

Laboratory methods that are performed in National Reference Laboratory for Rabies

Detection of antibody

- FAVN – modify with immunoperoxidase detection of virus
(WHO, 1996; OIE 2000)
- ELISA – blocking system using biotinylated goat polyclonal antibodies for detection anti G protein antibodies
(SVI Zvolen)



Detection of TTC

- Fluorescent microscopy
(EURL for Rabies virus 5th Workshop for Rabies, Nancy, France)



Introduction

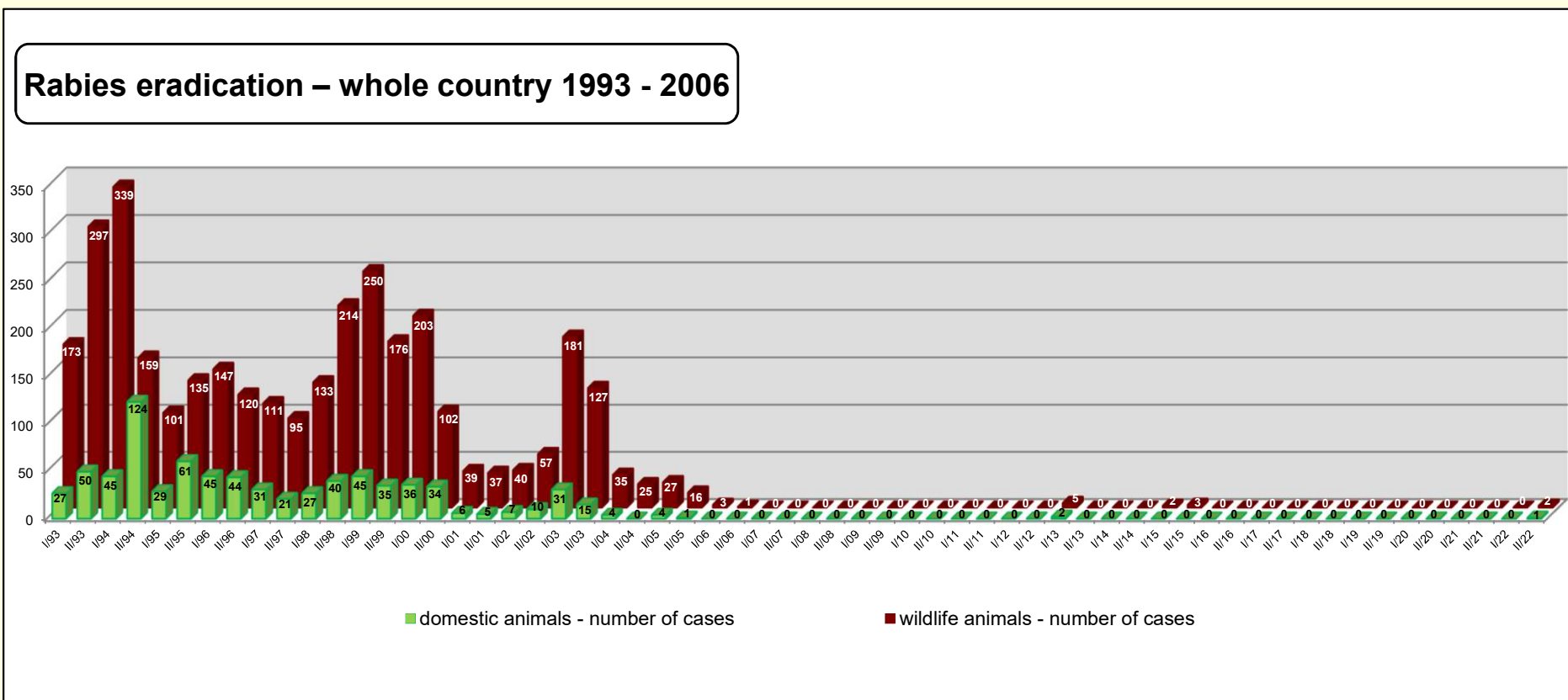
Foxes represented 95,5 % of all positive wildlife animals



**In Slovakia foxes are only reservoir species for Rabies virus.
Infection of other species is accidental and self-limited.**

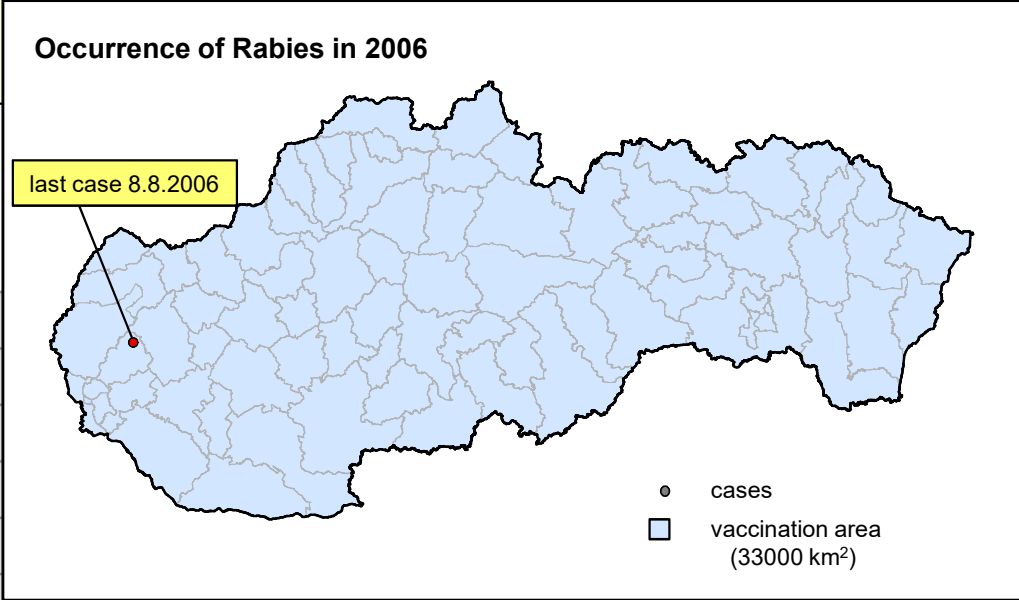
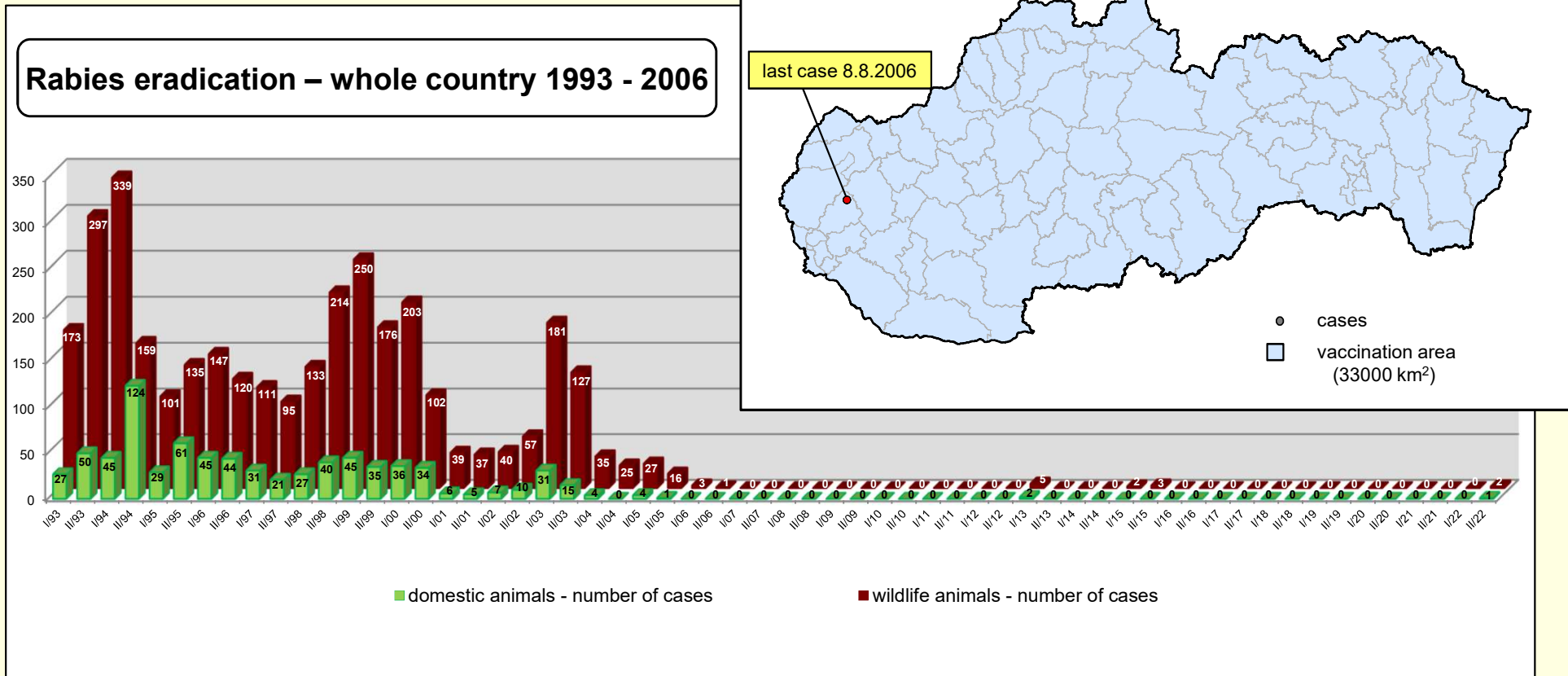
Introduction

Occurrence of Rabies in Slovakia during 1993 – 2022



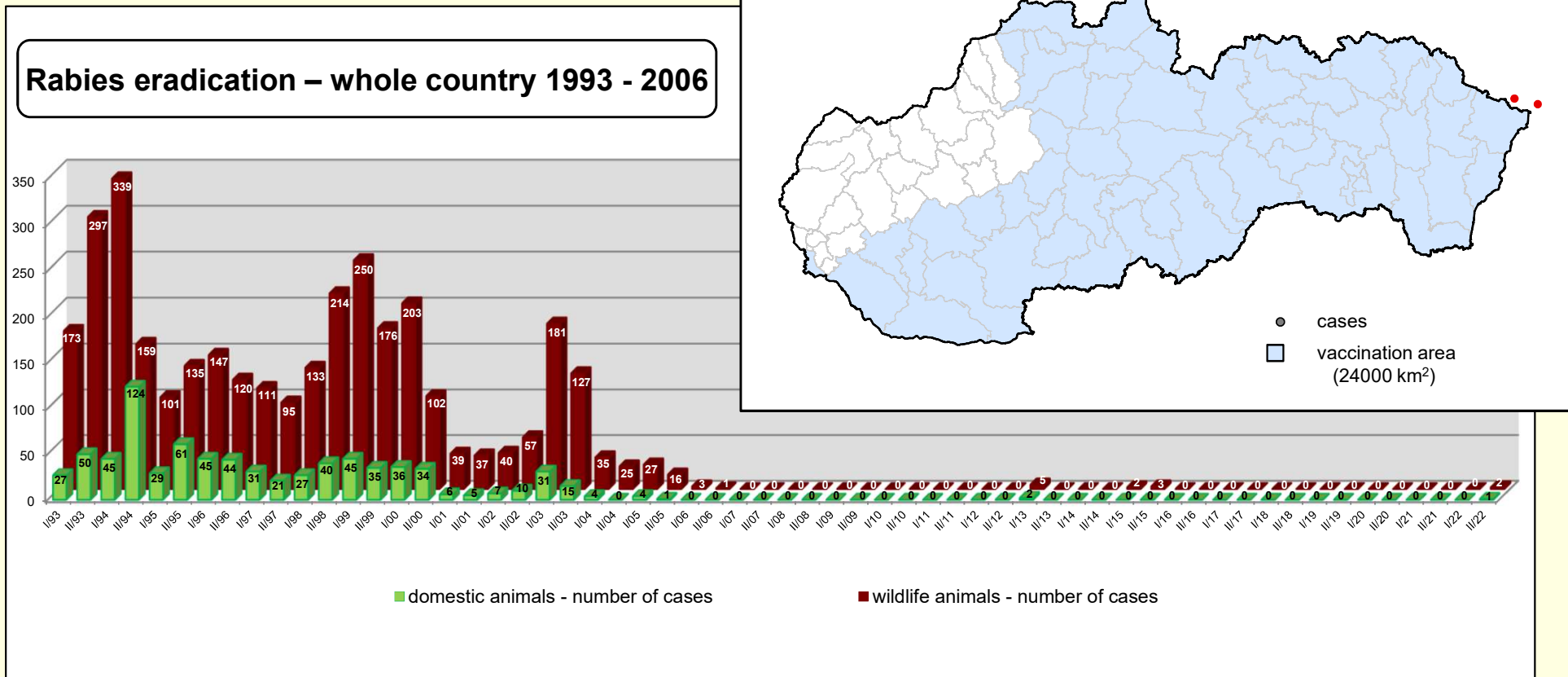
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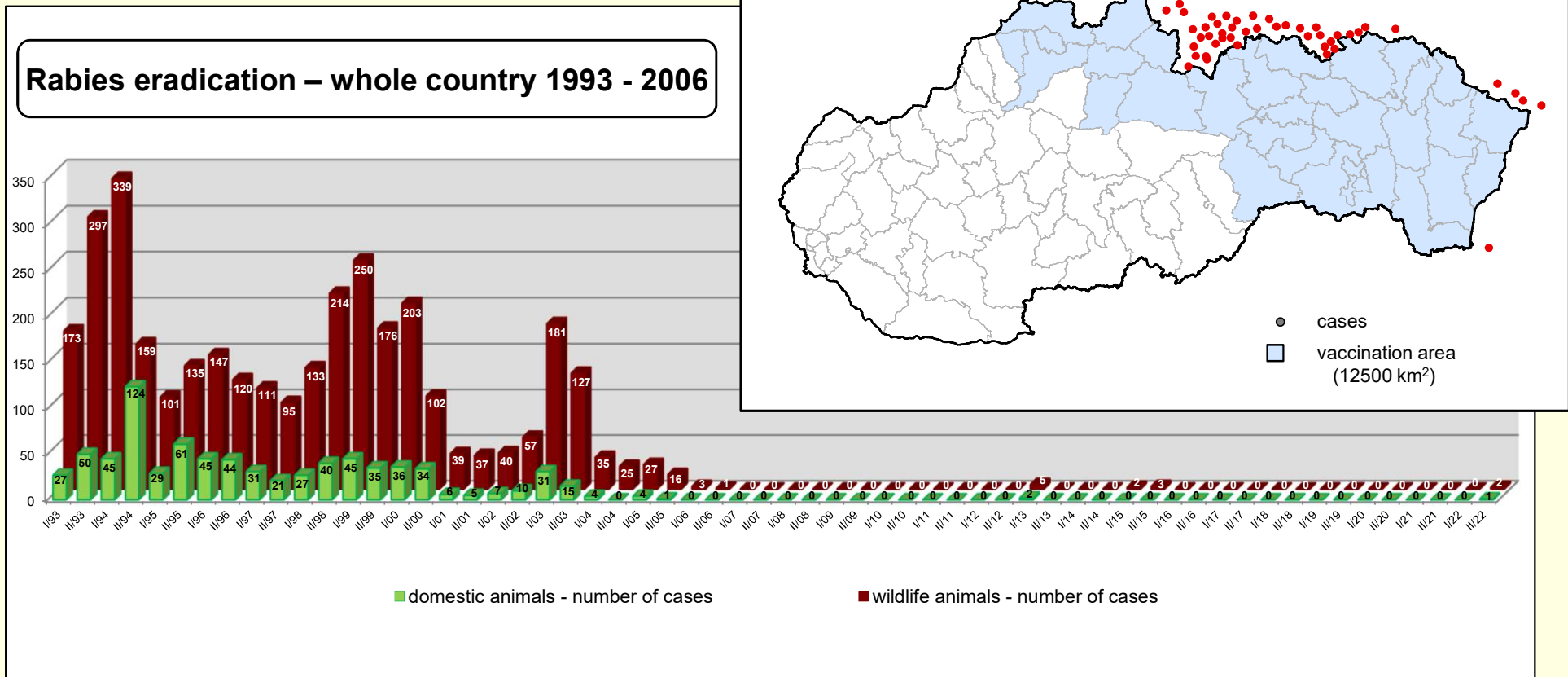
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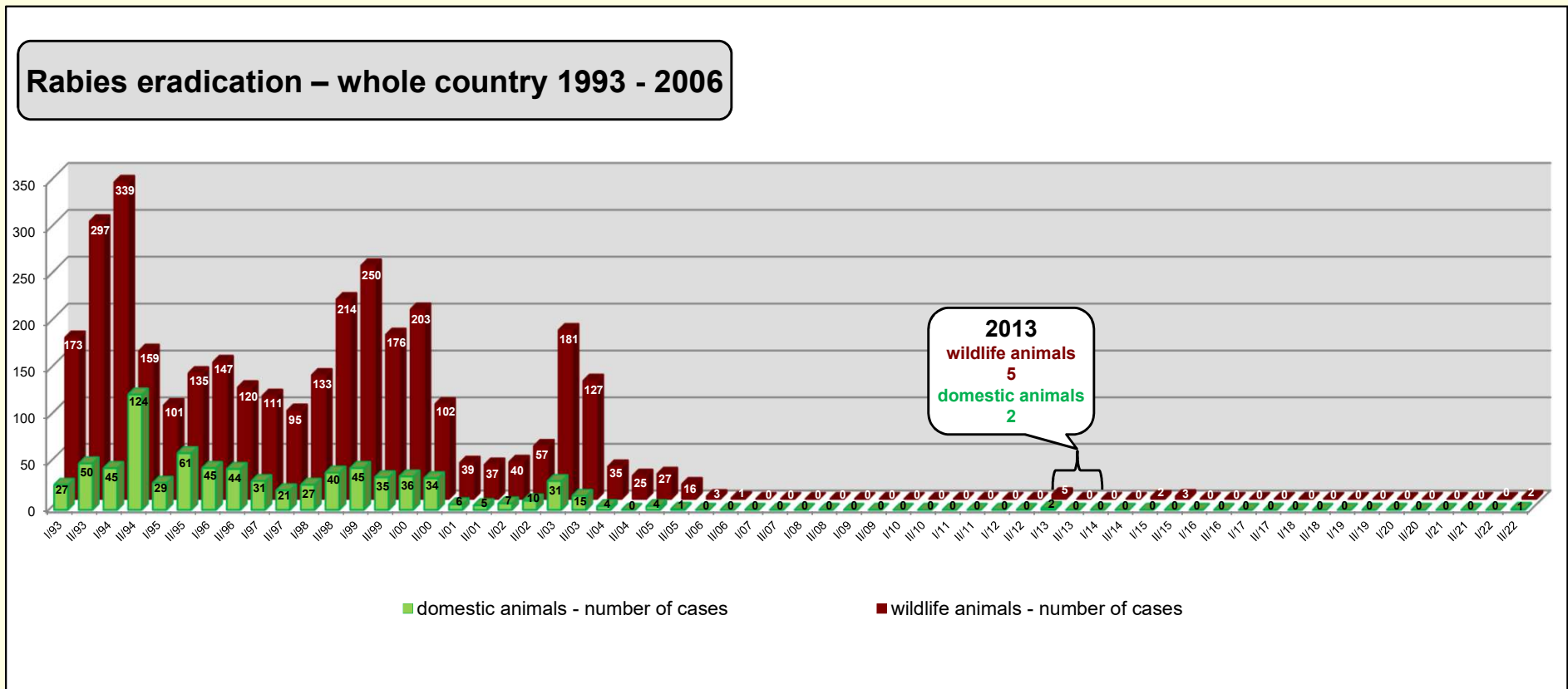
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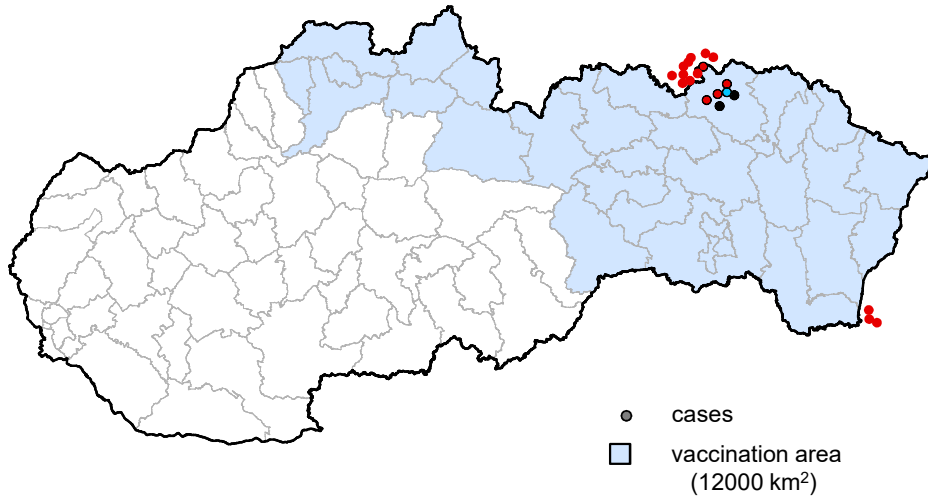
Occurrence of Rabies in Slovakia during 1993 – 2022



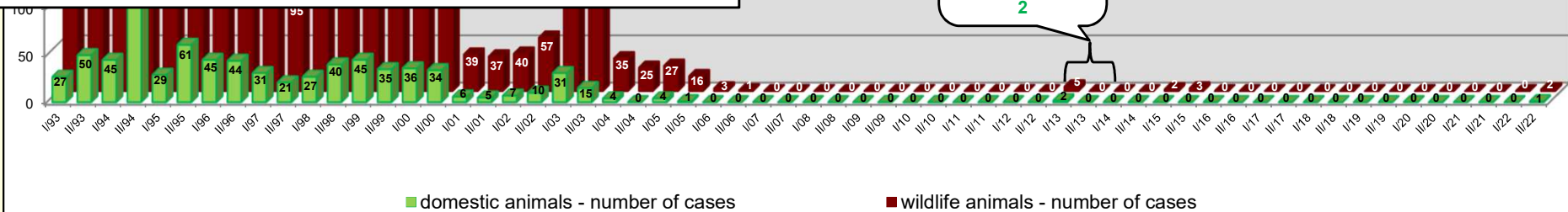
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Occurrence of Rabies in Slovakia during 1993 – 2022

Occurrence of Rabies in 2013

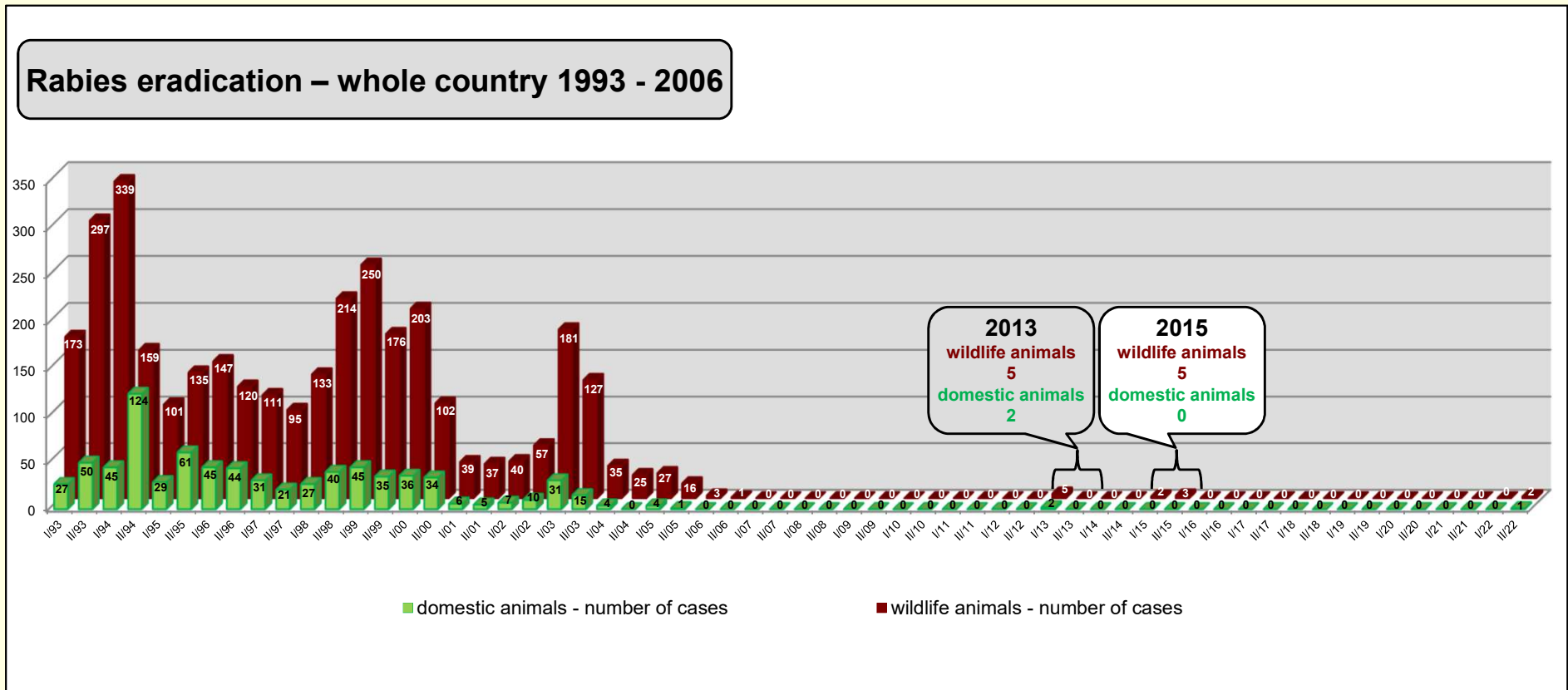


No	Date	Animal	District	Land register
1.	8.1.2013	fox	Bardejov	Petrová
2.	11.3.2013	fox	Bardejov	Kružlov
3.	18.3.2013	fox	Bardejov	Stebník
4.	23.4.2013	fox	Bardejov	Rokytov
5.	7.5.2013	marten	Bardejov	Bardejov
6.	7.5.2013	dog	Bardejov	Bardejov
7.	13.5.2013	dog	Bardejov	Bardejov



Introduction

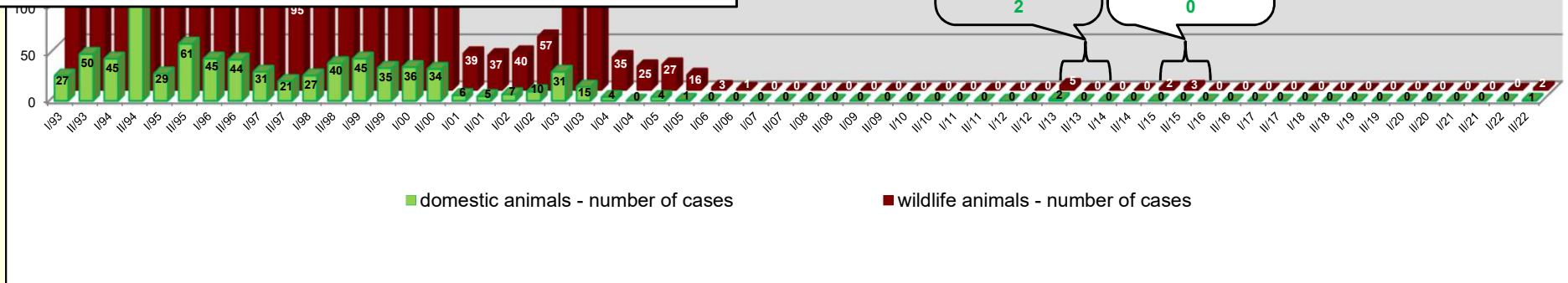
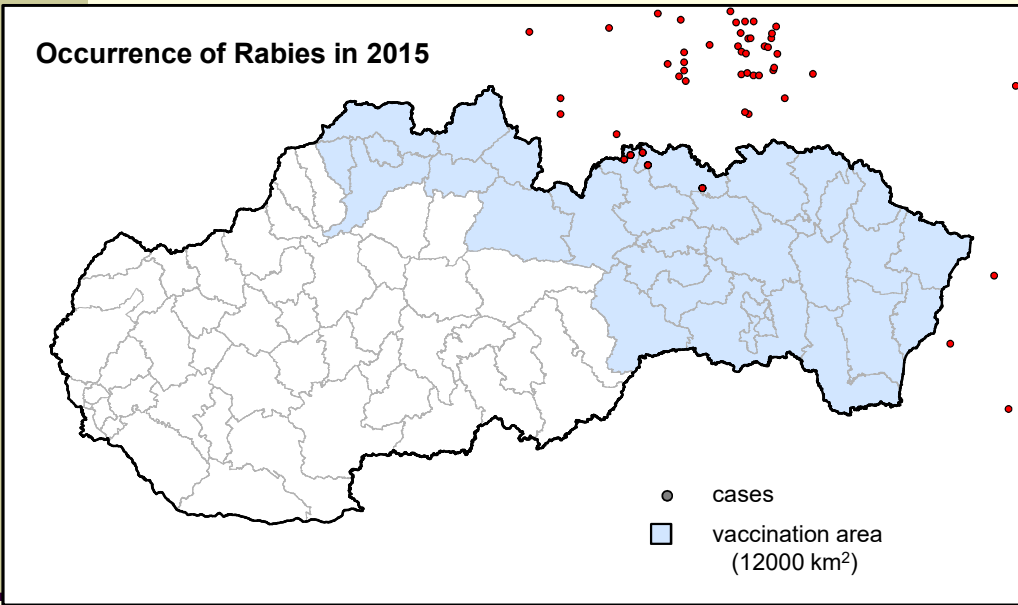
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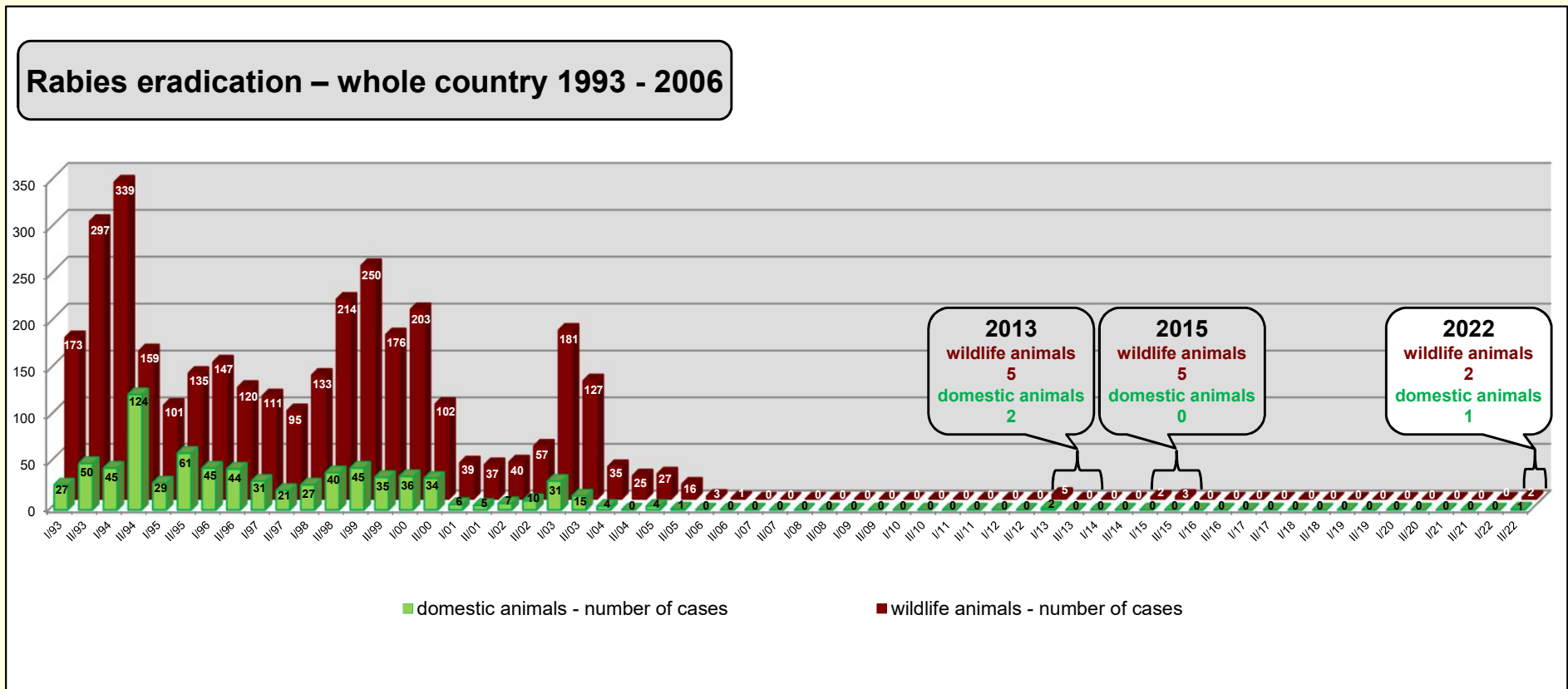
Occurrence of Rabies in Slovakia during 1993 – 2022

No	Date	Animal	District	Land register
1.	5.1.2015	fox	Stará Ľubovňa	Plaveč
2.	26.6.2015	fox	Stará Ľubovňa	Veľký Lipník
3.	17.7.2015	fox	Kežmarok	Spišská Stará Ves
4.	19.8.2015	fox	Kežmarok	Matiašovce
5.	2.9.2015	fox	Stará Ľubovňa	Haligovce



Introduction

Occurrence of Rabies in Slovakia during 1993 – 2022

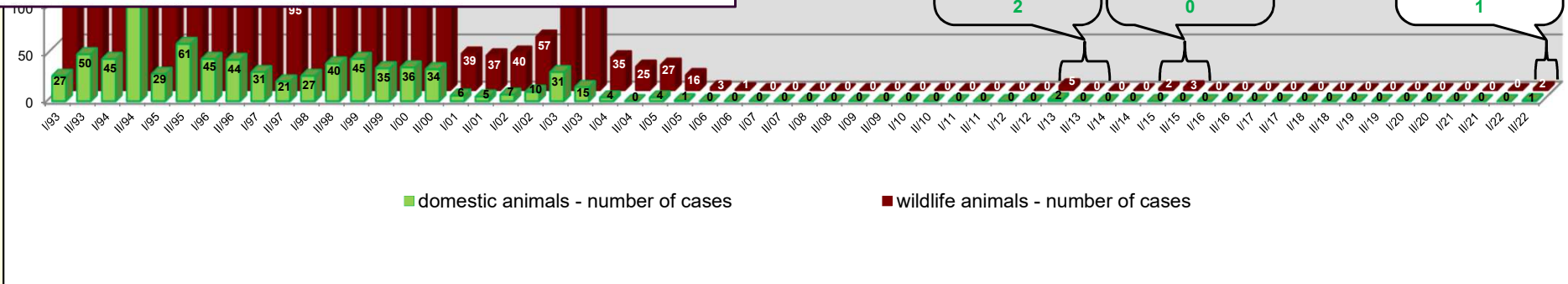
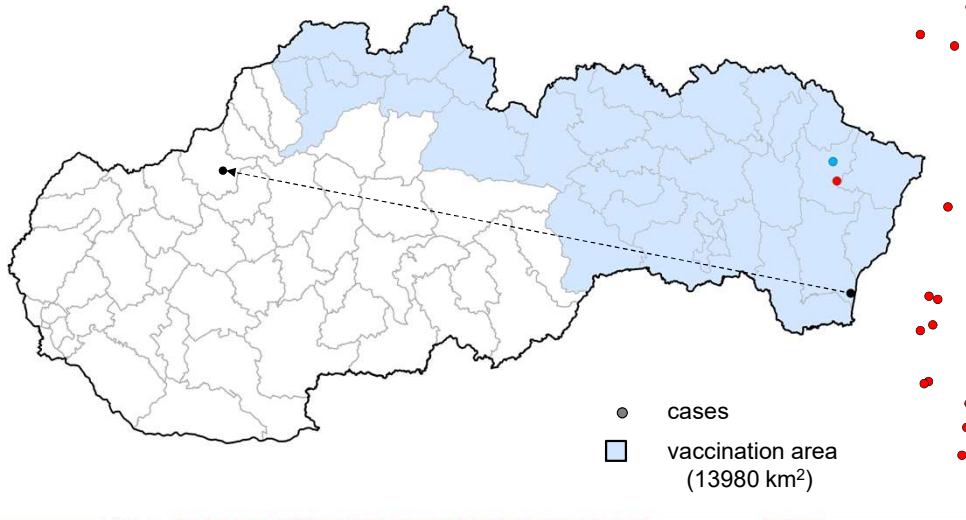


Introduction

Occurrence of Rabies in Slovakia during 1993 – 2022

No	Date	Animal	District	Land register
1.	30.9.2022	badger	Humenné	Jablož
2.	14.12.2022	dog	Michalovce	Veľké Slemence
3.	29.12.2022	fox	Humenné	Rovné

Occurrence of Rabies in 2022



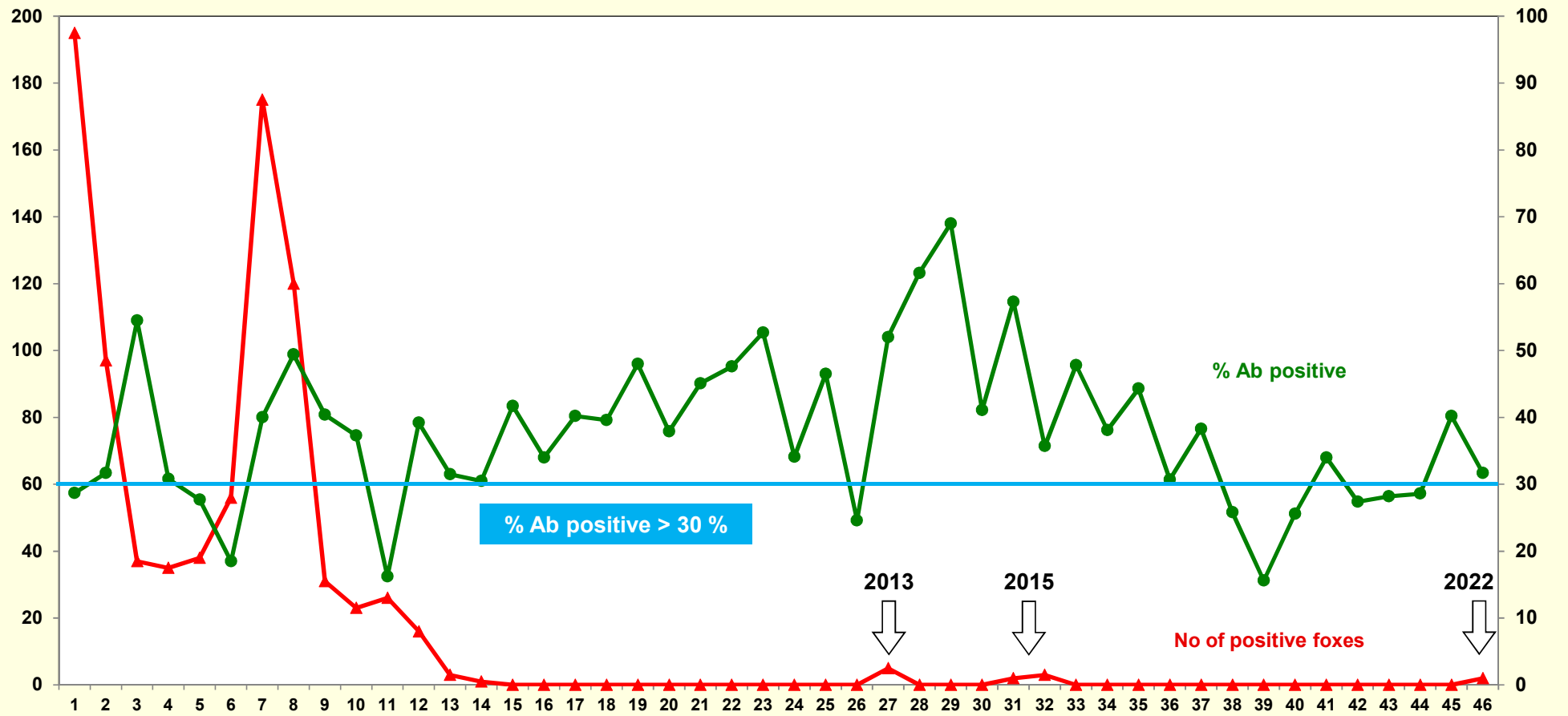
2013
wildlife animals 5
domestic animals 2

2015
wildlife animals 5
domestic animals 0

2022
wildlife animals 2
domestic animals 1

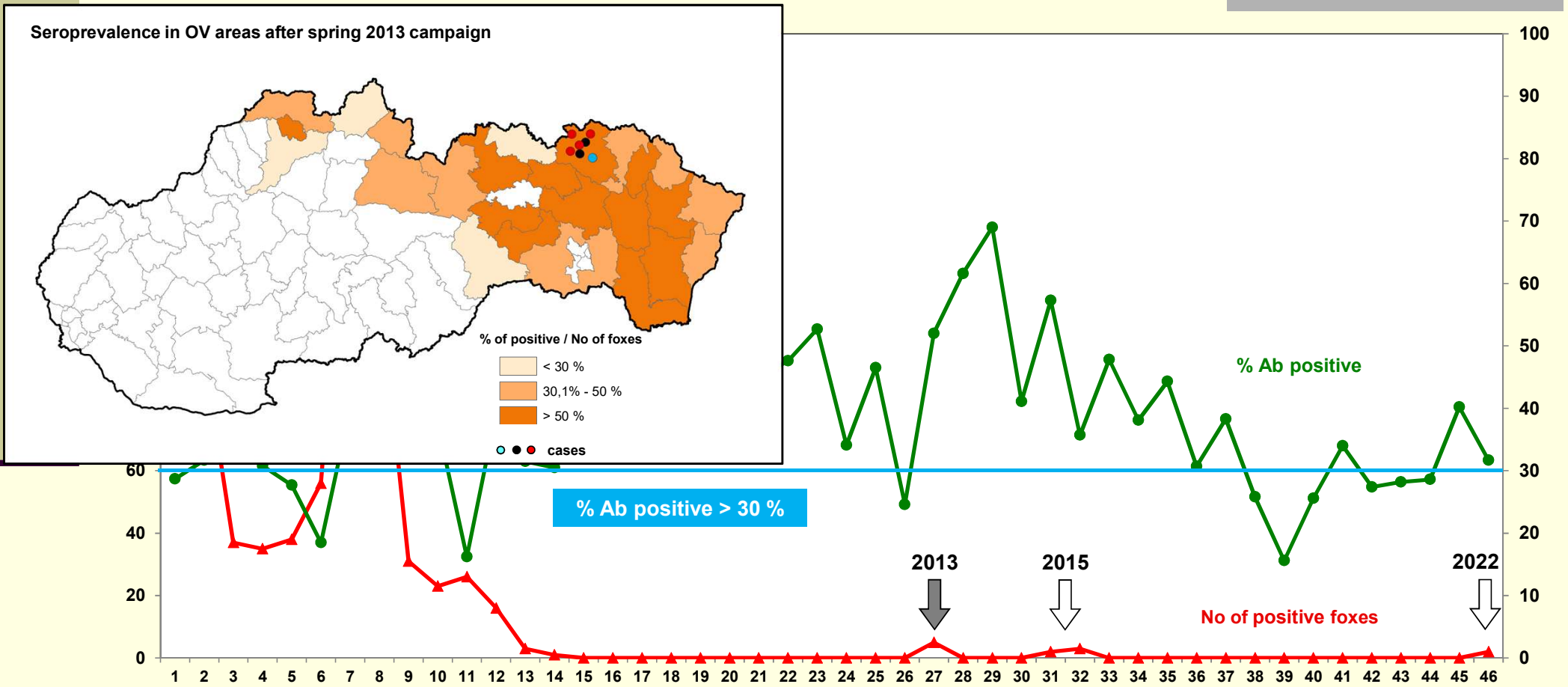
Discussion

Estimation of seroprevalence cut off in term effectiveness of OV



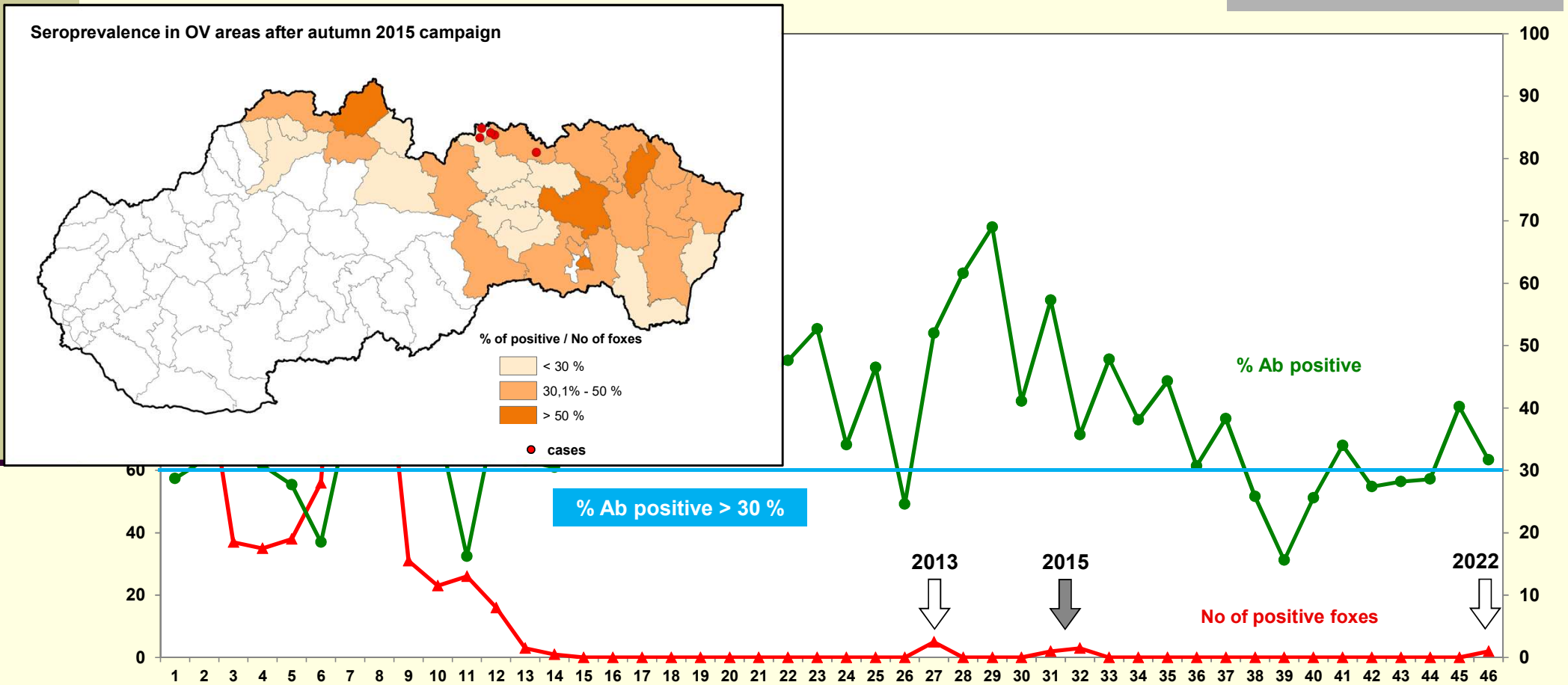
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Estimation of seroprevalence cut off in term effectiveness of OV



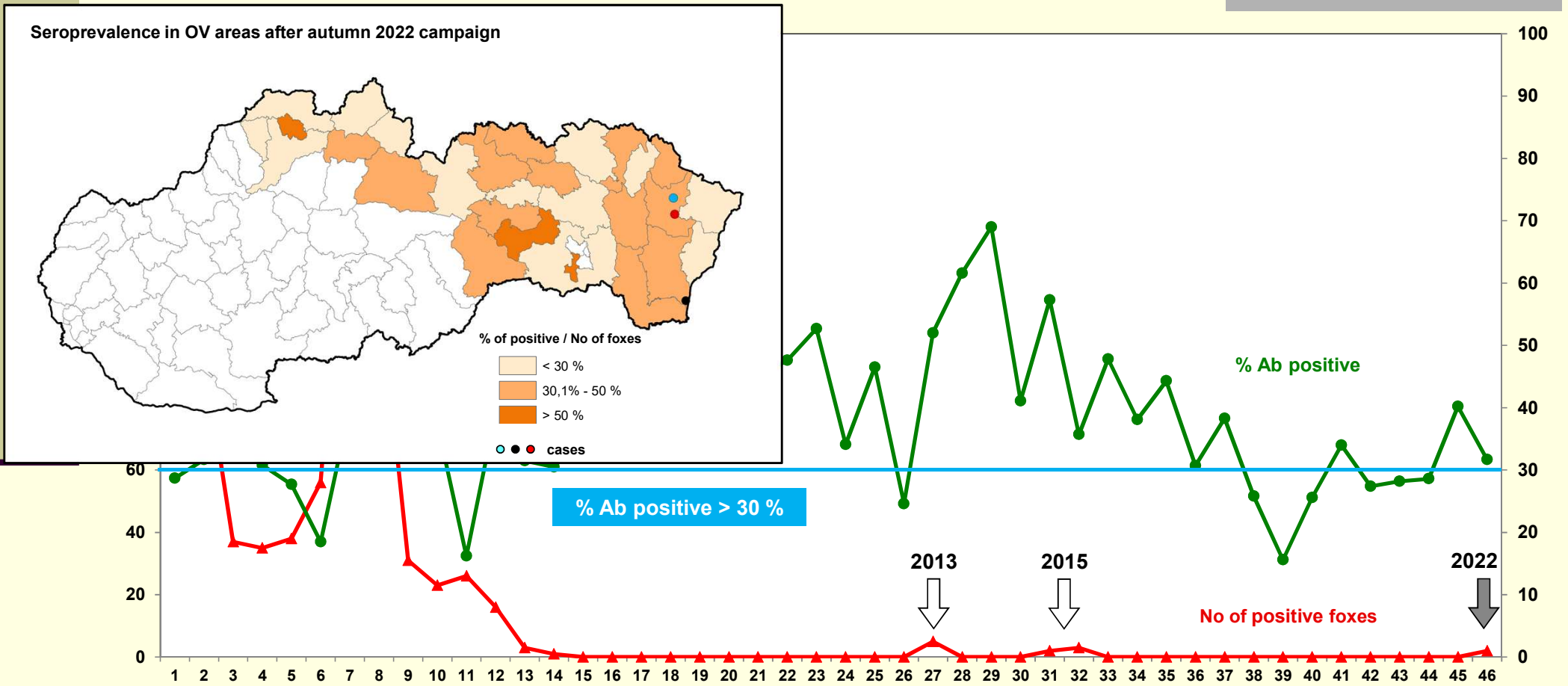
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Estimation of seroprevalence cut off in term effectiveness of OV



Discussion

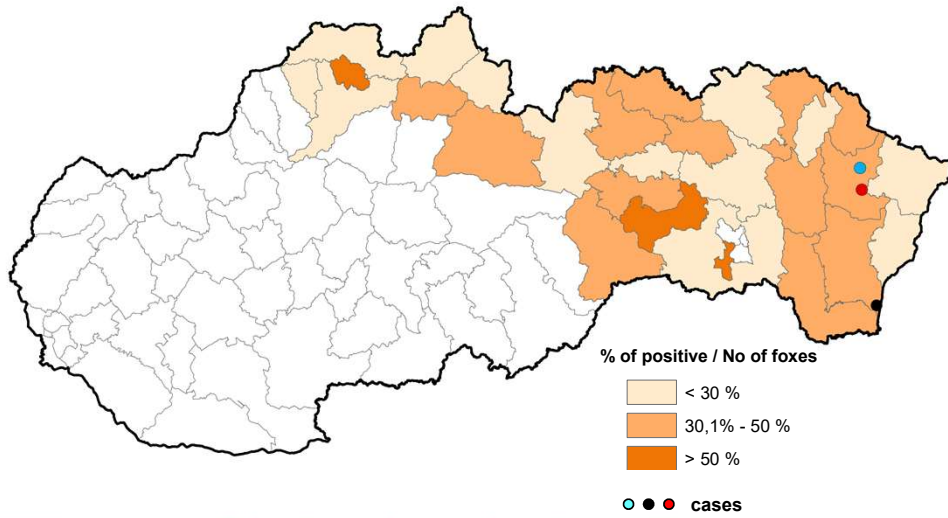
Estimation of seroprevalence cut off in term effectiveness of OV



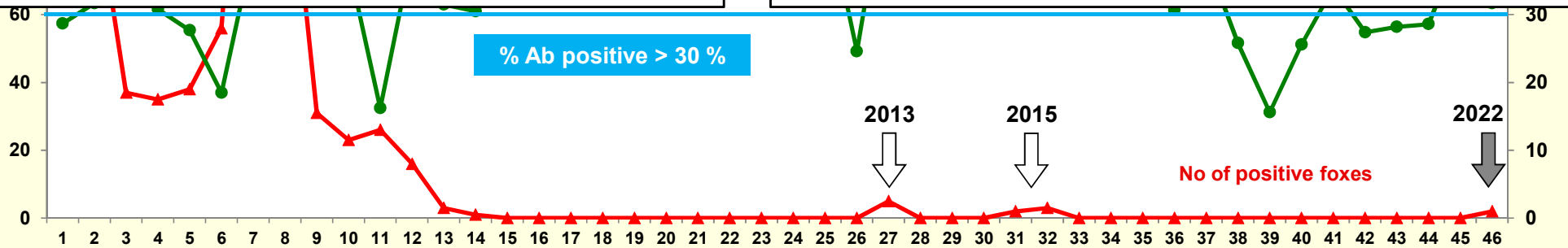
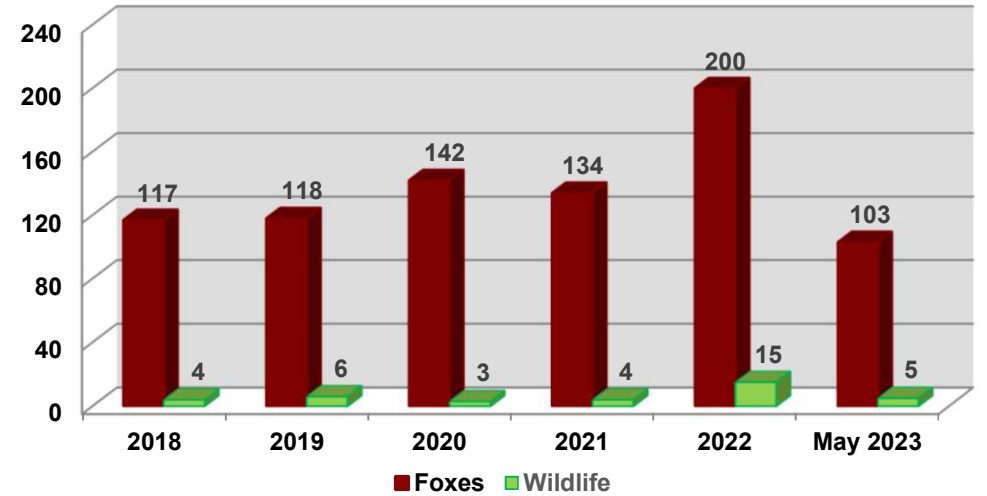
Discussion

Estimation of seroprevalence cut off in term effectiveness of OV

Seroprevalence in OV areas after autumn 2022 campaign



Number of foxes and wildlife submitted for Rabies diagnosis in affected and neighbouring districts



Sequence analyses of Slovakian field strains

Phylogenetic analyses

- Whole genome by Sanger sequencing
- N gene preliminary phylogenetic analysis

using Clustal V method from MegAlign Lasergene of DNASTAR Lasergene ver11 package program

RV GENOME















non-segmented negative-stranded RNA genome app 12 000 nts



Sequence analyses of Slovakian field strains

How many strains were used ?

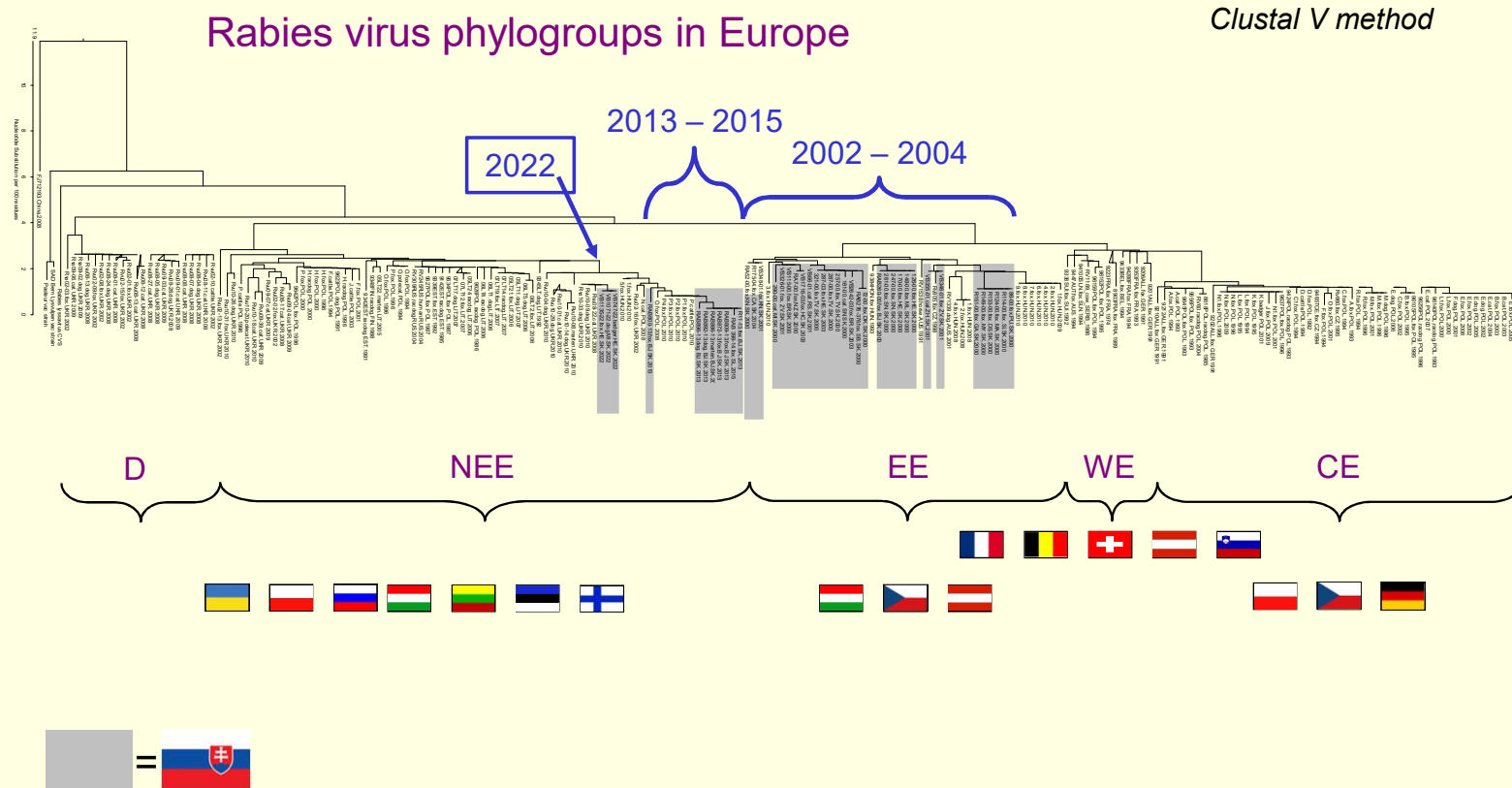
- 38 strains of Rabies virus isolated from red fox (34), cats (4), dogs (3), marten (1), badger (1) during years 2000-2022 in Slovakia.
- other strains previously published in GenBank :

	Poland	75
	Ukraine	41
	Russia	2
	Lithuania	16
	Estonia	3
	Finland	1
	Hungary	11
	Austria	3
	Slovenia	1
	Czech	3
	Germany	5
	France	4
	Belgium	1
	Switzerland	1
	SAD Bern vaccine strain (Lysvulpen)	1
	Pasteur vaccine strain	1
	Challenge vaccine standard	1

together
213 strains
in phylogenetic tree

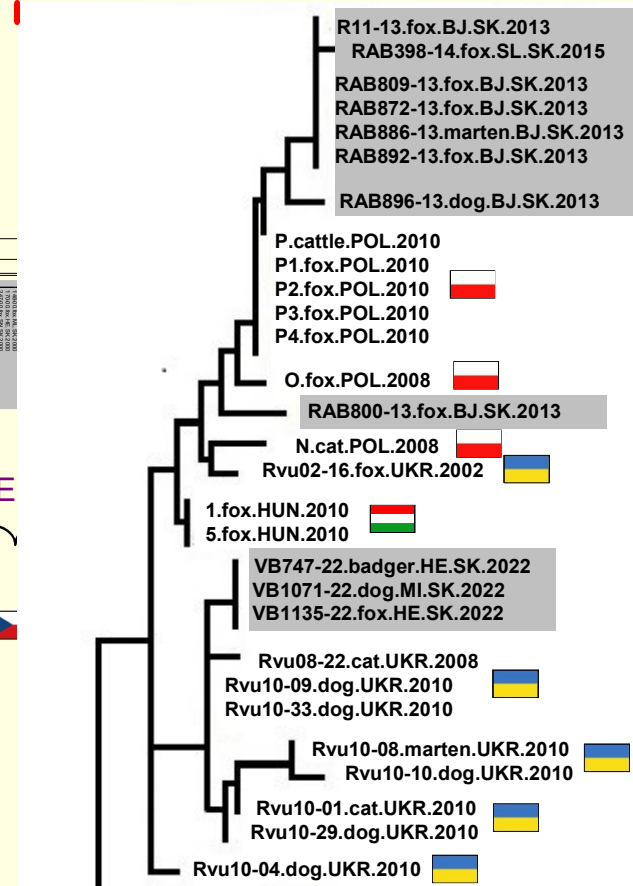
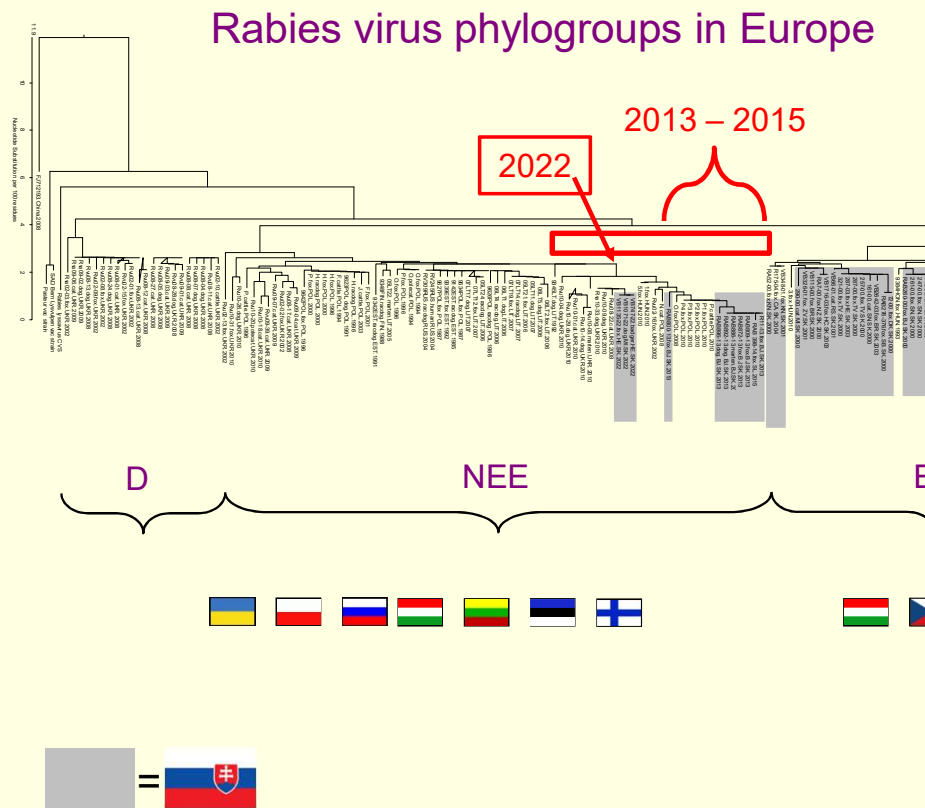
Sequence analyses of Slovakian field strains

Phylogenetic relationships between the part of N gene sequences



Sequence analyses of Slovakian field strains

Phylogenetic relationships between the part of N gene sequences



Conclusions

- After obtaining Rabies free status, in Slovakia we found three introductions of the disease from neighbouring countries.
- Sequence analyses showed that the all following introductions were independent from previous occurrence of Rabies in whole country. The introductions of Rabies in 2013 and 2015 were connected with occurrence in Poland. The last introduction in 2022 was different from previous and mostly related to strains circulated in Ukraine.
- Based on our seroprevalence studies performed in frame of control OV we suppose, that present Rabies will be restricted on bordering regions with low tendency to spread further to inland regions.
- But due to high level of movement the refugees through Ukraine border and impossible control of Rabies in wildlife in Ukraine, we should expect the other new introductions.



Thank you for your attention !