

Evaluation of rabies control campaigns in Bosnia and Herzegovina after the reemergence of rabies in 2020

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The aim of the presentation:

- Short review and gap assesment of the ORV campaign in Bosnia and Herzegovina,
- Assesment and gap analysis of NRL for rabies trough the analysis of the last case of rabies in dog.

Rabies in Bosnia and Herzegovina

BIH is implementing general measures in rabies control

- Awareness raising
- Vaccination of dogs older than 3 months
- Oral rabies vaccination of wild animals

In the territory of Bosnia and Herzegovina, as well as in Europe and Asia, the main reservoirs of rabies are red foxes (*Vulpes vulpes*).

Oral rabies vaccination in BiH



ORALNA VAKCINACIJA LISICA PROTIV BJESNILA U BiH



Ovaj je projekt finansiran od strane Evropske unije



Oral rabies vaccination in BIH

- ORV of foxes in Bosnia and Herzegovina started in spring 2011 and ended in 2018.
- 14 campaigns were carried out continuously until 2018. (1.200.000 bites per campaign).
- 15. campaign in late 2021.

- Two vaccines were used: Lysvulpen and Fuchsoral
- Campaign resulted in drastically decrease of rabies cases and headed towards the declaration of a country free of rabies.
- In 2014, the last case of rabies was recorded in a fox through active post vaccination monitoring of foxes.

Rabies monitoring

Active monitoring

- In order to monitor the success of oral rabies vaccination, post-vaccination monitoring of oral vaccination was carried out through planned testing of culled animals in all vaccination areas.

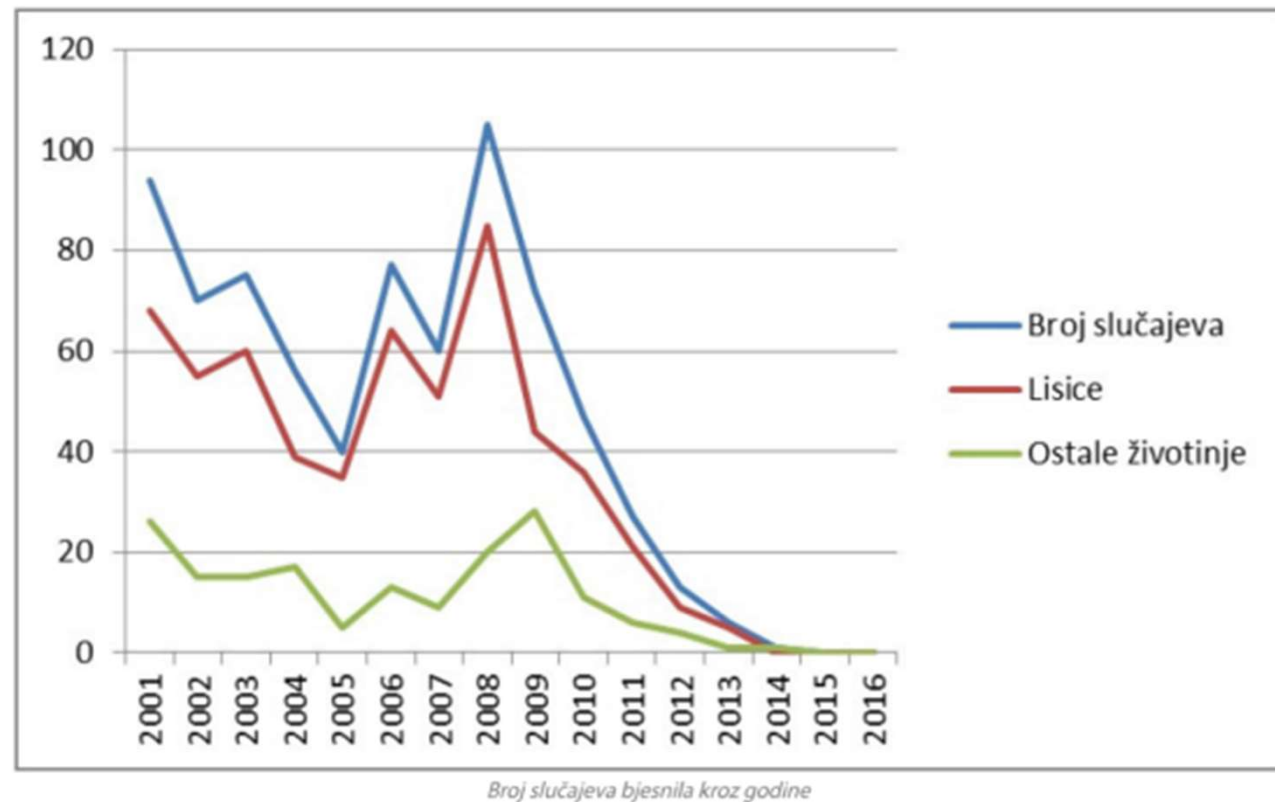
Tests implied:

- Assessment of the success of bait consumption by detecting a biomarker (tetracycline) in the teeth of the tested animal.
- Assessment of the success of immunization by testing for the presence of antibodies in tested animals
- dFAT for rabies status confirmation

Results of ORV „active” monitoring 2011 - 2017

Hunting season	Nr. Of samples planned	Processed nr. Of samples		Tetracycline intake		Seroconversion (ELISA)		FAT positive
		Samples nr	%	Samples nr	%	Samples nr	%	
2012/13	2065	120	6					
2013/14	2065	352	17	231	66	109	48	0
2014/15	2113	415	20	333	80	204	62	1
2015/16	2125	757	35	513	67	381	75	0
2016/17	2125	446	21	233	50	98	42	0
2022					35		23	0

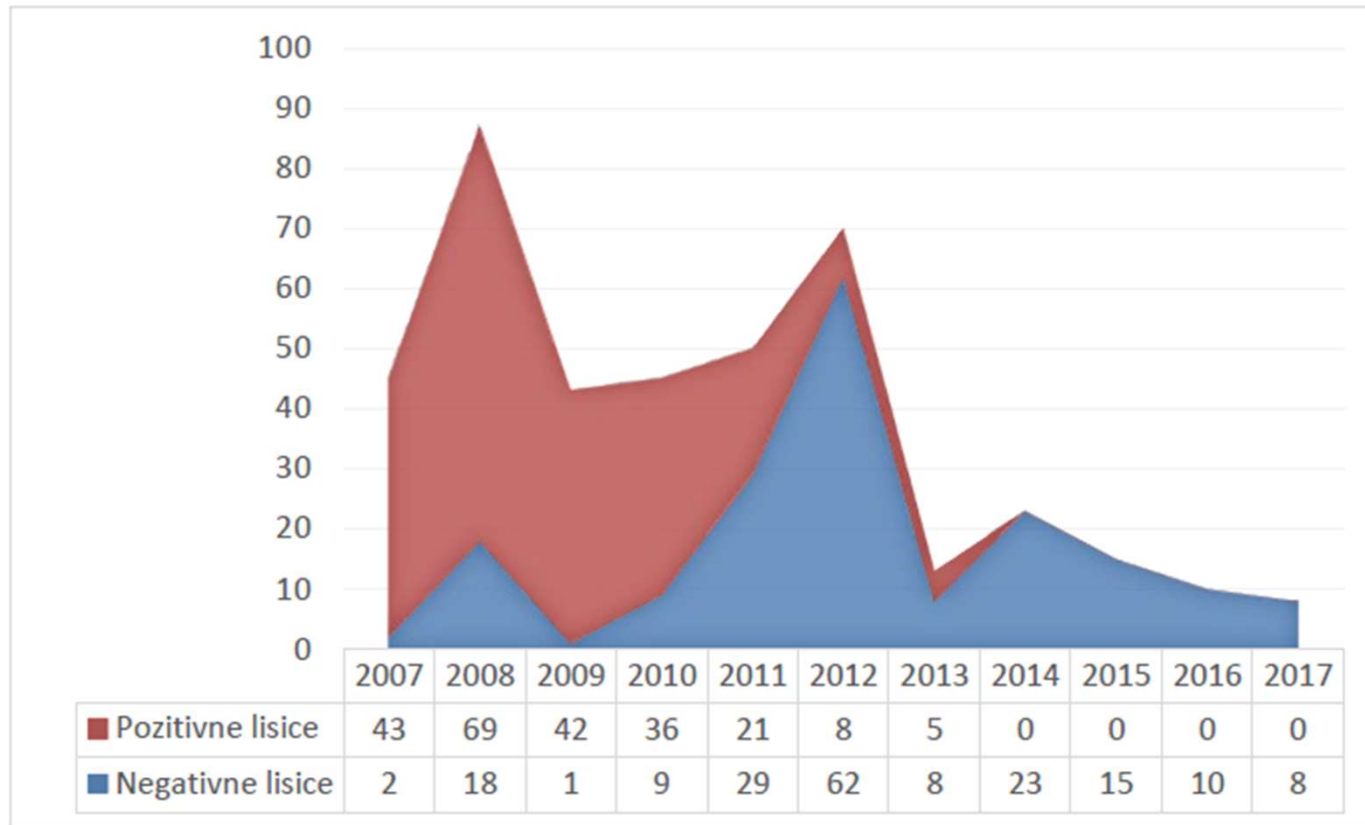
- In 2008 number Of rabies cases was 83,
- In 2012, one year after the onset of ORV **number of cases dropped to 6** one year after the onset of vaccination



Passive monitoring

- In Bosnia and Herzegovina, passive rabies surveillance is planned in such a way that all wild animals showing signs of rabies are shot and submitted for testing.
- Additionally, all foxes found dead in the hunting grounds or found dead on the road must be submitted for testing for the presence of rabies.

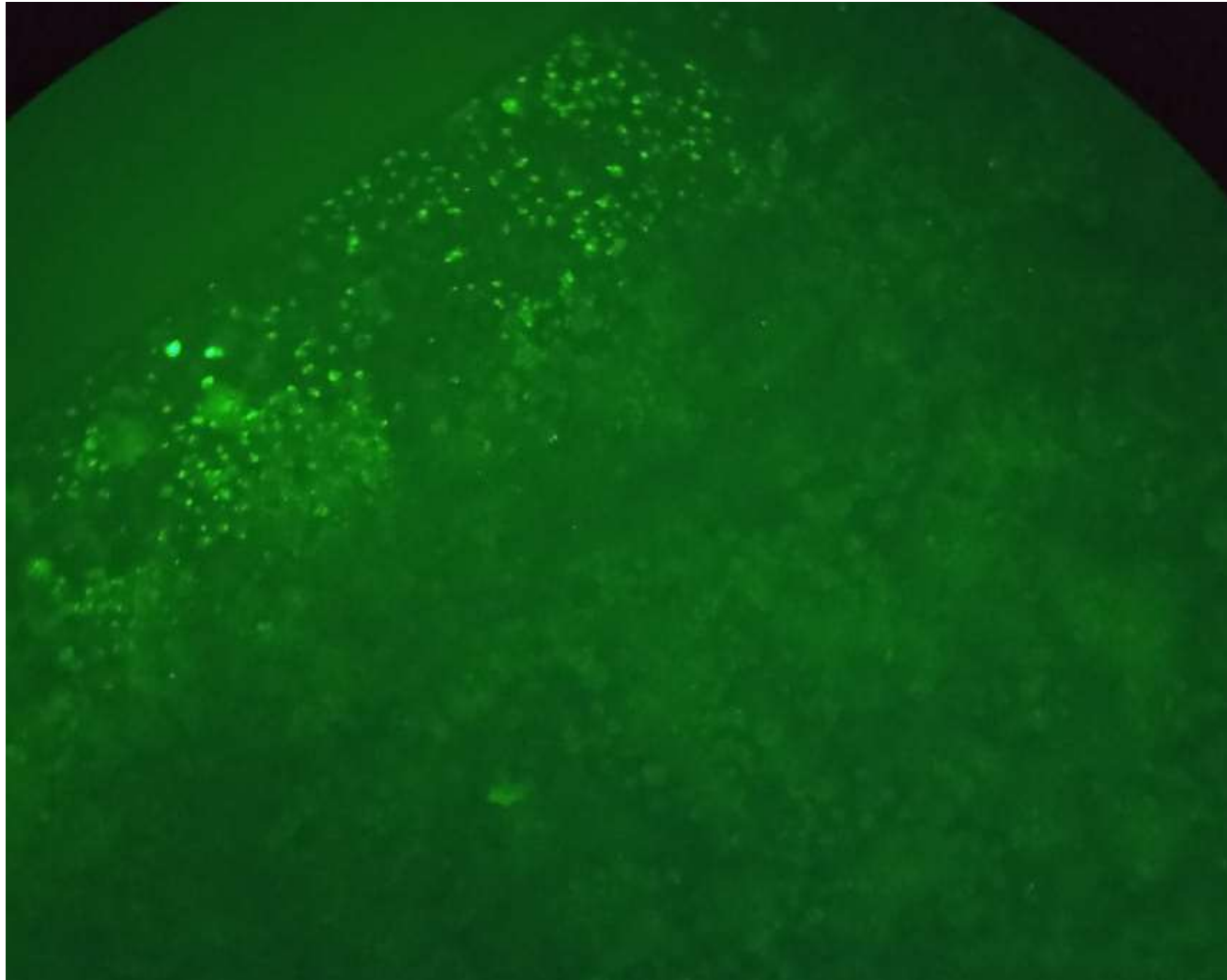
Results of passive monitoring



- Passive monitoring after 2012 drastically decreased,
- Number of testings has to increase at the end of eradication campaign

Case report of rabid dog

- May 29, 2020. dog, euthanized a day before with clinical signs suggestive to rabies, was delivered to Veterinary faculty in Sarajevo for rabies confirmation.
- National reference laboratory for rabies confirmed rabies infection same day using dFAT and RT-PCR test.
- Additionally in following days the virus has been isolated on N2a cell culture.



Positive brain sample inoculated on N2a cell culture, stained 3 days after inoculation with dFAT

- On June 2, 2020 at the request of the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska, an epizootiological investigation led by by dr. Oliver Stevanović (Veterinary institute of Republica Srpska) was carried out in the territory of the municipality of Srebrenica due to the outbreak of the infectious disease rabies.
- The investigation concluded low percentage of dog and cat vaccinations in the municipality, a large number of stray dogs and the absence of dog shelters
- In order to suppress and eradicate rabies in the given area, the same authority proposes emergency measures to prevent the spread of rabies, which were implemented within the following 14 days.

Measures

- a) **Identification by chipping of dogs** (unidentified dogs), registration of dogs and cats and vaccination of unvaccinated dogs and cats older than three months (priority is vaccination against rabies) or euthanasia of unvaccinated dogs and cats;
- b) Segregation, confinement and isolation of vaccinated animals suspected of having been in contact with an animal suffering from rabies, animals that have been in indirect contact with an animal suffering from rabies and placing them under surveillance for a period of three months, during which the owner they are obliged to report changes in the health status of animals under supervision to the veterinary organization;
- c) Keeping dogs and cats under control in a securely fenced area or keeping dogs on a leash and/or with a basket;
- g) Closing and prohibiting the movement of dogs and cats younger than three months;
- d) **Prohibition of traffic, exhibitions, events** and other forms of public display of dogs and cats;
- d) Collection of abandoned dogs and cats through the hygiene service in order to cause the animals as little pain and suffering as possible, and euthanasia of unvaccinated stray dogs and cats;
- e) Increased shooting of foxes and other wild carnivores that can be the source of the infectious disease rabies;
- h) Prohibition of trade in wild animals;
- z) Euthanasia of animals suffering from rabies, animals bitten by a sick animal, animals suspected to be suffering from rabies and animals that have not been vaccinated against the causative agent of rabies that were in direct contact with the diseased animal or vaccination of animals that have not been vaccinated against the causative agent rabies that were in direct contact with the diseased animal and placing it under surveillance for three months in quarantine, during which the owner or keeper is obliged to report changes in the health status of the monitored animal to the veterinary organization;
- i) **Prohibition of trade and slaughter** of animals suspected of rabies, sale or consumption of individual by-products of these animals, milk or other products derived from these animals, as well as skinning of these animals;
- j) Prohibition of skinning with foxes, wolves and other furs;
- k) harmless removal of animal carcasses from public areas;
- l) Disinfection of the room and area where the diseased animal was, as well as equipment that was in contact with a sick animal;
- lj) **Vaccination of animals** will be done by June 14, 2020. years;
- m) **Mandatory laboratory analyzes** for rabies of shot foxes and other wild carnivores that may be sources of the infectious disease rabies;
- n) As an exception to point v) of this point, the following herd guard dogs grazing with the herd, official dogs of the Police of the Republika Srpska, dogs of rescue teams and guide dogs of blind people can move freely outside of populated areas. , if the immunity period has expired after vaccination, and dogs can be used for hunting if they have been vaccinated twice a year against the causative agent of rabies, if they have been identified and if the immunity period has expired after the last vaccination

Measures

- ORV campaign should be continued, and started as soon as possible

Genetic characterization of the isolated rabies virus strain

- **To differentiate the wild from the vaccine strain**
- Due to pandemic conditions, almost a year after the case confirmation on **19 April 2021**, the EURL for rabies received a dog brain sample from the Veterinary Institute Laboratory for Virology and Serology of Sarajevo (Bosnia and Herzegovina)

Molecular biology

Sample preparation (VFS, Veterinary institute)

- Dog brain sample was stabilized on FTA[®] paper for typing and sent to the European Union Reference Laboratory for Rabies, Nancy, France

Preparation for sequencing (EURL, ANSES, Nancy, France)

1. RNA extraction
2. Amplification of the partial Nucleoprotein gene by conventional RT-PCR
3. Amplification of the full Nucleoprotein gene by conventional RT-PCR

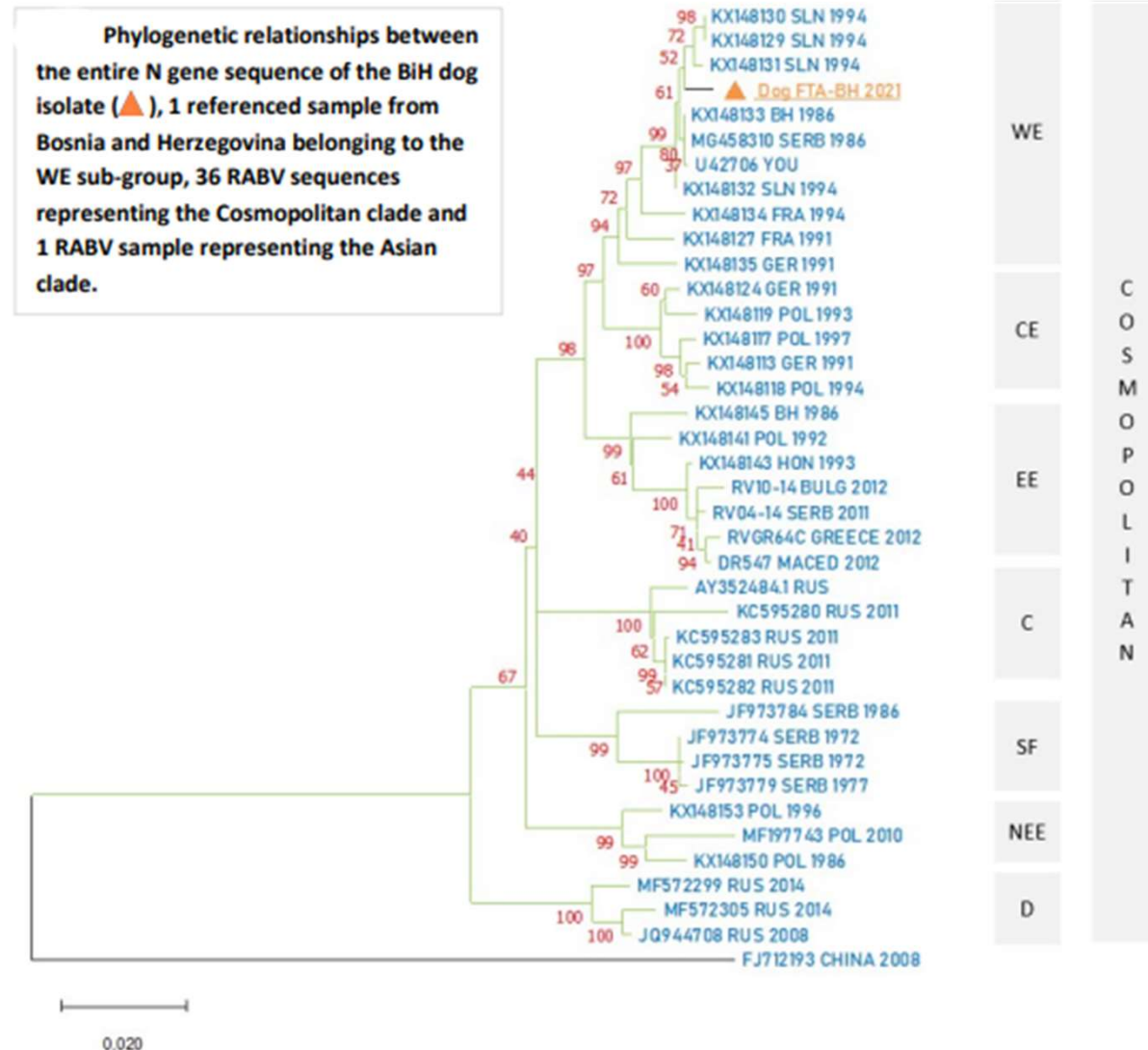
The sanger sequencing was performed by the company Eurofins genomics (Ebersberg, Germany).

Nucleotide analysis

- The sequences were assembled using the software Geneious Prime software platform (Geneious, France).
- Editing of the alignments was performed in MEGA-X.
- Percentage identities and similarity scores were determined in BIOEDIT.

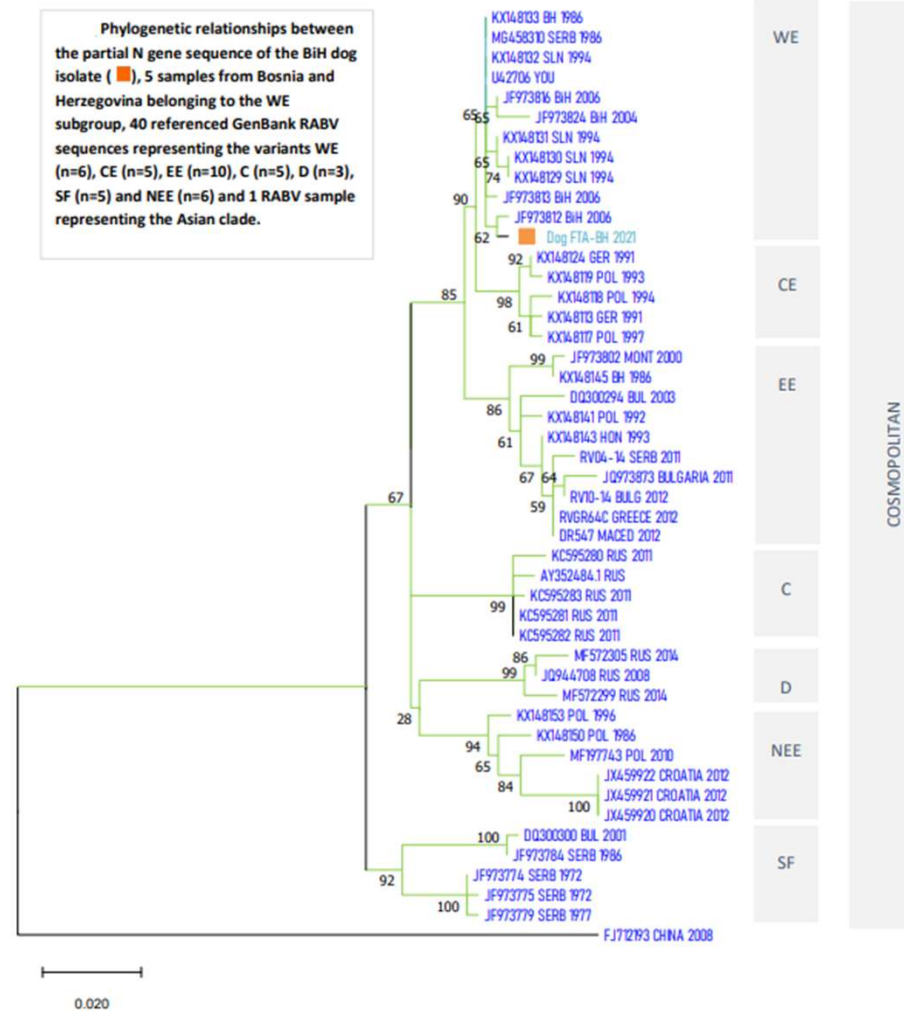
RESULT OF PHYLOGENETIC ANALYSIS OF THE BIH DOG SEQUENCE (1353-bp FRAGMENT) WITH REFERENCED RABV SEQUENCES

The phylogeny tree shows that the BiH dog sequence **belongs to the rabies sub-group WE** (bootstrap of 97) empirically representative of isolates from Western Europe [Germany (n=1) and France (n=2)], Slovenia (n=4), Serbia (n=1) and BiH (n=1).



PHYLOGENETIC ANALYSIS OF THE BIH DOG SEQUENCE (589-bp FRAGMENT) WITH REFERENCED RABV SEQUENCES

The phylogeny tree shows that the partial N gene sequence amplified from the BiH **dog** grouped within the **WE subgroup** (bootstrap of 90) formed by 5 referenced samples isolated in Bosnia and Herzegovina from 1986 to 2006, 4 samples from Slovenia isolated in 1994 and 1 from Serbia isolated in 1986.



Results of genetic characterization of the isolated rabies virus strain

- Sequencing of the sample confirms that the positive dog in Srebrenica was infected with a **wild rabies virus strain**.
- Genetic characterization of the rabies virus strain showed the sample grouped within the subgroup WE formed by Western European RABV samples.
- **NOT A VACCINE STRAIN**

Case analysis

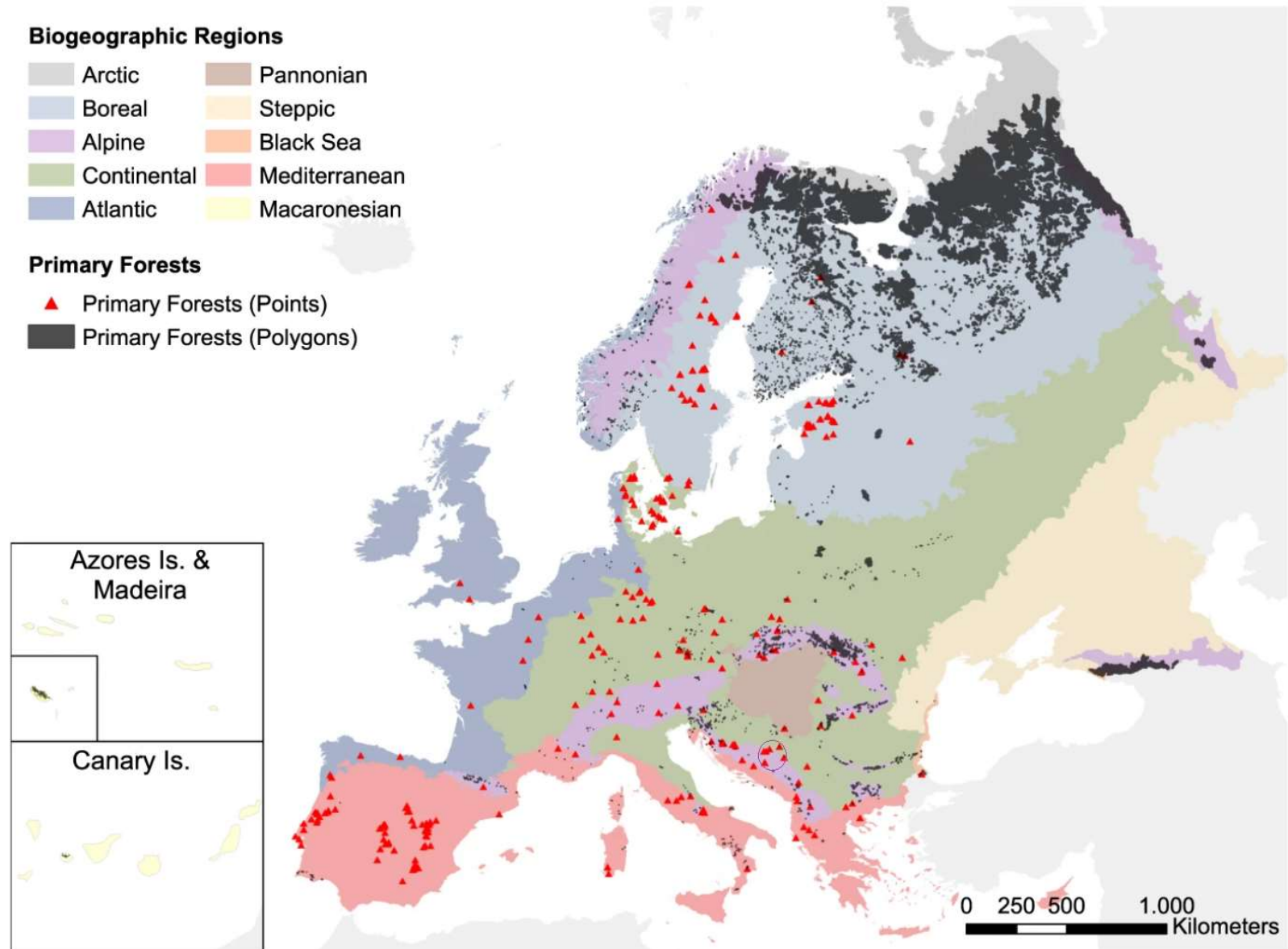
- We presume contact with infected wildlife.
- Oral rabies vaccination (ORV) stopped in 2018
- Adequate passive monitoring program was not implemented

Conclusions and recommendations

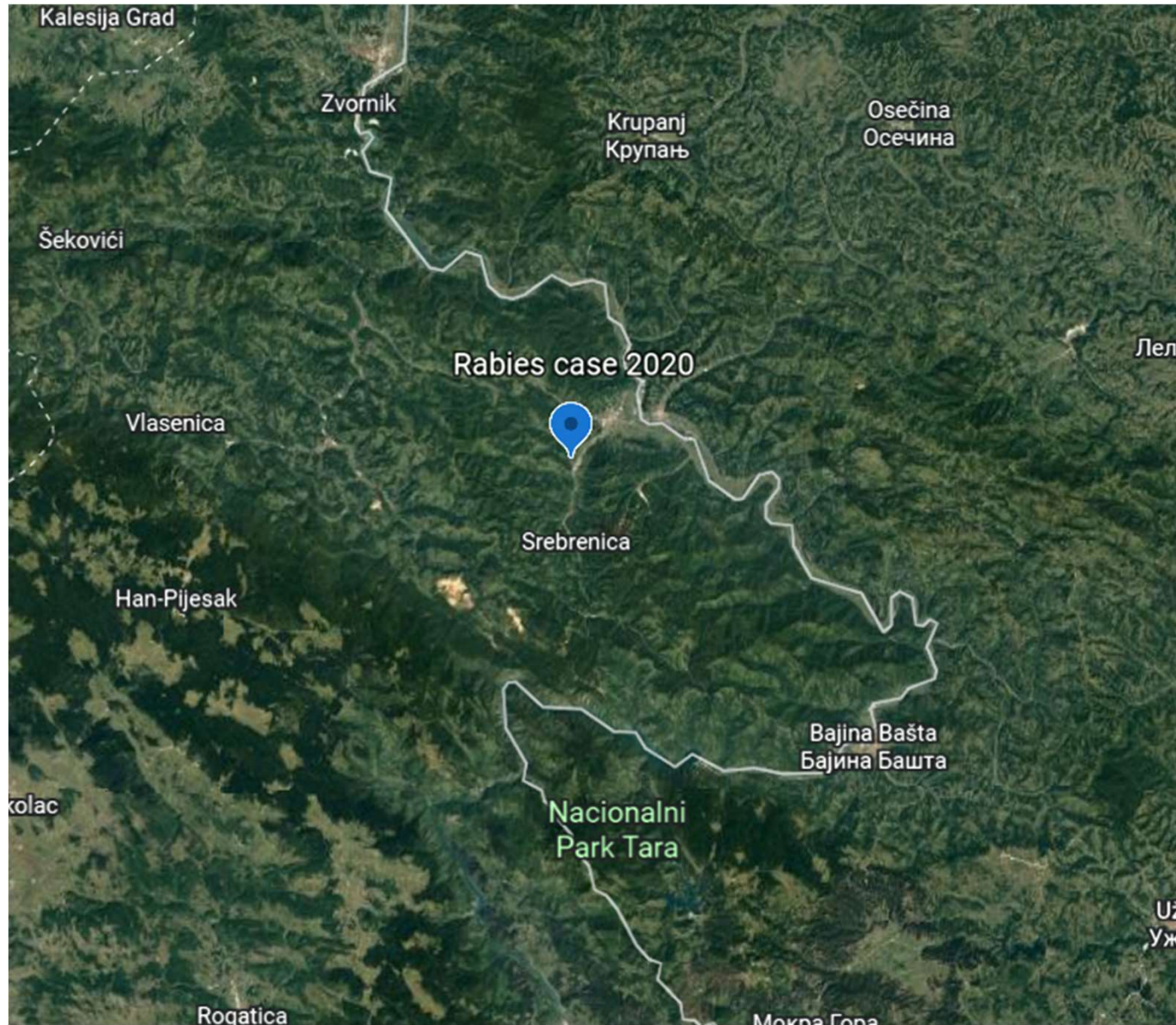
- Next planned ORV campaign was delayed and started in late 2021 (due to a plane crash)
- Bait distribution flights in the region were suspended during the investigation.
- In the close proximity of the outbreak there are several national parks, with limited access, where the volume of wood per hectare is up to 1.000 cubic meters (in the primary forest -old forests without significant disturbance or impact of Perućica national park)
- The average European volume of wood per hectare is 169 cubic meters.



From: [European primary forest database v2.0](#)



Overview of the primary forest patches contained in the EPFD v2.0. Both points and polygons were magnified to improve visibility.



- This case indicates that, despite of large-scale ORV campaigns, rabies in Bosnia and Herzegovina is not yet eliminated.
- No. of bites distributed in the regions with dense forests should be increased
- In recent years the number of processed rabies samples was low (active and passive surveillance), and the necessity of enhanced surveillance of wildlife in Bosnia and Herzegovina and neighboring Serbia is of utmost importance.

GAP analysis

Veterinary service response response to disease outbreak

- Veterinary service in BIH is well aware of the potential risks of disease spread,
- Fast implementation of given measures
- Good collaboration between entities (RS and FBIH) in emergency cases

GAP analysis

Nation reference laboratory

In last 10 years implemented new techniques:

- RT-PCR, qRT-PCR
- MIT has been replaced with cell culture
- Missing a continuous contract with a sequencing service provider
- Uncertain financing for passive monitoring and laboratory maintenance

GAP analysis

ORV (active monitoring)

- When continuously financed good implementation is guaranteed
- Depending on foreign donations

GAP analysis

Passive monitoring

- Was never founded by donations
- Before 2011. good public awareness
- Planned passive monitoring after the offset of ORV was not conducted
- Public awareness campaign about the importance of the testing after the offset of ORV was not conducted.

- **Thank you for your attention!**
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