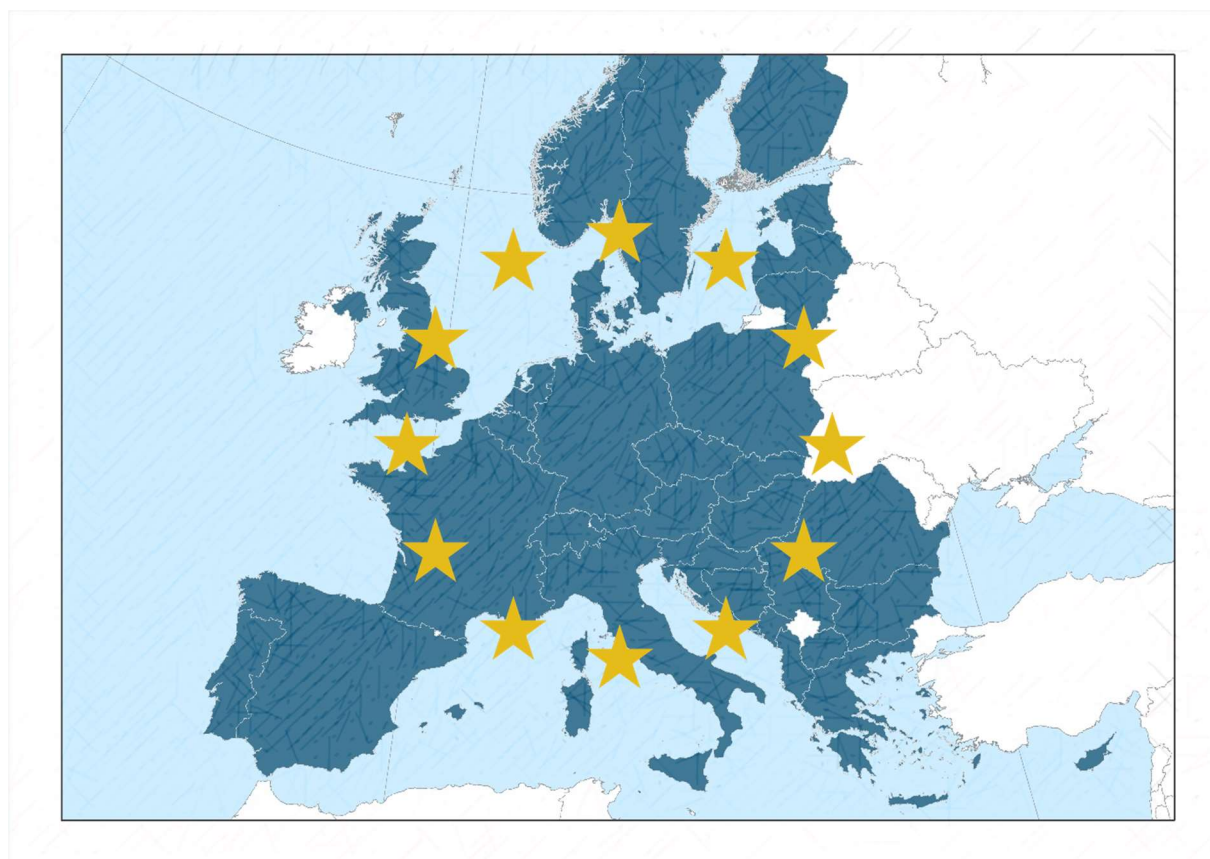


REVIEW OF THE ANALYSIS RELATED TO RABIES

DIAGNOSIS AND FOLLOW-UP OF ORAL

VACCINATION PERFORMED IN NRLS IN 2020



EURL for Rabies

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Report authored by: Emmanuelle Robardet

Validated by: Franck Boué

Data Submitted and validated by:

NRL of Albania: Valentin Shtjefni
NRL of Austria: Zoltán Bagó
NRL of Belgium: Sanne Terryn
NRL of Bosnia and Herzegovina - Sarajevo: Toni Eterovic
NRL of Bulgaria: Reneta Petrova
NRL of Croatia: Ivana Lojkić
NRL of Cyprus: Vasiliki Christodoulou
NRL of Czech Republic: Vlastimil Křivda
NRL of Denmark: Thomas Bruun Rasmussen
NRL of Estonia: Katrin Mähar
NRL of Finland: Tiina Nokireki
NRL of France: Alexandre Servat
NRL of Germany: Thomas Müller
NRL of Greece: Konstantia Tasioudi
NRL of Hungary: Peter Malik
NRL of Italy: Paola De Benedictis
NRL of Latvia: Žanete Zommere
NRL of Lithuania: Ingrida Jaceviciene
NRL of Luxembourg: Serge Losch
NRL of Montenegro: Nikola Pejovic
NRL of Netherlands (The): Phaedra Eblé
NRL of North Macedonia: Iskra Cvetkovikj
NRL of Norway: Irene Ørpetveit
NRL of Poland: Marcin Smreczak
NRL of Portugal: Isabel Almeida
NRL of Romania: Vlad Vuta
NRL of Serbia - Belgrade: Vesna Milićević
Pasteur Institute of Novi Sad (Serbia): Nenad Vranjes
NRL of Slovakia: Slavomir Jerg
NRL of Slovenia: Peter Hostnik
NRL of Spain: Juan E. Echevarría
NRL of Sweden: Viktor Ahlberg
NRL of Switzerland: Zaroni Reto
NRL of United Kingdom: Lorraine M. McElhinney

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An annual activity online questionnaire was submitted to all National Reference Laboratories (NRLs) on last February 2021 to collect and collate data on methods used and results of tests carried out in the European Union in the frame of rabies control programmes (Commission regulations (EU) N° 737/2008 and N°415/2013).

This document reviews the 2020 analysis performed in 25 European Union member states and in 8 third countries involved in a rabies control programme.

1 GENERAL DATA

In 2020, the European Union National Reference Laboratories (NRLs) network for Rabies included 27 member states. Twenty-five NRLs participated in the investigation. To ensure a better overview, some third countries of interest or involved in Oral Rabies Vaccination (ORV) programmes were invited to take part in the study. Nine laboratories (from Albania, Bosnia Herzegovina, United Kingdom, Montenegro, Republic of North Macedonia, 2 laboratories from Serbia, Switzerland and Norway) were added in the dataset. Finally, the survey enrolled 34 laboratories (Figure 1).

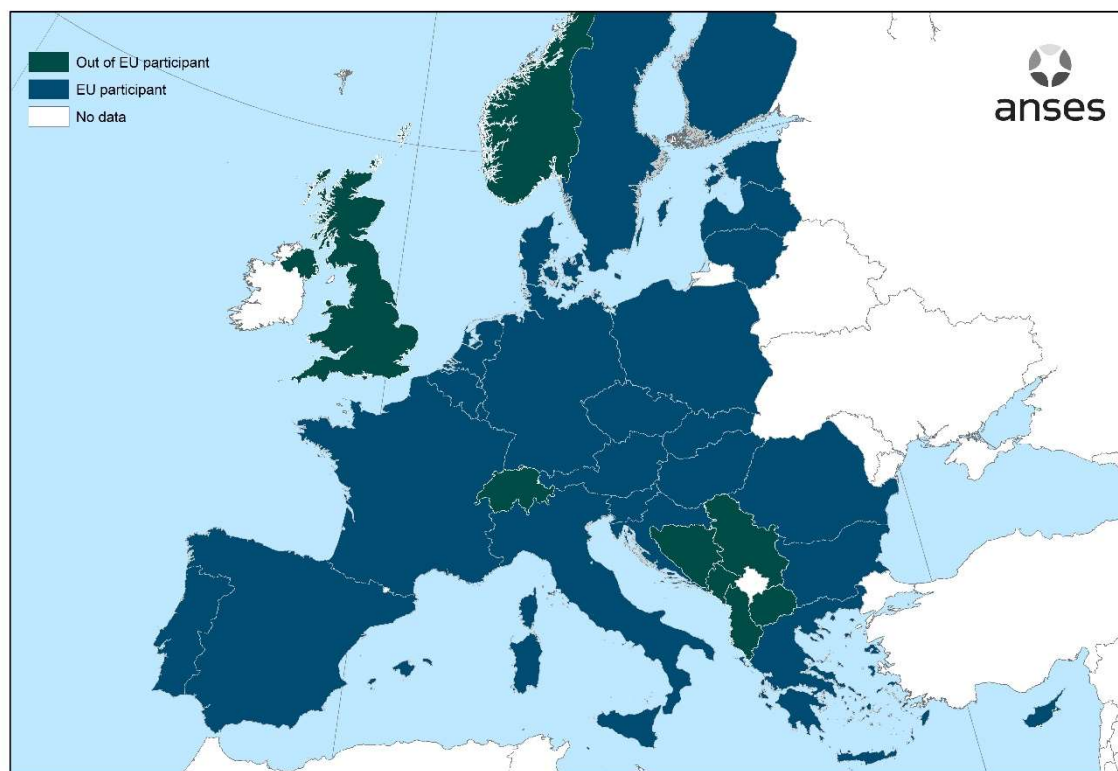


Figure 1: Map of the participating countries in the 2020 review.

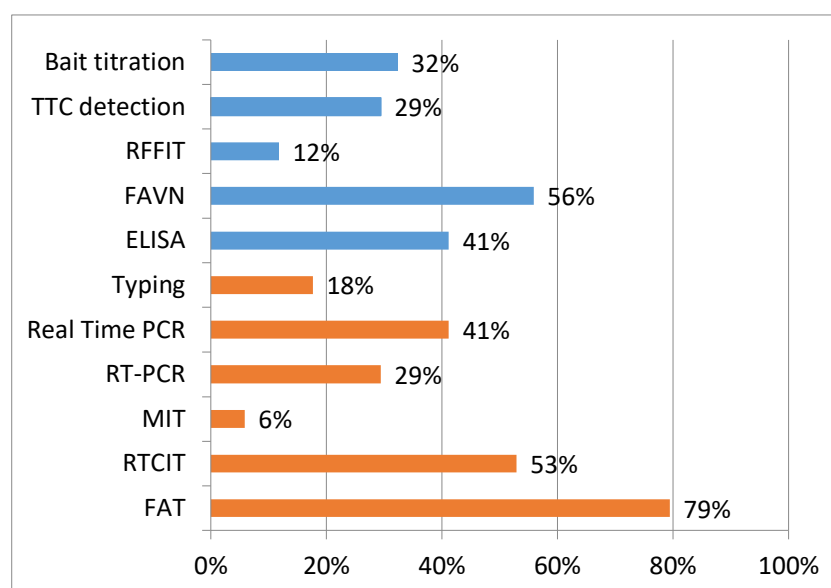
2 QUALITY ASSURANCE

In 2020, 31 on 34 laboratories (91%) of which 24/25 EU NRLs declared being accredited laboratory according to the ISO EN 17025 standards.

Accrediated laboratory for rabies are commonly accredited for various combinations of techniques. The most widely used techniques operated under quality mangement sytem are the FAT (79% of laboratories accredited, 88% for EU NRLs), the FAVN test (56%; 65% for EU NRLs) and the RTCIT (53%; 62 %for EU NRLs) (Figure 2).

Thirty-eight percent (13/34) of participating national laboratories and 11/25 EU NRLs (44%) are working in BSL3 facilities.

a)



b)

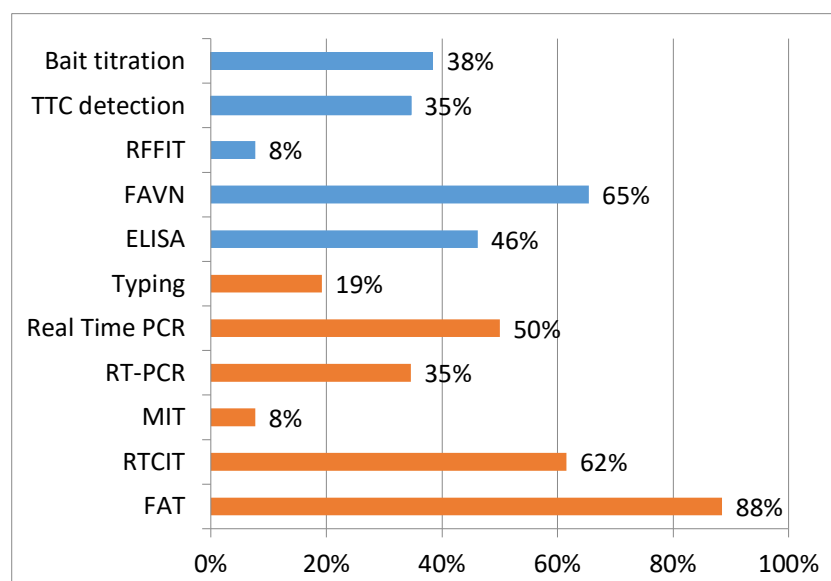


Figure 2: Percentage of laboratories (a. All laboratories; b. EU NRLs) accredited for the different techniques related to rabies field (diagnosis techniques in orange and monitoring techniques in blue).

3 RABIES LABORATORY NETWORK AND DIAGNOSIS DECISION TREE

Among participating laboratories, 6/33 (18%) laboratories are heading a regional laboratory network where rabies diagnosis analysis are also implemented (Figure 3). In three countries (Bosnia-Herzegovina, France and Serbia), rabies diagnosis in animals is performed in a different laboratory, in case of animal involving a human exposure mainly.

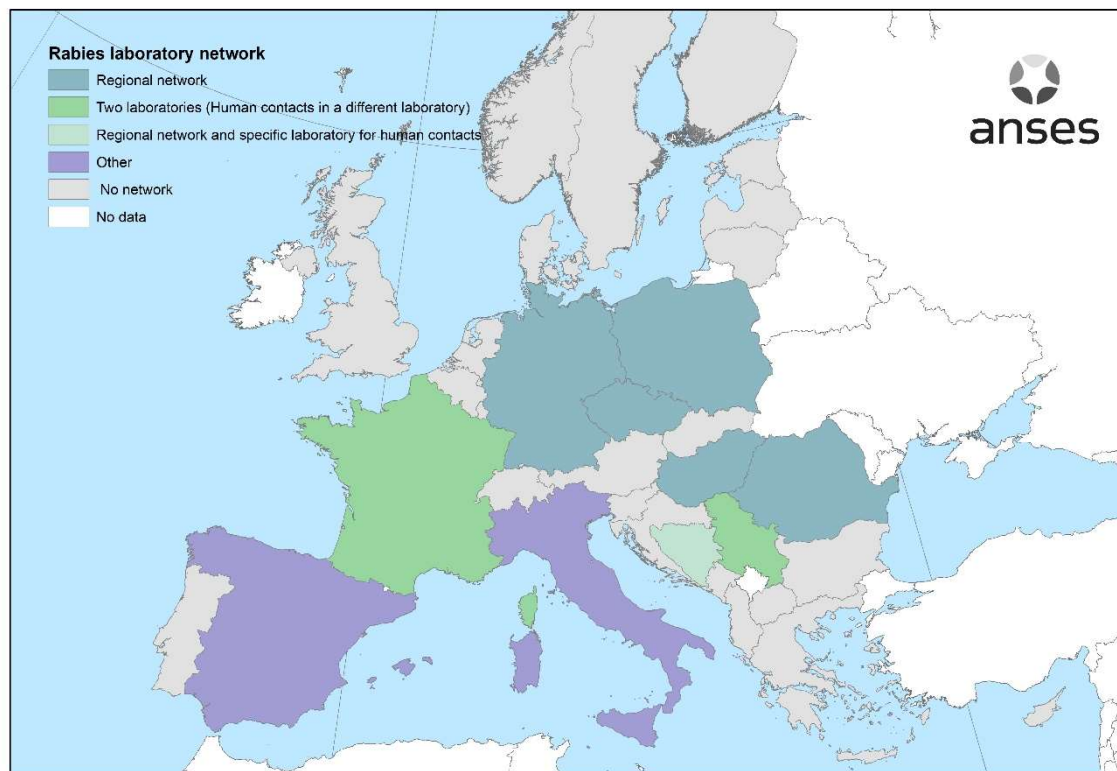


Figure 3: Organisation of rabies laboratory networks.

According to the result survey, most of the confirmatory tests used for rabies diagnosis are molecular biology tools. First test assessed is generally the FAT (23/31), the Real Time RT-PCR tests is commonly used as first confirmatory test (11/27) while the second confirmatory test is more commonly the conventional RT-PCR (8/18). The third confirmatory test, when used, is most commonly the RTCIT (3/13).

Globally, the chain of diagnosis tests used by laboratories of this review vary widely. As example, for the diagnosis of a sample enrolled in human exposure, no less than 23 different chains of tests have been announced (Table 1) showing that a high disparity of rabies diagnosis decision trees coexists within the network.

Table 1: Successive confirmatory tests used by participating laboratories in case of human exposure.

Chain of tests	First test	Second test	Third test	Fourth test	N of laboratories
1	FAT	Real Time RT-PCR			4
2	FAT	Real Time RT-PCR	Conventional RT-PCR	RTCIT	3
3	FAT	Real Time RT-PCR	Conventional RT-PCR		3
4	FAT	RTCIT	Real Time RT-PCR	Conventional RT-PCR	2
5	FAT	RTCIT			2
6	FAT	RTCIT	Conventional RT-PCR	Other	1
7	FAT	RTCIT	Conventional RT-PCR	Real Time RT-PCR	1
8	FAT	RTCIT	MIT		1
9	FAT	RTCIT	Real Time RT-PCR		1
10	FAT	Conventional RT-PCR	RTCIT	Other	1
11	FAT	Conventional RT-PCR	RTCIT	Real Time RT-PCR	1
12	FAT	Conventional RT-PCR			1
13	FAT	MIT	RTCIT	Conventional RT-PCR	1
14	FAT	Other	Conventional RT-PCR	MIT	1
15	FAT	Real Time RT-PCR	RTCIT		1
16	FAT	Real Time RT-PCR	MIT		1
17	FAT				1
18	Conventional RT-PCR	FAT	RTCIT		1
19	Real Time RT-PCR	FAT	RTCIT		1
20	Real Time RT-PCR	RTCIT			1
21	Real Time RT-PCR	Conventional RT-PCR	FAT		1
22	Real Time RT-PCR	Real Time RT-PCR			1
23	Real Time RT-PCR				1

4 RABIES DIAGNOSIS IN THE FRAME OF RABIES SURVEILLANCE IN MAMMALS EXCLUDING BATS (PASSIVE SURVEILLANCE ONLY)

The FAT (OIE, 2018; WHO; 2018a) remains the most commonly used technique (representing 81% of the total amount of diagnostic tests performed during the year and used by 88% of laboratories) (Table 2 for all mammals excluding bats, Table 3 for wildlife excluding bats, Table 4 for domestic animals).

The Real Time is the second technique of choice used by laboratories (58% of laboratories and 6% of the total amount of diagnosis tests performed during the year) while RTCIT still represent the second most used technique when considering the total number of analysis (10%) (Table 2). Still 12% of laboratories (n=4) are using the MIT in their rabies diagnosis process, although, for ethical reasons, it is recommended whenever possible to replace MIT by another confirmatory technique (OIE, 2018).

Number of animals analysed in the frame of rabies surveillance programme (bats excluded) varied from 0 to 3 568 samples at country level. Thirteen positive cases were identified for a total of 19 402 FAT representing 0.0007% of positive within FAT.

Table 2: Number of tests performed per country (NRL and regional laboratories data) in 2020 in the frame of rabies diagnosis (**mammals excluding bats and passive surveillance only**)

(Green box: number of tests; red box: number of positive cases;

*: including one EBLV-1 strain; **: including one WCBV strain). ^h: For Hungarian NRL, MIT is no longer routinely used since 2020.

Code iso2	Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
AL	Albania	0	0	0	0	0	0	0
AT	Austria	201	37	0	37	37	0	0
BA	Bosnia and Herzegovina	22	0	0	6	0	0	1
BE	Belgium	76	0	0	0	0	0	0
BG	Bulgaria	178	0	0	0	0	0	0
CH	Switzerland	99	82	0	0	0	0	0
CY	Cyprus	0	0	0	0	0	0	0
CZ	Czechia	2889	0	0	111	7	0	0
DE	Germany	3568	0	0	0	107	0	0
DK	Denmark	0	0	0	0	2	0	0
EE	Estonia	634	10	0	0	154	0	0
ES	Spain	54	0	0	52	51	1	1
FI	Finland	141	56	0	0	0	0	0
FR	France	1338	2	0	2	2	2	2*
GB	United Kingdom	12	0	0	0	4	0	0
GR	Greece	773	0	0	0	115	0	0
HR	Croatia	476	0	0	5	0	0	0
HU	Hungary	872	436	7 ^h	0	534	0	0
IT	Italy	2747	500	0	396	47	14	1**
LT	Lithuania	230	73	0	0	10	0	0
LU	Grand Duchy of Luxembourg	67	0	0	0	0	0	0
LV	Latvia	1128	0	0	0	48	0	0
ME	Montenegro	8	0	0	0	0	0	0
MK	North Macedonia	10	2	0	2	2	0	0
NL	The Netherlands	9	0	0	0	9	0	0
NO	Norway	0	0	0	0	4	0	0
PL	Poland	2563	1147	0	14	3	7	7
PT	Portugal	3	3	0	3	0	0	0
RO	Romania	653	4	153	5	0	5	5
RS	Republic of Serbia	69	0	34	0	0	0	0
SE	Sweden	10	0	0	0	57	0	0
SI	Slovenia	232	25	0	0	0	0	0
SK	Slovakia	340	0	0	0	150	0	0
	Total (n analysis)	19402	2377	204	633	1343	29	17
	Total (% analysis)	81%	10%	1%	3%	6%	0%	
	Total (n laboratories)	29	13	4	11	19	5	
	Total (% laboratories)	88%	39%	12%	33%	58%	15%	

Table 3: Number of tests performed per country (NRL and regional laboratories data) in 2020 in the frame of rabies diagnosis (**wild animals excluding bats and passive surveillance only**)
(Green box: number of tests; red box: number of positive cases).

Code iso2	Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
AL	Albania	0	0	0	0	0	0	0
AT	Austria	153	4	0	4	4	0	0
BA	Bosnia and Herzegovina	11	0	0	3	0	0	0
BE	Belgium	50	0	0	0	0	0	0
BG	Bulgaria	166	0	0	0	0	0	0
CH	Switzerland	26	0	0	0	0	0	0
CY	Cyprus	0	0	0	0	0	0	0
CZ	Czechia	2782	0	0	25	1	0	0
DE	Germany	3450	0	0	0	72	0	0
DK	Denmark	0	0	0	0	1	0	0
EE	Estonia	317	5	0	0	14	0	0
ES	Spain	14	0	0	14	14	0	0
FI	Finland	120	35	0	0	0	0	0
FR	France	41	0	0	0	0	0	0
GB	United Kingdom	0	0	0	0	0	0	0
GR	Greece	748	0	0	0	96	0	0
HR	Croatia	404	0	0	0	0	0	0
HU	Hungary	333	64	1	0	99	0	0
IT	Italy	2176	154	0	72	15	9	0
LT	Lithuania	193	47	0	0	5	0	0
LU	Grand Duchy of Luxembourg	67	0	0	0	0	0	0
LV	Latvia	1103	0	0	0	28	0	0
ME	Montenegro	2	0	0	0	0	0	0
MK	North Macedonia	5	0	0	0	0	0	0
NL	The Netherlands	1	0	0	0	1	0	0
NO	Norway	0	0	0	0	3	0	0
PL	Poland	1408	262	0	7	2	5	5
PT	Portugal	0	0	0	0	0	0	0
RO	Romania	365	2	54	1	0	1	1
RS	Republic of Serbia	32	0	5	0	0	0	0
SE	Sweden	1	0	0	0	2	0	0
SI	Slovenia	181	0	0	0	0	0	0
SK	Slovakia	202	0	0	0	23	0	0
Total		14351	573	60	126	380	15	6

Table 4: Number of tests performed per country (NRL and regional laboratories data) in 2020 in the frame of rabies diagnosis (**domestic animals**)

(Green box: number of tests; red box: number of positive cases; *: one imported case included; μ : one EBLV-1 case included; $\mu\mu$: one WCBV case included).

Code iso2	Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
AL	Albania	0	0	0	0	0	0	0
AT	Austria	48	33	0	33	33	0	0
BA	Bosnia and Herzegovina	11	0	0	3	0	0	1
BE	Belgium	26	0	0	0	0	0	0
BG	Bulgaria	12	0	0	0	0	0	0
CH	Switzerland	73	82	0	0	0	0	0
CY	Cyprus	0	0	0	0	0	0	0
CZ	Czechia	107	0	0	86	6	0	0
DE	Germany	118	0	0	0	35	0	0
DK	Denmark	0	0	0	0	1	0	0
EE	Estonia	317	5	0	0	140	0	0
ES	Spain	40	0	0	38	37	1	1*
FI	Finland	21	21	0	0	0	0	0
FR	France	1297	2	0	2	2	2	2* μ
GB	United Kingdom	12	0	0	0	4	0	0
GR	Greece	25	0	0	0	19	0	0
HR	Croatia	72	0	0	5	0	0	0
HU	Hungary	539	372	6	0	435	0	0
IT	Italy	571	346	0	324	32	5	1 $\mu\mu$
LT	Lithuania	37	26	0	0	5	0	0
LU	Grand Duchy of Luxembourg	0	0	0	0	0	0	0
LV	Latvia	25	0	0	0	20	0	0
ME	Montenegro	6	0	0	0	0	0	0
MK	North Macedonia	5	2	0	2	2	0	0
NL	The Netherlands	8	0	0	0	8	0	0
NO	Norway	0	0	0	0	1	0	0
PL	Poland	1155	885	0	7	1	2	2
PT	Portugal	3	3	0	3	0	0	0
RO	Romania	288	2	99	4	0	4	4
RS	Republic of Serbia	37	0	29	0	0	0	0
SE	Sweden	9	0	0	0	55	0	0
SI	Slovenia	51	25	0	0	0	0	0
SK	Slovakia	138	0	0	0	127	0	0
Total		5051	1804	134	507	963	14	11

Sampling effort in the frame of rabies surveillance has been estimated by dividing the number of FAT tests (wildlife and domestic animals separately) by the total area (km²) of the country multiplied by 100. This provided a surveillance indicator of the number of samples analysed for 100 km² in each country (for wildlife excepted bats and for domestic animals). As the surveillance system depends upon the

epidemiological situation in the country, we divided countries in four groups according to the rabies situation or implementation or not of oral vaccination programme (Table 5).

Table 5: Number of FAT tests performed in the frame of rabies surveillance programmes (mammals excluding bats and passive surveillance only) per country for 100 km² in wildlife (excluding bats) and in domestic animals. Countries are classified in groups according to their rabies situation or implementation of oral vaccination programmes or not.

Wildlife			Domestic Animals		
Country	Wildlife sampling	Group	Country	Domestic animals sampling	Group
Poland	0.45	A	Poland	0.37	A
Romania	0.16	A	Romania	0.12	A
Latvia	1.71	B	Estonia	0.70	B
Croatia	0.71	B	Hungary	0.58	B
Estonia	0.70	B	Slovakia	0.28	B
Greece	0.57	B	Croatia	0.13	B
Slovakia	0.41	B	Lithuania	0.06	B
Hungary	0.36	B	Republic of Serbia	0.04	B
Lithuania	0.30	B	Latvia	0.04	B
Bulgaria	0.15	B	Greece	0.02	B
Finland	0.04	B	Bulgaria	0.01	B
Republic of Serbia	0.04	B	Finland	0.01	B
North Macedonia	0.02	C	Montenegro	0.04	C
Bosnia and Herzegovina	0.02	C	North Macedonia	0.02	C
Montenegro	0.01	C	Bosnia and Herzegovina	0.02	C
Germany	0.97	D	Germany	0.03	D
Czechia	3.53	E	Slovenia	0.25	E
Grand Duchy of Luxembourg	2.59	E	France	0.20	E
Slovenia	0.89	E	Italy	0.19	E
Italy	0.72	E	Switzerland	0.18	E
Austria	0.18	E	Czechia	0.14	E
Belgium	0.16	E	Belgium	0.09	E
Switzerland	0.06	E	Austria	0.06	E
France	0.01	E	The Netherlands	0.02	E
Spain	0.00	E	Spain	0.01	E
The Netherlands	0.00	E	United Kingdom	0.00	E
Sweden	0.00	E	Portugal	0.00	E
Cyprus	0.00	E	Sweden	0.00	E
Denmark	0.00	E	Cyprus	0.00	E
United Kingdom	0.00	E	Denmark	0.00	E
Norway	0.00	E	Grand Duchy of Luxembourg	0.00	E
Portugal	0.00	E	Norway	0.00	E
Albania	0.00	E	Albania	0.00	E

The groups range according following criteria:

Group A: Countries with at least one endemic positive case in the year n⁻¹ (2019).

Group B: Countries excluded from group A (no endemic positive case in the year n⁻¹) with at least one endemic positive case in a bordering country in the year n⁻¹ (2019) and conducting ORV in 2020.

Group C: Countries excluded from group A and B (with no endemic positive case in a bordering country in the year n⁻¹) but conducting ORV in 2020.

Group D: Countries excluded from group A, B, C with at least one endemic positive case in a bordering country in the year n⁻¹ (2019) and not conducting ORV in 2020.

Group E: Countries excluded from groups A, B, C, D and E, not involved in ORV programmes.

5 RABIES CASES IN MAMMALS EXCLUDING BATS

In 2020, 5 of the 33 (15%) participating countries identified at least a positive endemic case (Figure 4) while two countries identified an imported dog case (France and Spain).

Thirteen endemic RABV cases including 12 in EU were recorded (Bosnia Herzegovina, Poland and Romania) showing an increase of detected RABV endemic cases compared to 2019 (5 in EU in 2019). Particularity of 2020 is the diversity of lyssavirus species detected, as one EBLV-1 case was detected in France and one WCBV case was detected in Italy both in a cat. Following a retrospective study of death due to encephalitis of unknown origin, one EBLV-1 human case with death dated in 2019 has been identified in France as EBLV-1 origin in 2020. This is the first human case in the European Union of EBLV-1 origin.

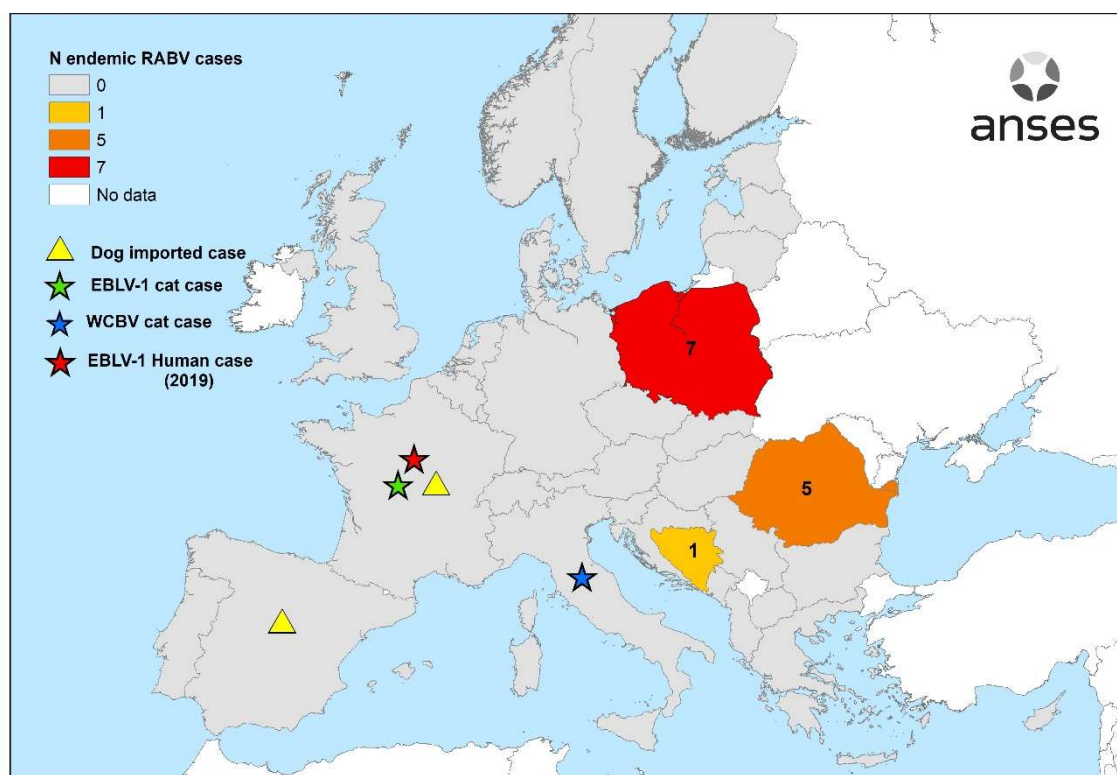


Figure 4: Number of reported rabies cases per country in mammals excluding bats in 2020. The location of isolated symbols is not exact but arbitrarily positioned in the center of the concerned country.

6 RABIES CASES IN BATS

Eighteen countries (55%) performed rabies diagnosis on bats.

First line rabies diagnosis technique used to identify a positive case is the FAT (9 countries) or a PCR technique (9 countries).

The number of samples tested throughout 2020 varied from 1 to 416 tests for FAT and varied from 1 to 564 tests for PCR (Table 6). The most implicated countries in rabies surveillance in bats are principally located in Western Europe where a total of 34 bat cases were detected (compared to 39 detected in 2019) (Figure 5).

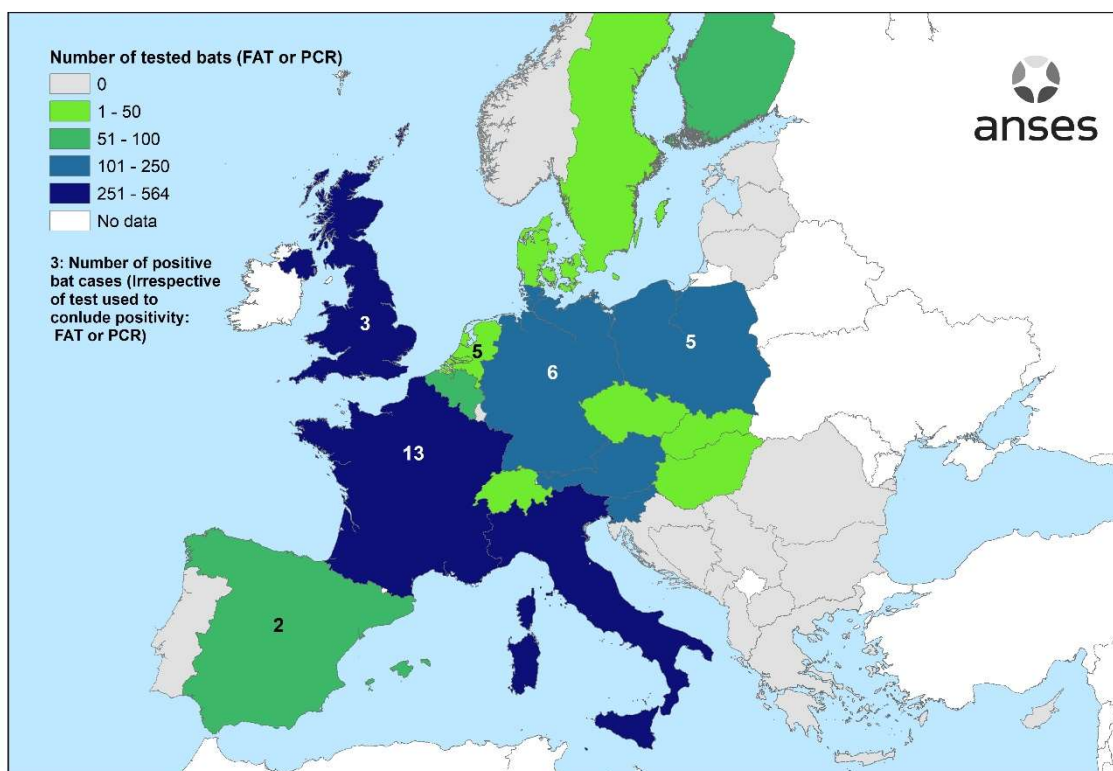


Figure 5: Number of bats tested by FAT or PCR (maximum of tests whatever the category) per country in 2020 in the frame of passive surveillance programme and number of associated positive cases.

Table 6: Number of tests performed per country (NRLs and regional laboratories data) in 2020 in the frame of passive surveillance on bats (Green box: number of tests; red box: number of positive cases)

Code iso2	Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
AL	Albania	0	0	0	0	0	0	0
AT	Austria	191	4	0	5	5	0	0
BA	Bosnia and Herzegovina	0	0	0	0	0	0	0
BE	Belgium	0	0	0	0	94	0	0
BG	Bulgaria	0	0	0	0	0	0	0
CH	Switzerland	11	0	0	0	0	0	0
CY	Cyprus	0	0	0	0	0	0	0
CZ	Czechia	8	0	0	6	0	0	0
DE	Germany	84	0	0	0	129	0	6
DK	Denmark	0	0	0	0	13	0	0
EE	Estonia	0	0	0	0	0	0	0
ES	Spain	65	0	0	83	82	2	2
FI	Finland	78	0	0	0	0	0	0
FR	France	416	2	0	13	13	13	13
GB	United Kingdom	315	1	0	5	156	3	3
GR	Greece	0	0	0	0	0	0	0
HR	Croatia	0	0	0	0	0	0	0
HU	Hungary	9	7	0	0	9	0	0
IT	Italy	18	0	0	564	462	0	0
LT	Lithuania	0	0	0	0	0	0	0
LU	Grand Duchy of Luxembourg	0	0	0	0	0	0	0
LV	Latvia	0	0	0	0	0	0	0
ME	Montenegro	0	0	0	0	0	0	0
MK	North Macedonia	0	0	0	0	0	0	0
NL	The Netherlands	12	0	0	0	35	0	5
NO	Norway	0	0	0	0	0	0	0
PL	Poland	200	74	0	6	1	5	5
PT	Portugal	0	0	0	0	0	0	0
RO	Romania	0	0	0	0	0	0	0
RS	Republic of Serbia	0	0	0	0	0	0	0
SE	Sweden	0	0	0	0	7	0	0
SI	Slovenia	0	0	0	150	150	0	0
SK	Slovakia	1	0	0	0	1	0	0
Total		1408	88	0	832	1157	23	34

7 ORAL VACCINATION MONITORING

7.1 Oral Vaccination

Thirteen countries implemented oral vaccination campaigns in 2020 (Table 7 and Figure 6). All countries performed two ORV campaigns within the year (one in spring and one in autumn) except Finland, Montenegro and Republic of Serbia (one campaign, over december 2019-january 2020 for Serbia). A total of 30,933,760 baits were distributed over 1,227,612 km² (644 535 km² in autumn and 122 7612 km² in spring). Bait titration of vaccine batches before their release in the field was carried out by 11/12 countries (in their own or in another laboratory), one laboratory did not provided data. All the virus titres of the 89 batches were found satisfactory. Eighty five percent (11/13) of countries used Lysvulpen oral vaccines. No vaccine-induced cases were detected in 2020.

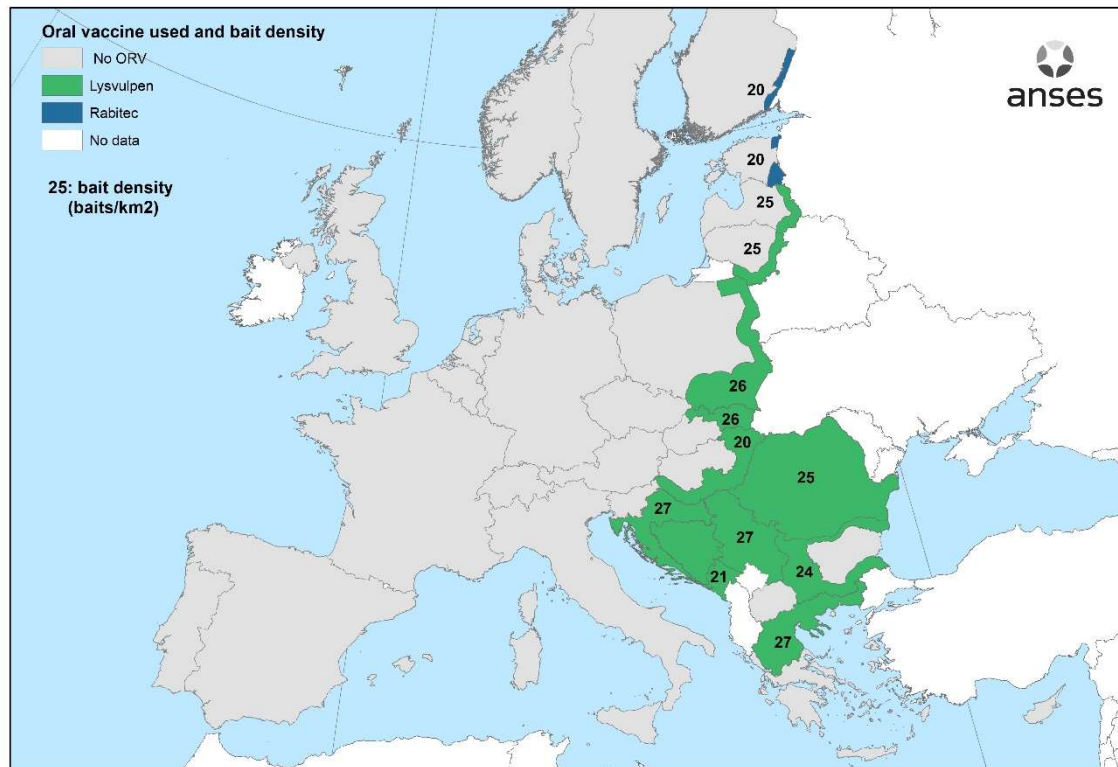


Figure 6: Oral vaccines used in the oral rabies vaccination and corresponding overall bait density per country. ORV area limitation kindly provided by the European Commission.

Table 7: Oral vaccination campaigns performed in European countries and number of oral vaccine batches analysed for titration in NRLs.

Code iso2	Country	Number of campaign	Vaccine Bait used	Total vaccinated area (km ²) (spring + autumn)	Total number of baits distributed (spring + autumn)	Bait density (baits/km ²)	N batches analysed
AL	Albania	NA	NA	NA	NA	NA	NA
AT	Austria						
BA	Bosnia and Herzegovina	1	Lysvulpen	NA	NA	NA	NA
BE	Belgium						
BG	Bulgaria	2	Lysvulpen	120,329	2,875,800	24	10
CH	Switzerland						
CY	Cyprus						
CZ	Czechia						
DE	Germany						
DK	Denmark						
EE	Estonia	2	Rabitec	12,200	244,800	20	ND
ES	Spain						
FI	Finland	1	Rabitec	9,000	180,000	20	1
FR	France						
GB	United Kingdom						
GR	Greece	2	Lysvulpen	109,596	2,991,495	27	12
HR	Croatia	2	Lysvulpen	106,658	2,827,100	27	9
HU	Hungary	2	Lysvulpen	83,940	1,678,800	20	5
IT	Italy						
LT	Lithuania	2	Lysvulpen	40,800	1,010,000	25	4
LU	Grand Duchy of Luxembourg						
LV	Latvia	2	Lysvulpen	38,490	961,800	25	4
ME	Montenegro	1	Lysvulpen	12,859	275,000	21	1
MK	North Macedonia						
NL	The Netherlands						
NO	Norway						
PL	Poland	2	Lysvulpen	178,368	4,659,578	26	12
PT	Portugal						
RO	Romania	2	Lysvulpen	426,000	10,823,211	25	28
RS	Republic of Serbia	1	Lysvulpen	60,996	1,671,000	27	0
SE	Sweden						
SI	Slovenia						
SK	Slovakia	2	Lysvulpen	28,376	735,176	26	3
Total		22		1,227,612	30,933,760	24	89

7.2 Rabies diagnosis performed on samples collected in the frame of ORV monitoring.

In the frame of ORV programmes, a target of 4 animals per 100 km² (two per campaign) collected in vaccinated areas has been established to evaluate the efficiency of ORV campaigns, by performing serological and bait uptake analysis (EC, 2002; EFSA, 2015). All laboratories are performing rabies diagnosis on this sampling scheme. Most of laboratories performed more diagnosis on wildlife samples collected in the frame of ORV monitoring (random sampling of 4 animals per 100 km²) than on wildlife samples issued from rabies surveillance programme (indicator animals i.e. animals found dead, or road-killed, or showing symptoms suggestive of rabies) (Figure 7).

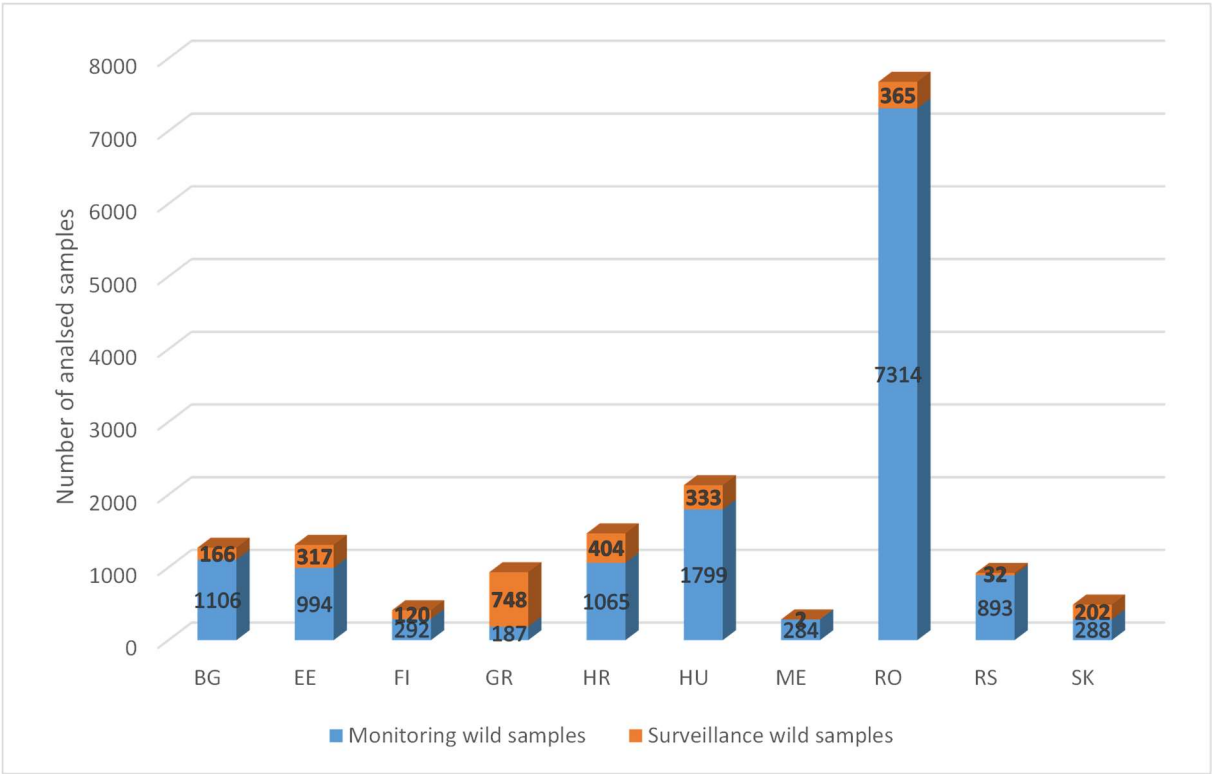


Figure 7: Number of diagnosis performed on wildlife in the frame of ORV monitoring (Hunted animals from ORV areas) and in the frame of passive surveillance (Indicators animals).

7.3 Percentage of seroconversion in the target population

Percentages of seroconversion ranged from 22% to 56% with a median of 34% (Figure 8). As in 2019, such data suggest that none of the countries reached the minimum 70% recommended vaccination coverage of the WHO (2018b).

Various tests are used for the serological analysis of wildlife within Europe: 11/13 laboratories (85%) used an ELISA commercial kit (8 laboratories used the BioPro kit, 5 used the Bio-Rad kit). In laboratory group not using ELISA commercial kits, Croatia used an mFAVN test and Slovakia used a “home made” ELISA test.

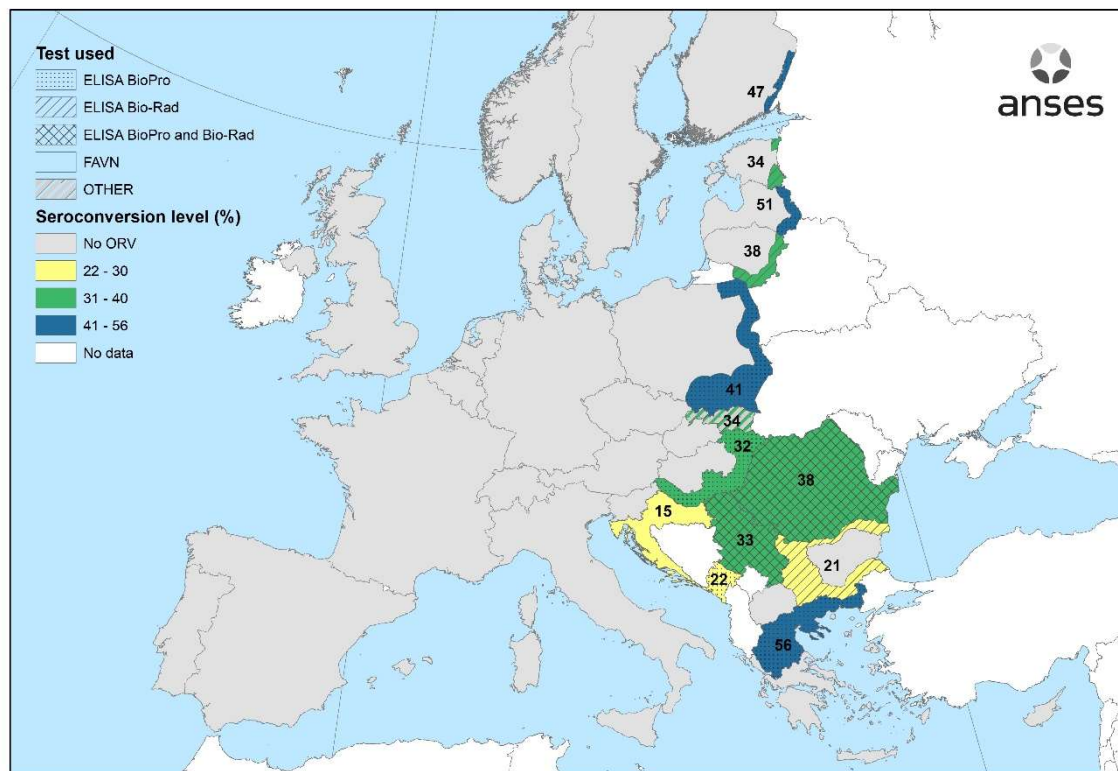


Figure 8: Proportion of sero-conversion in the target population and type of test used in 2020. ORV area limitation kindly provided by the European Commission.

7.4 Percentage of tetracycline presence in target population

The proportion of animals identified positive for the presence of tetracycline in teeth (bait uptake level) ranged from 58% to 87% with a median of 77% (Figure 9). 9/13 countries reported a bait uptake that exceeded 70%, which is in accordance with the minimum 70% recommended vaccination coverage of the WHO (2018b).

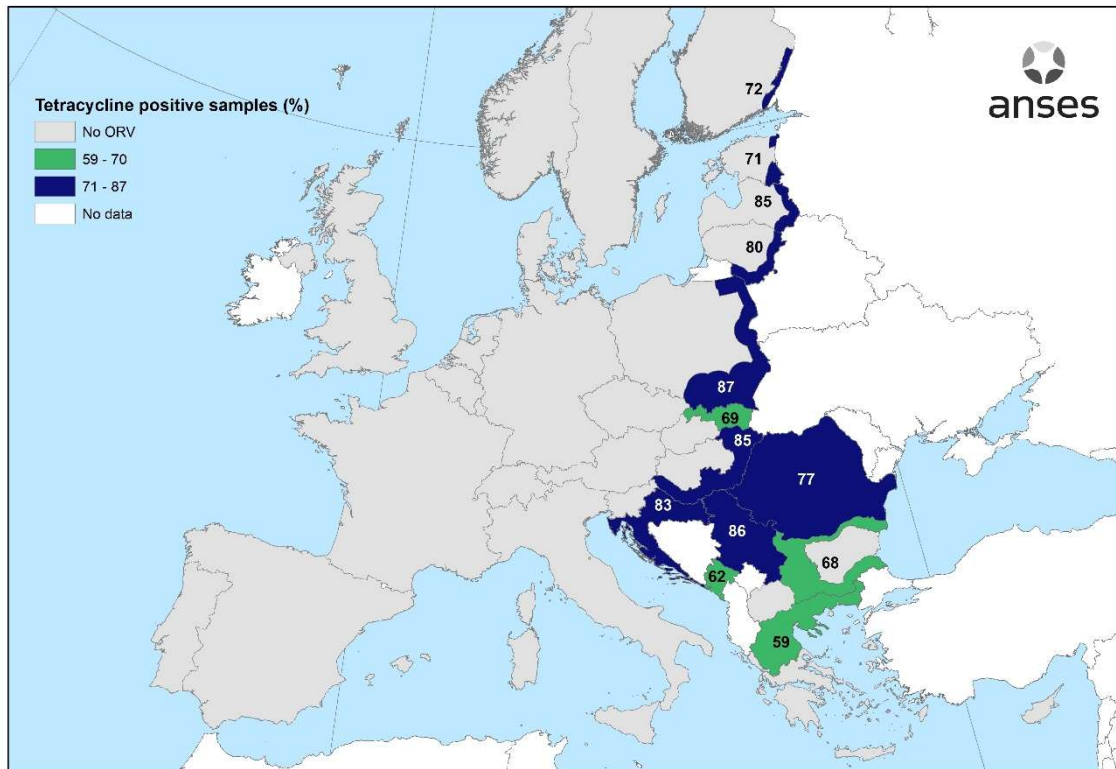


Figure 9: Proportion of positive samples for tetracycline presence in the target population in 2020. ORV area limitation kindly provided by the European Commission.

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