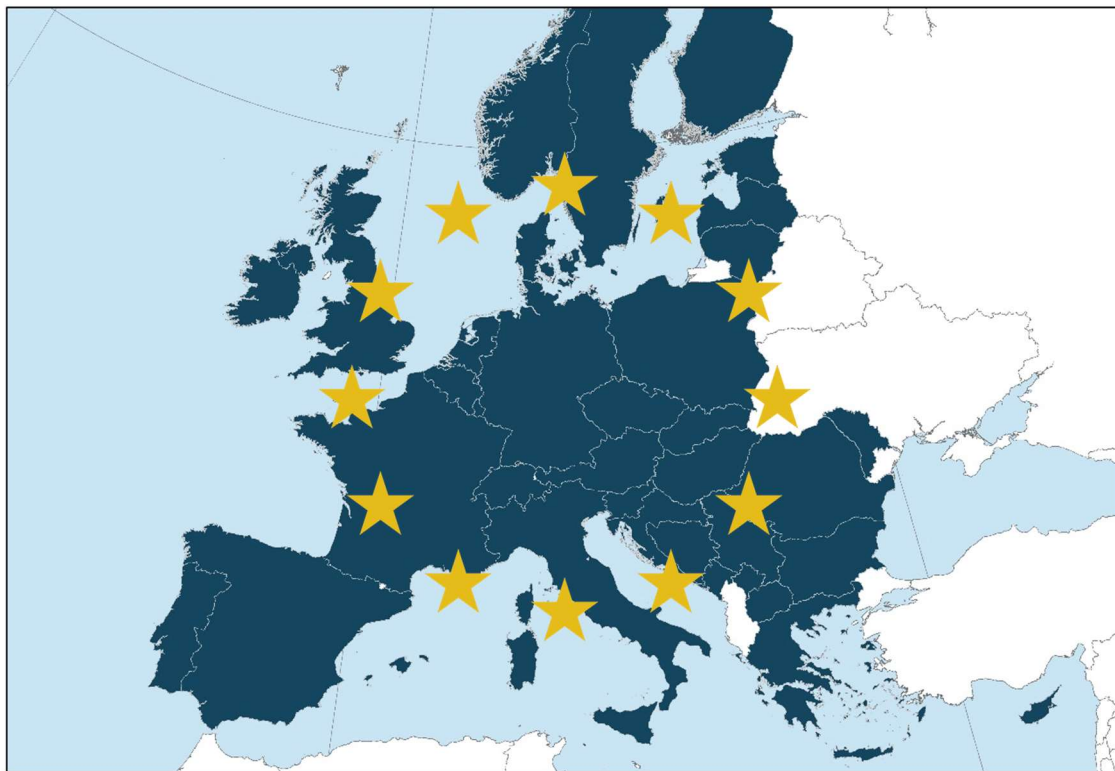


REVIEW OF THE ANALYSIS RELATED TO RABIES

DIAGNOSIS AND FOLLOW-UP OF ORAL

VACCINATION PERFORMED IN NRLS IN 2019



E. Robardet and F. Cliquet

August 2020

TABLE OF CONTENTS

1	GENERAL DATA.....	1
2	QUALITY ASSURANCE	2
3	RABIES LABORATORY NETWORK AND DIAGNOSIS DECISION TREE	3
4	RABIES DIAGNOSIS IN THE FRAME OF RABIES SURVEILLANCE IN MAMMALS EXCLUDING BATS (PASSIVE SURVEILLANCE ONLY).....	5
5	RABIES CASES IN MAMMALS EXCLUDING BATS	10
6	RABIES CASES IN BATS	11
7	ORAL VACCINATION MONITORING.....	13
7.1	ORAL VACCINATION.....	13
7.2	PERCENTAGE OF SEROCONVERSION IN THE TARGET POPULATION.....	15
7.3	PERCENTAGE OF TETRACYCLINE PRESENCE IN TARGET POPULATION	16

An annual activity online questionnaire was submitted to all National Reference Laboratories (NRLs) on last February 2020 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 2019 analysis performed in 27 European Union member states and in 8 third countries involved in a rabies control programme.

1 GENERAL DATA

In 2019, the European Union National Reference Laboratories (NRLs) network for Rabies included 28 member states. Twenty-seven 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 Bosnia Herzegovina, Kosovo, Moldavia, Montenegro, Republic of North Macedonia, 2 laboratories from Serbia, Switzerland and Norway) were added in the dataset. Finally, the survey enrolled 36 laboratories (Figure 1).

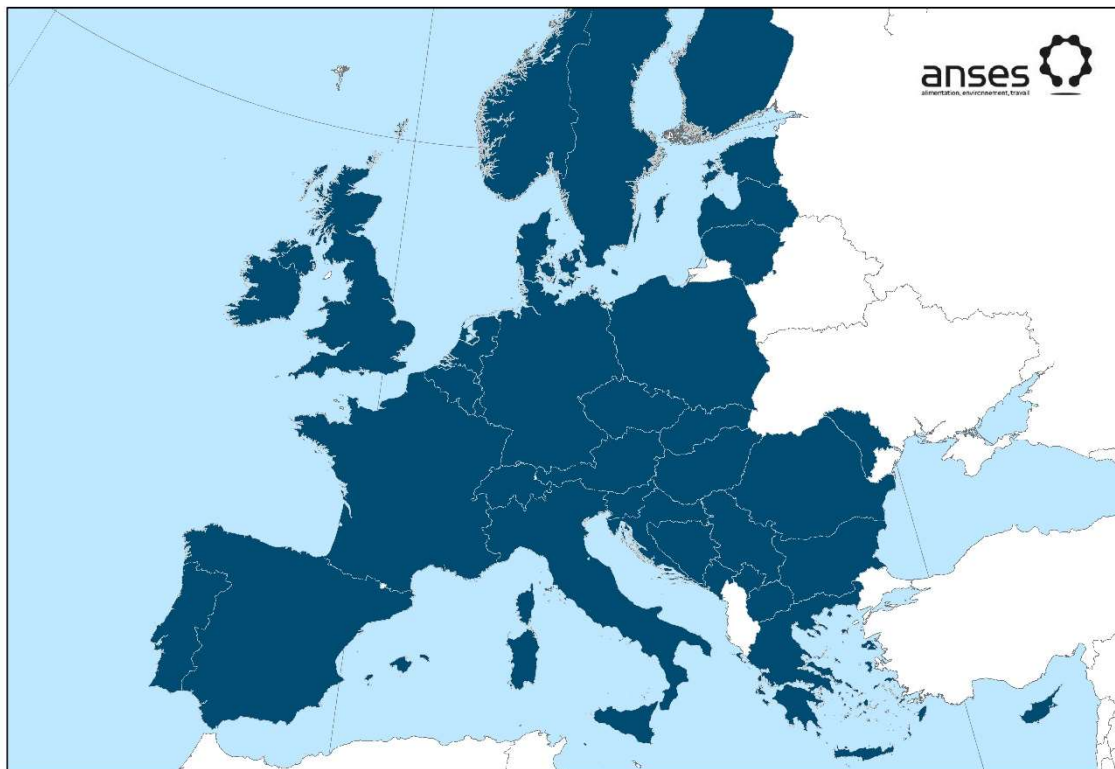


Figure 1: Map of the participating countries in the 2019 review

2 QUALITY ASSURANCE

In 2019, 33 on 36 laboratories (92%) of which 26/27 EU NRLs were accredited according to the ISO EN 17025 standards.

Each laboratory is accredited for various combinations of techniques. The most widely used techniques under quality assurance system management are the FAT (75% of laboratories accredited), the FAVN test (50%) and the RTCIT (50%) (Figure 2).

Thirty-nine percent (14/36) participating national laboratories and 13/27 EU laboratories (48%) are working in BSL3 facilities.

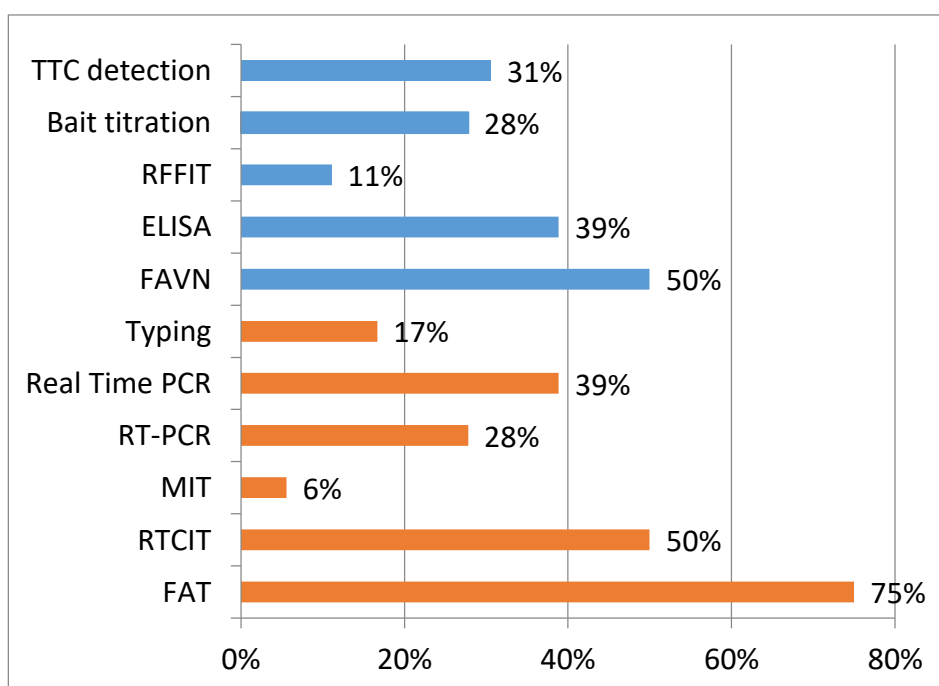


Figure 2: Percentage of laboratories accredited for the different techniques related to rabies field (diagnosis techniques in orange and monitoring techniques in blue).

3 RABIES LABORATORY NETWORK AND DIAGNOSIS DECISION TREE

Among participating laboratories, 12/36 (33%) laboratories are heading a regional laboratory network where rabies diagnosis analysis are also implemented. In two countries (France and Serbia), rabies diagnosis in animals is performed in a different laboratory in case of animal involving a human exposure.

The conclusions given by the laboratories for a rabies diagnosis test are either positive (the animal was infected) either negative (the animal was not infected). Among the participants, 47% of laboratories (17/36) have an additional result, i.e. consider the status 'inconclusive' for the results (FAT and/or RTCIT and/or RT-PCR and/or Real Time RT-PCR) in rabies diagnosis tests in their NRL and in regional laboratories (2/10).

Confirmatory tests are more commonly used in case of inconclusive result or in certain circumstances (imported animals, animals for which there was a human exposure, the OIE indeed recommends to confirm any primary positive result on samples at the origin of a human exposure). In case with human exposure, three laboratories did not perform a confirmatory test in their laboratory. In cases without human exposure, seven laboratories did not perform a confirmatory test.

According to the result survey, most of the confirmatory tests are molecular biology tools. First test assessed is generally the FAT (24/31), the Real Time RT-PCR tests is commonly used as first confirmatory test (13/28) 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 (2/6).

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 21 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 laboratories
1	FAT	Real Time RT-PCR	Conventional RT-PCR	RTCIT	2
2	FAT	Real Time RT-PCR	MIT	RTCIT	1
3	FAT	Real Time RT-PCR	Conventional RT-PCR		3
4	FAT	Real Time RT-PCR	RTCIT		3
5	FAT	Real Time RT-PCR			3
6	FAT	Conventional RT-PCR	RTCIT	MIT	1
7	FAT	Conventional RT-PCR	RTCIT	Real Time RT-PCR	1
8	FAT	Conventional RT-PCR			1
9	FAT	RTCIT	Real Time RT-PCR		2
10	FAT	RTCIT	Conventional RT-PCR		1
11	FAT	RTCIT	Conventional RT-PCR	Real Time RT-PCR	1
12	FAT	RTCIT			1
13	FAT	MIT			3
14	FAT				1
15	Real Time RT-PCR	Real Time RT-PCR	Conventional RT-PCR	FAT	1
16	Real Time RT-PCR	Conventional RT-PCR			1
17	Real Time RT-PCR	RTCIT			1
18	Real Time RT-PCR				1
19	RTCIT	Conventional RT-PCR	Real Time RT-PCR		1
20	RTCIT	Real Time RT-PCR			1
21	RTCIT				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 86% 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 (54% of laboratories and 4% 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 (9%) (Table 2). Still 11% 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 4 876 samples at country level. Nine positive cases in EU (including three cases of human imported rabies and one dog imported rabies case) and 97 (including one case of human imported rabies) out of EU were identified for a total of 27 578 FAT (0.004%).



Table 2: Number of tests performed per country (NRL and regional laboratories data) in 2019 in the frame of rabies diagnosis (**mammals excluding bats and passive surveillance only**) (Green box: number of tests; red box: number of positive cases; ND: No data; *: imported case). For number of cases in Romania: 1 case detected in the frame of the monitoring of ORV.

Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
Austria	247	33	0	34	34	0	
Belgium	44	0	0	0	0	0	
Bosnia and Herzegovina	20	0	0	0	0	0	
Bulgaria	902	0	0	0	0	0	
Croatia	488	0	0	44	0	0	
Cyprus	0	0	0	0	0	0	
Czechia	3193	0	0	189	0	0	
Denmark	5	1	0	0	7	0	
Estonia	237	0	0	0	28	0	
Finland	181	76	0	0	45	0	
France	3422	3	9	8	8	6	
Germany	3835	0	0	0	120	0	
Greece	789	0	0	0	157	0	
Grand Duchy of Luxembourg	115	0	0	0	0	0	
Hungary	1077	0	492	0	424	0	
Ireland	0	0	0	0	0	0	
Italy	4876	755	0	604	39	35	1 human*
Republic of Kosovo	0	0	0	0	0	0	
Latvia	1131	48	0	0	80	0	1 human*
Lithuania	245	77	0	0	0	0	
Montenegro	11	0	0	0	0	0	
North Macedonia	17	2	0	0	2	0	
Norway	1	0	0	0	20	0	1 human*
Poland	3240	1787	0	11	12	1	1
Portugal	0	0	0	6	6	0	
Republic of Moldova	0	0	0	0	0	0	96
Republic of Serbia	132	0	54	0	0	0	
Romania	888	10	547	3	0	3	4
Slovakia	374	0	0	0	176	0	
Slovenia	1377	35	0	0	0	0	
Spain	126	0	0	122	118	4	1 human* + 1 dog*
Sweden	3	0	0	0	39	0	
Switzerland	123	92	0	0	0	0	
The Netherlands	8	0	0	0	8	0	
United Kingdom	471	7	0	4	203	4	
Total (n analysis)	27578	2926	1102	1025	1526	53	
Total (% analysis)	81%	9%	3%	3%	4%	0%	
Total (n laboratories)	30	13	4	10	19	6	
Total (% laboratories)	86%	37%	11%	29%	54%	17%	



Table 3: Number of tests performed per country (NRL and regional laboratories data) in 2019 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; ND: No data; *: vaccine induced case)

Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
Austria	196	5	0	6	6	0	
Belgium	13	0	0	0	0	0	
Bosnia and Herzegovina	3	0	0	0	0	0	
Bulgaria	887	0	0	0	0	0	
Croatia	374	0	0	5	0	0	
Cyprus	0	0	0	0	0	0	
Czechia	3043	0	0	39	0	0	
Denmark	2	0	0	0	2	0	
Estonia	207	0	0	0	28	0	
Finland	90	20	0	0	45	0	
France	2066	0	0	8	8	6	
Germany	3735	0	0	0	71	0	
Greece	757	0	0	0	138	0	
Grand Duchy of Luxembourg	112	0	0	0	0	0	
Hungary	451	0	84	0	98	0	
Ireland	0	0	0	0	0	0	
Italy	4146	221	0	137	6	0	
Republic of Kosovo	0	0	0	0	0	0	
Latvia	1096	26	0	0	53	0	
Lithuania	200	41	0	0	0	0	
Montenegro	4	0	0	0	0	0	
North Macedonia	15	0	0	0	0	0	
Norway	1	0	0	0	17	0	
Poland	1682	321	0	7	4	1	1
Portugal	0	0	0	0	0	0	
Republic of Moldova	0	0	0	0	0	0	58
Republic of Serbia	49	0	6	0	0	0	
Romania	392	1	254	2	0	2	3
Slovakia	229	0	0	0	38	0	
Slovenia	1313	7	0	0	0	0	
Spain	45	0	0	42	40	3	
Sweden	0	0	0	0	2	0	
Switzerland	41	10	0	0	0	0	
The Netherlands	0	0	0	0	0	0	
United Kingdom	466	7	0	4	201	4	
Total	21615	659	344	250	757	16	62



Table 4: Number of tests performed per country (NRL and regional laboratories data) in 2019 in the frame of rabies diagnosis (**domestic animals**) (Green box: number of tests; red box: number of positive cases; ND: No data; *: vaccine induced case)

Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
Austria	51	28	0	28	28	0	
Belgium	31	0	0	0	0	0	
Bosnia and Herzegovina	17	0	0	0	0	0	
Bulgaria	15	0	0	0	0	0	
Croatia	114	0	0	39	0	0	
Cyprus	0	0	0	0	0	0	
Czechia	150	0	0	150	0	0	
Denmark	3	1	0	0	5	0	
Estonia	30	0	0	0	0	0	
Finland	91	56	0	0	0	0	
France	1356	3	9	0	0	0	
Germany	100	0	0	0	49	0	
Greece	32	0	0	0	19	0	
Grand Duchy of Luxembourg	3	0	0	0	0	0	
Hungary	626	0	408	0	326	0	
Ireland	0	0	0	0	0	0	
Italy	730	534	0	467	33	35	
Republic of Kosovo	0	0	0	0	0	0	
Latvia	35	22	0	0	27	0	
Lithuania	45	36	0	0	0	0	
Montenegro	7	0	0	0	0	0	
North Macedonia	2	2	0	0	2	0	
Norway	0	0	0	0	3	0	
Poland	1558	1466	0	4	8	0	
Portugal	0	0	0	6	6	0	
Republic of Moldova	0	0	0	0	0	0	38
Republic of Serbia	83	0	48	0	0	0	
Romania	496	9	293	1	0	1	1
Slovakia	145	0	0	0	138	0	
Slovenia	64	28	0	0	0	0	
Spain	81	0	0	80	78	1	
Sweden	3	0	0	0	37	0	
Switzerland	82	82	0	0	0	0	
The Netherlands	8	0	0	0	8	0	
United Kingdom	5	0	0	0	2	0	
Total	5963	2267	758	775	769	37	39



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	Sampling wildlife	Groupe	Country	Sampling domestic animals	Groupe
Latvia	1.70	A	Poland	0.50	A
Poland	0.54	A	Romania	0.21	A
Romania	0.17	A	Republic of Serbia	0.09	A
Republic of Serbia	0.06	A	Latvia	0.05	A
Republic of Moldova	0.00	A	Republic of Moldova	0.00	A
Bulgaria	0.80	B	Hungary	0.67	B
Croatia	0.66	B	Croatia	0.20	B
Greece	0.57	B	Lithuania	0.07	B
Hungary	0.48	B	Estonia	0.07	B
Estonia	0.46	B	Finland	0.03	B
Lithuania	0.31	B	Greece	0.02	B
North Macedonia	0.06	B	Bulgaria	0.01	B
Finland	0.03	B	North Macedonia	0.01	B
Republic of Kosovo	0.00	B	Republic of Kosovo	0.00	B
Slovenia	6.48	C	Slovenia	0.32	C
Slovakia	0.47	C	Slovakia	0.30	C
Montenegro	0.03	C	Montenegro	0.05	C
Bosnia and Herzegovina	0.01	D	Bosnia and Herzegovina	0.03	D
Grand Duchy of Luxembourg	4.33	E	Italy	0.24	E
Czechia	3.86	E	France	0.21	E
Italy	1.38	E	Switzerland	0.20	E
Germany	1.05	E	Czechia	0.19	E
France	0.32	E	Grand Duchy of Luxembourg	0.12	E
Austria	0.23	E	Belgium	0.10	E
UK	0.19	E	Austria	0.06	E
Switzerland	0.10	E	Germany	0.03	E
Belgium	0.04	E	The Netherlands	0.02	E
Spain	0.01	E	Spain	0.02	E
Denmark	0.00	E	Denmark	0.01	E
Norway	0.00	E	UK	0.00	E
Cyprus	0.00	E	Sweden	0.00	E
Ireland	0.00	E	Cyprus	0.00	E
Portugal	0.00	E	Ireland	0.00	E
Sweden	0.00	E	Norway	0.00	E
The Netherlands	0.00	E	Portugal	0.00	E



The groups are the following:

Group A: Countries with at least one positive case in the year n^{-1} (2018).

Group B: Countries excluded from group A (no positive case in the year n^{-1}) with at least one positive case in a bordering country in the year n^{-1} (2018) and conducting ORV in 2019.

Group C: Countries excluded from group A and B (with no positive case in a bordering country in the year n^{-1}) but conducting ORV in 2019.

Group D: Countries excluded from group A, B, C with at least one positive case in a bordering country in the year n^{-1} (2018) and not conducting ORV in 2019.

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 2019, 3 of the 35 (9%) participating countries identified at least a positive endemic case (Figure 3).

The number of detected cases within the European Union reached the lowest reported annual figures never observed. Eight endemic animal cases and one human imported case were indeed recorded in 2018 in the EU while 5 endemic animal cases and 5 imported cases were recorded in 2019 (four human and one dog cases).

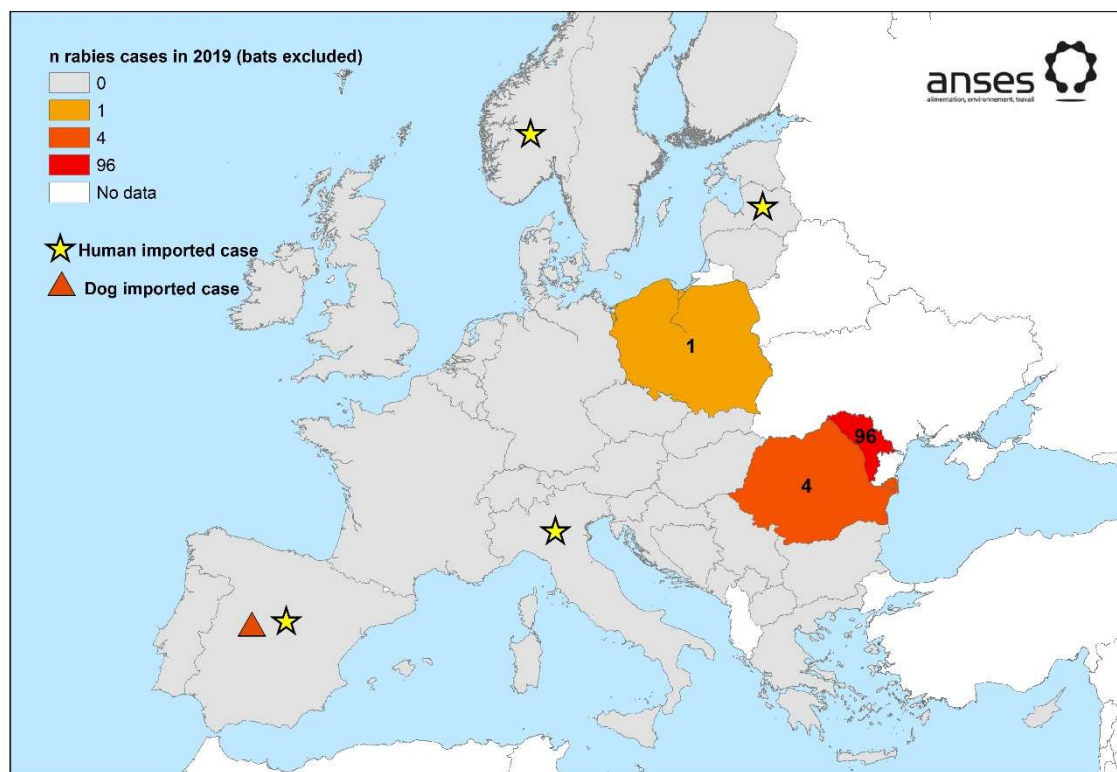


Figure 3: Number of reported rabies cases per country in mammals excluding bats in 2019.

6 RABIES CASES IN BATS

Twenty countries (55%) performed rabies diagnosis on bats (Figure 4 and Table 6). The number of samples tested by FAT throughout 2019 varied from 1 to 663 tests within the year according to the country. The most implicated countries in rabies surveillance in bats are principally located in Western Europe where a total of 39 bat cases were detected.

Rabies diagnosis technique commonly used to identify a positive case is the FAT while some countries used molecular biology techniques as primary diagnostic test (Belgium, Denmark, Germany, the Netherlands, Norway, Portugal and Sweden).

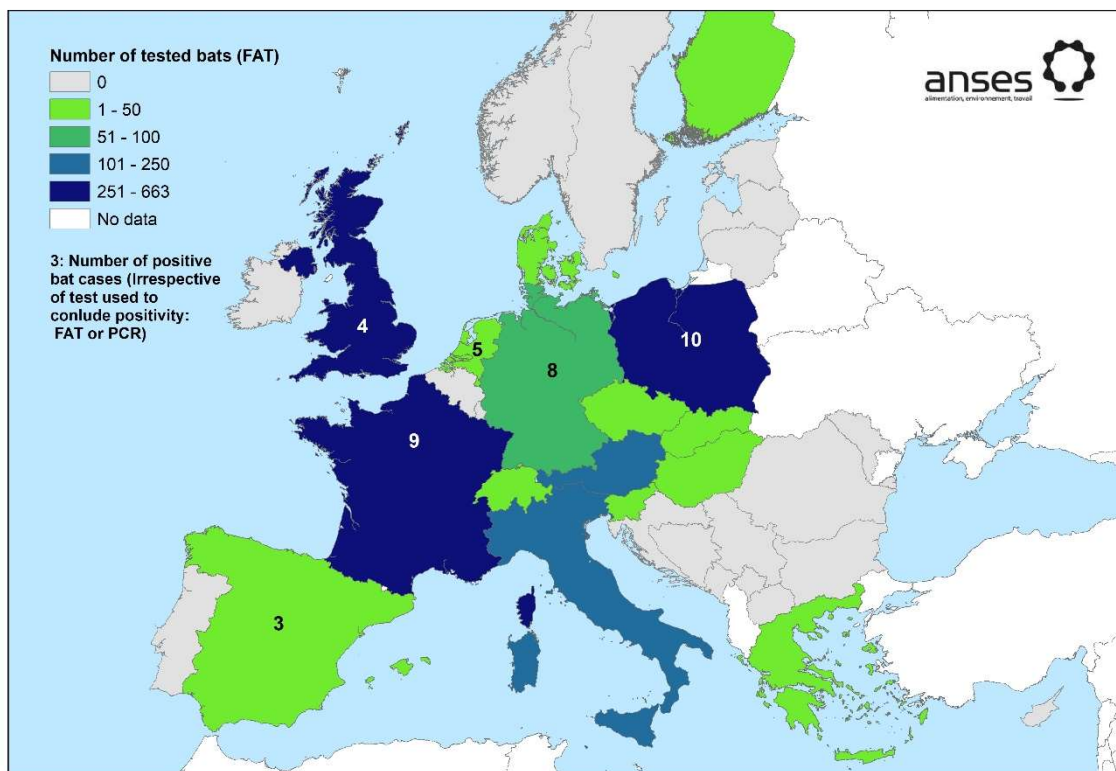


Figure 4: Number of bats tested by FAT per country in 2019 in the frame of passive surveillance programme and number of associated positive cases.

Table 6: Number of tests performed per country (NRLs and regional laboratories data) in 2019 in the frame of passive surveillance on bats (Green box: number of tests; red box: number of positive cases)

Country	FAT	RTCIT	MIT	RT-PCR	RealTime	Typing	n cases
Austria	112	3	0	3	3	0	
Belgium	0	0	0	0	30	0	
Bosnia and Herzegovina	0	0	0	0	0	0	
Bulgaria	0	0	0	0	0	0	
Croatia	0	0	0	0	0	0	
Cyprus	0	0	0	0	0	0	
Czechia	14	0	0	6	0	0	
Denmark	1	0	0	0	11	0	
Estonia	0	0	0	0	0	0	
Finland	40	0	0	0	40	0	
France	663	8	0	8	8	7	9
Germany	75	8	0	0	450	16	8
Greece	1	0	0	1	1	0	
Grand Duchy of Luxembourg	0	0	0	0	0	0	
Hungary	16	0	6	0	16	0	
Ireland	0	0	0	0	0	0	
Italy	192	42	0	157	0	57	
Republic of Kosovo	0	0	0	0	0	0	
Latvia	0	0	0	0	0	0	
Lithuania	0	0	0	0	0	0	
Montenegro	0	0	0	0	0	0	
North Macedonia	0	0	0	0	0	0	
Norway	0	0	0	0	5	0	
Poland	298	105	0	11	8	10	10
Portugal	0	0	0	66	0	0	
Republic of Moldova	0	0	0	0	0	0	
Republic of Serbia	0	0	0	0	0	0	
Romania	0	0	0	0	0	0	
Slovakia	1	0	0	0	1	0	
Slovenia	1	0	0	2	2	0	
Spain	29	0	0	29	27	3	3
Sweden	0	0	0	0	3	0	
Switzerland	18	4	0	0	0	0	
The Netherlands	12	0	0	0	59	0	5
United Kingdom	466	7	0	4	201	4	4
Total	1939	177	6	287	865	97	39



Table 7: Oral vaccination campaigns performed in European countries and number of oral vaccine batches analysed for titration in NRLs. ND: no data

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
Austria						
Belgium						
Bosnia and Herzegovina						
Bulgaria	2	Lysvulpen	122,295	2,852,000	23	ND
Croatia	2	Lysvulpen	106,588	2,665,850	25	7
Cyprus						
Czechia						
Denmark						
Estonia	2	SPBN GasGas	12,200	244,800	20	ND
Finland	1	SPBN GasGas	9,000	180,000	20	1
France						
Germany						
Greece	2	Lysvulpen	109,483	2,983,316	27	9
Grand Duchy of Luxembourg						
Hungary	2	Lysvulpen	83,940	1,678,800	20	6
Ireland						
Italy						
Republic of Kosovo	1	Lysvulpen	7,700	300,000	39	ND
Latvia	2	Lysvulpen	38,490	961,800	25	5
Lithuania	2	Lysvulpen	40,800	1,010,000	25	4
Montenegro	2	Lysvulpen	27,000	550,000	20	2
North Macedonia	2	Lysvulpen	50,000	1,000,000	20	5
Norway						
Poland	2	Lysvulpen	195,984	5,045,868	26	11
Portugal						
Republic of Moldova						
Republic of Serbia	2	Lysvulpen	121,992	3,342,000	27	9
Romania	2	Lysvulpen	426,000	10,668,000	25	26
Slovakia	2	Lysvulpen	26,806	667,550	25	2
Slovenia	2	Fuschoral + SPBN GasGas	30,000	760,000	25	7
Spain						
Sweden						
Switzerland						
The Netherlands						
United Kingdom						
Total	30		1,408,278	34,909,984	25	94



7.2 Percentage of seroconversion in the target population

Percentages of seroconversion ranged from 14% to 54% with a median of 37% (Figure 6). As in 2018, 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: 13/15 laboratories (87%) used an ELISA commercial kit (10 laboratories used the BioPro kit, 3 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.

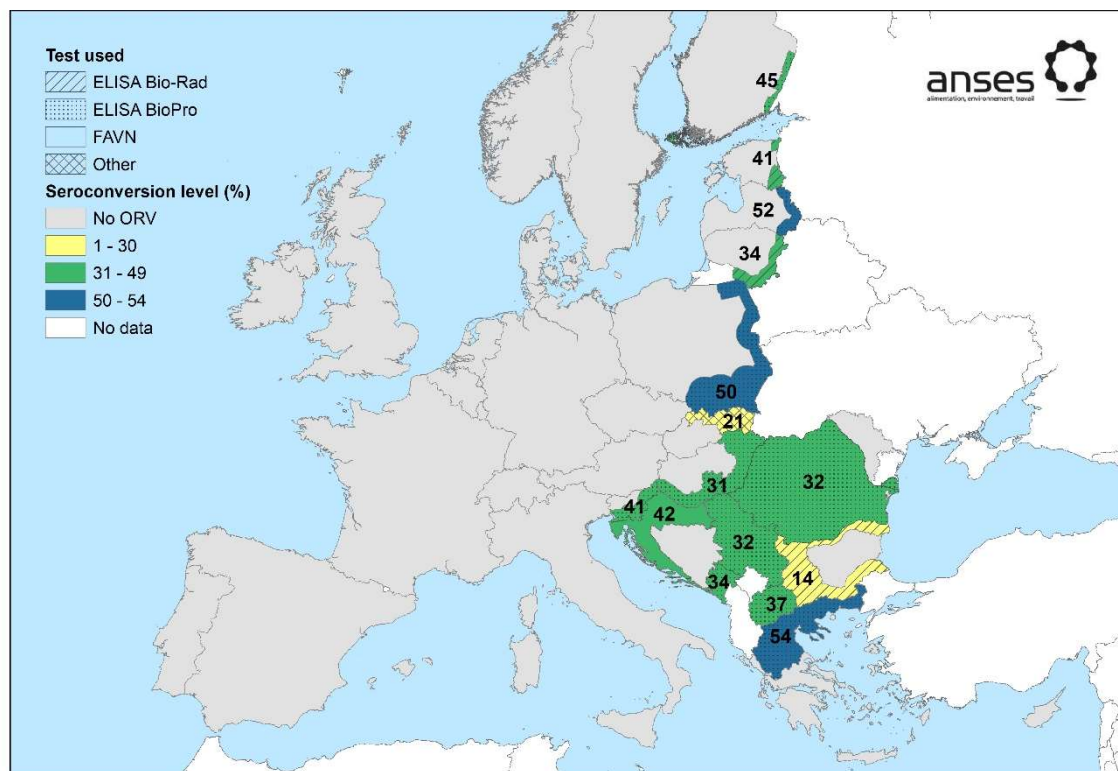


Figure 6: Proportion of sero-conversion in the target population and type of test used in 2019. ORV area limitation kindly provided by the European Commission.

7.3 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 90% with a median of 74% (Figure 7). 10/15 countries (Croatia, Estonia, Finland, Greece, Latvia, Lithuania, North Macedonia, Poland, Serbia, Slovakia) reported a bait uptake that exceeded 70%, which is in accordance with the minimum 70% recommended vaccination coverage of the WHO (2018b).

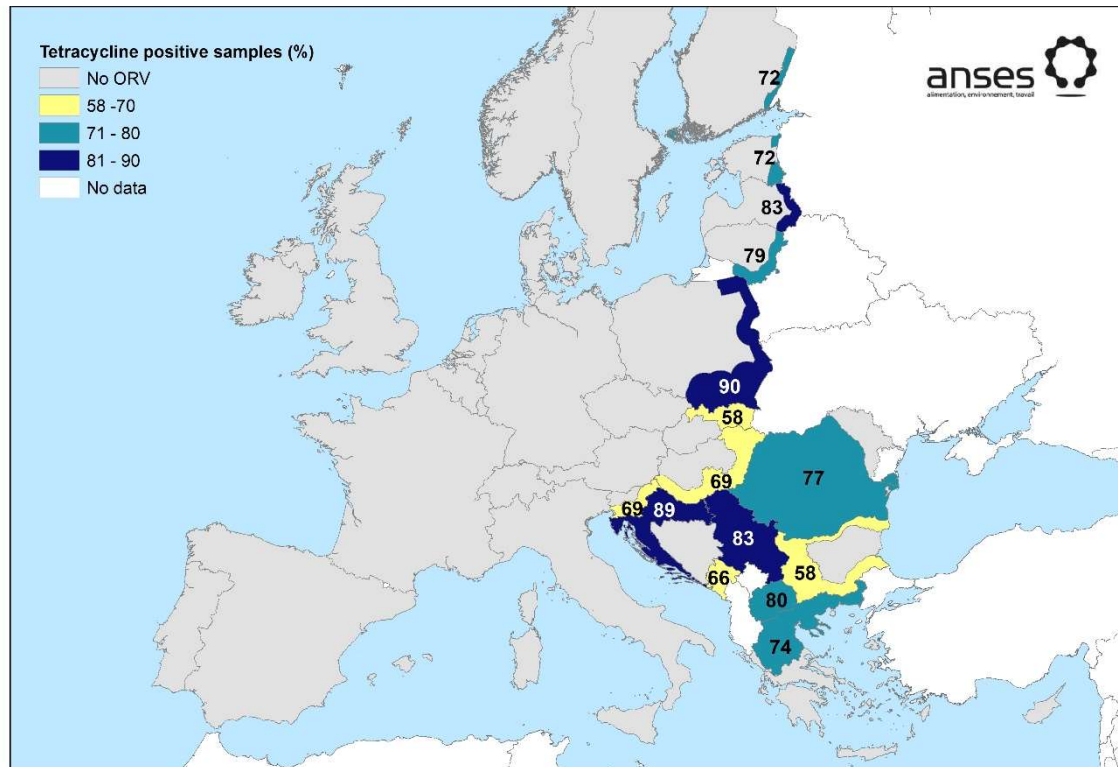


Figure 7: Proportion of positive samples for tetracycline presence in the target population in 2019. ORV area limitation kindly provided by the European Commission

ACKNOWLEDGMENTS

We would like to thank all the participants in this review for kindly providing the data of the analysis performed in their laboratory and in regional laboratories of their own network.

COPYRIGHT

The information found in this report is made available to the rabies NRL network. This information is protected by the Berne Convention for the Protection of Literary and Artistic Works, by other international agreements and by French national laws on copyright and derived rights. Any private, public or commercial use of information from ANSES's documents must identify ANSES as the source of the information used.

REFERENCES

Scientific Panel on Animal Health and Welfare (AHAW) (2015). Update on oral vaccination of foxes and raccoon dogs against rabies. EFSA Journal. 70p.

URL : http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4164.pdf

European Commission (2002). The oral vaccination of foxes against rabies. Report of the Scientific Committee on Animal Health and Animal Welfare. 55p.

URL: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out80_en.pdf

OIE (2018). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.17. Rabies. Paris. 35p. URL: <http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/>

WHO (2018a). Laboratory techniques in rabies. 5th edition ed, Edited by Charles E. Rupprecht, Anthony R. Fooks, Bernadette Abela-Ridder, Geneva. 289p.

URL Volume 1 : <https://apps.who.int/iris/bitstream/handle/10665/310836/9789241515153-eng.pdf?sequence=1&isAllowed=y>;

URL Volume: 2: <https://apps.who.int/iris/bitstream/handle/10665/310837/9789241515306-eng.pdf?sequence=1&isAllowed=y>

WHO (2018b). WHO Expert Consultation on rabies. Third report. World Health Organization technical report series 1012. Geneva. 183p

(<https://apps.who.int/iris/bitstream/handle/10665/272364/9789241210218-eng.pdf?sequence=1&isAllowed=y>)

