REVIEW OF THE ANALYSIS RELATED TO RABIES DIAGNOSIS AND FOLLOW-UP OF ORAL VACCINATION PERFORMED IN NRLS IN 2017

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An annual activity online questionnaire was submitted to all National Reference Laboratories (NRLs) on last February 2018 to collect and collate data on methods used and results of tests carried out in the Community in the frame of rabies control programmes (Commission regulations (EU) N° 737/2008 and N°415/2013).

This document reviews the 2017 analysis performed in 25 NRLs from the European Union and in 5 NRLs from third countries involved in a rabies control programme.

1 GENERAL DATA

In 2017, the European National Reference Laboratories network for Rabies included 28 laboratories from the European Union. Twenty five 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. Five laboratories (from Kosovo, Former Yugoslav Republic of Macedonia, Montenegro, Serbia and Norway) were added in the dataset. At the end, a total of 30 countries were included in this survey (Figure 1).

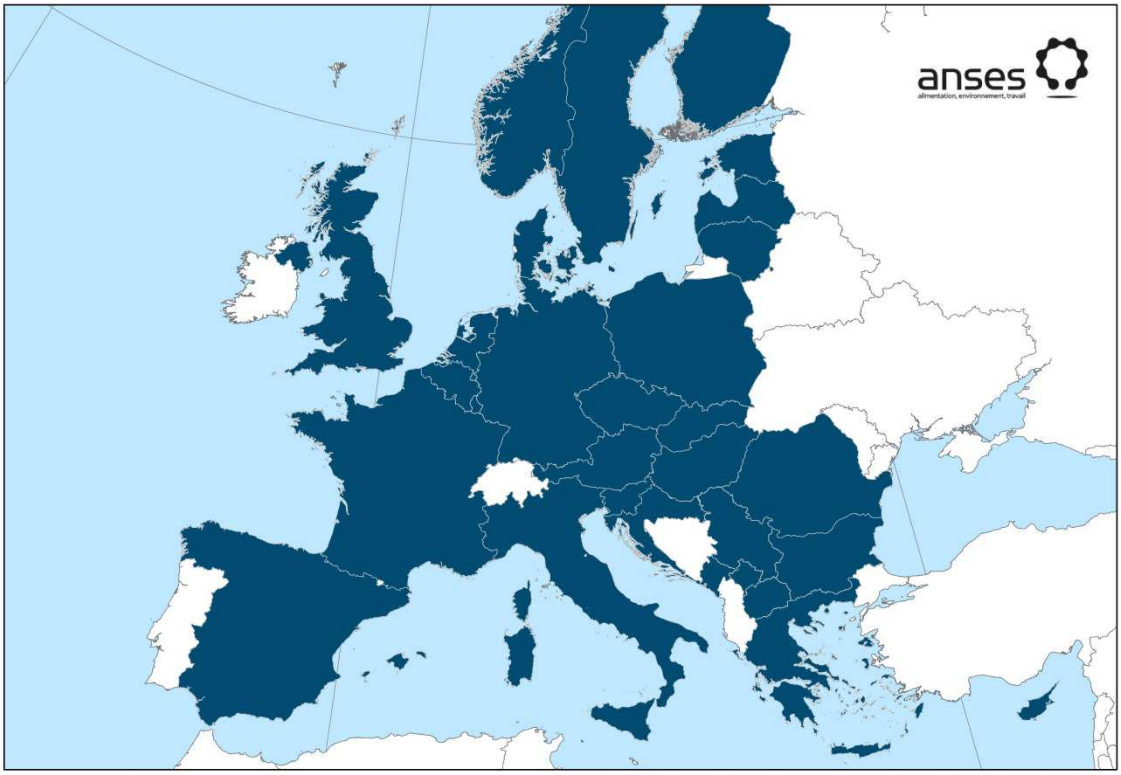

Figure 1: Map of the participating countries in the 2017 review

2 QUALITY ASSURANCE

In 2017, 29 on 30 laboratories (97%) were accredited according to the ISO EN 17025 system.
Each laboratory is accredited for various combinations of techniques. As in previous years, the most widely used techniques under quality assurance system management are the gold standard FAT (89% of laboratories accredited) and the FAVN test (61%) (Figure 2).
Forty-seven percent participating national laboratories and 12/13 EU laboratories are working in BSL3 facilities.

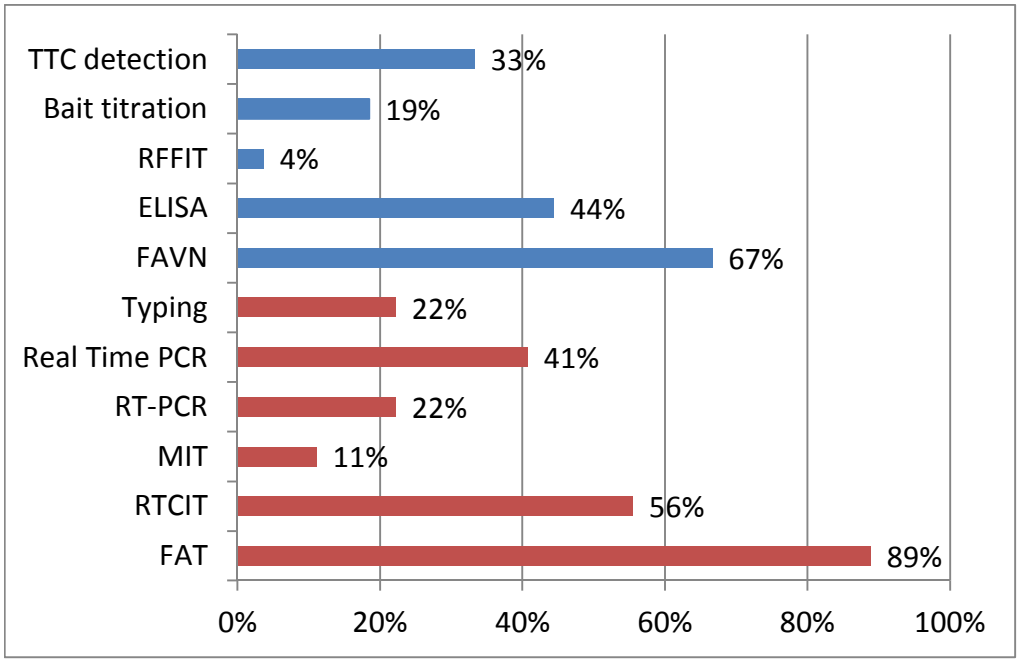

Figure 2: Percentage of laboratories accredited for the different techniques related to rabies field (diagnosis techniques in red and monitoring techniques in blue).

3 RABIES LABORATORIES NETWORK AND DIAGNOSIS DECISION TREE

Within participating laboratories, 8 laboratories are heading a regional laboratories network where analyses are also implemented. In two countries (France and Serbia), rabies diagnosis in animals is performed in a different laboratory in case of human contact.

Among the participants, 33% of laboratories (10/30) declared considering the status 'inconclusive' for the results for FAT or RTCIT in rabies diagnosis tests.
Confirmatory tests are used in case of imported cases (18/30), of Inconclusive FAT (14/30) or negative FAT (14/30).

According to the result survey, most of the confirmatory tests are molecular biology tools (Table 1). Real Time RT-PCR tests is commonly used as first confirmatory test.

Table 1: Number of tests Confirmatory tests used by participating laboratories
(most frequent techniques are in red).**FAT being not a confirmatory test, the data have been reported as declared by participants but in italic. Such result could indeed be the consequence of a misunderstanding. The questionnaire will be consequently clarified next year.*

CONFIRMATORY TEST USED IN CASE OF HUMAN CONTACT		CONFIRMATORY TEST USED IN OTHER CASES	
First confirmatory test:		First confirmatory test:	
*FAT**	*9*	*FAT**	*8*
RTCIT	7	RTCIT	6
Conventional RT-PCR	1	Conventional RT-PCR	1
Real Time RT-PCR	10	Real Time RT-PCR	12
Second confirmatory test:		Second confirmatory test:	
RTCIT	7	*FAT**	*1*
MIT	2	MIT	1
Conventional RT-PCR	8	RTCIT	6
Real Time RT-PCR	6	Conventional RT-PCR	10
		Real Time RT-PCR	5
Third confirmatory test:		Third confirmatory test:	
RTCIT	3	RTCIT	3
Conventional RT-PCR	3	Conventional RT-PCR	3
Real Time RT-PCR	4	Real Time RT-PCR	3
Other	1	Other	2

Considering the different combinations of answers, a disparity of rabies diagnosis decision tree coexists within the network.

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

As expected, the FAT gold standard technique (OIE, 2013; WHO; 1996) is the most commonly used technique (representing 82% of the total amount of diagnostic tests performed during the year and used by 100% of laboratories) (Table 2).

The RTCIT is the second most widely used technique (50% of laboratories and 10% of the total amount of diagnosis tests performed during the year) and is often used as confirmatory test (Table 2). Real Time and RT-PCR techniques are used by 50% and 37% of laboratories respectively. Still 20% of laboratories (n=6) are using the MIT techniques in their rabies diagnosis process, although, for ethical reasons, it is recommended whenever possible that RTCIT replace MIT. Four of laboratories using MIT are also using the virus isolation on cells (RTCIT) (Table 2).

Number of animals analysed in the frame of rabies surveillance programme (bats excluded) varied from 0 to 4054 samples at country level (Figure 3). Globally, 11 positive cases were identified for a total of 28 924 FAT (0.0004%).

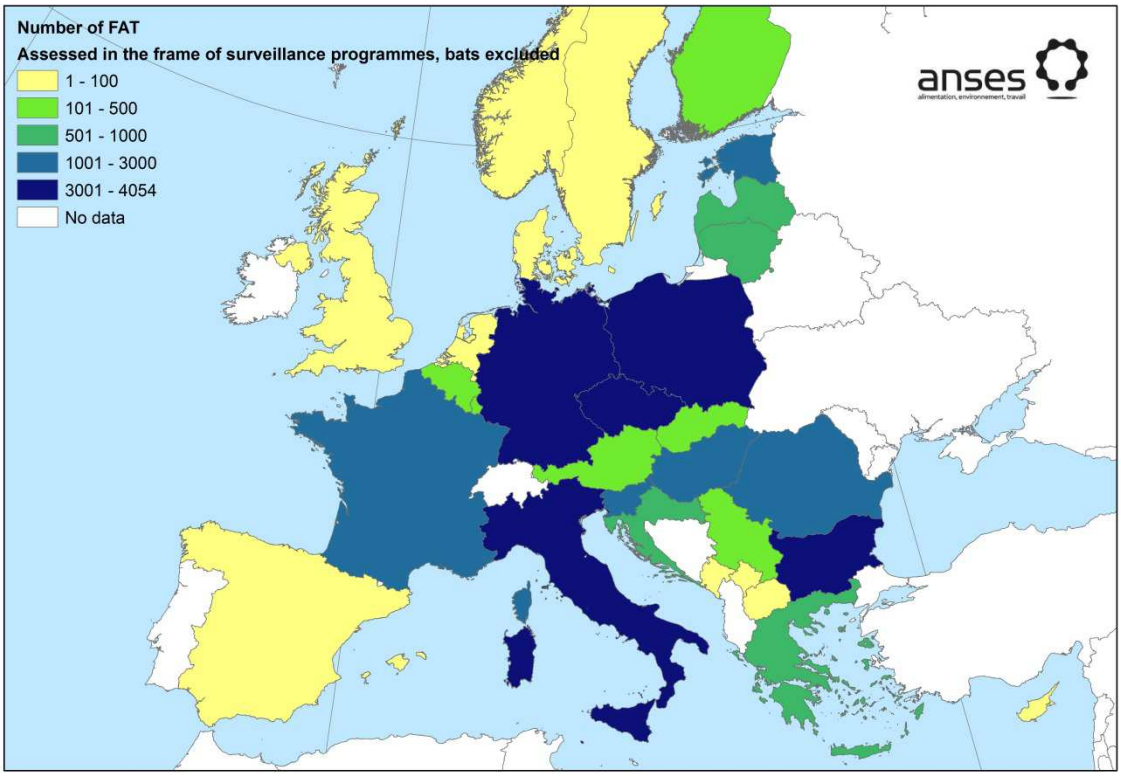

Figure 3: Number of FAT performed per country under passive surveillance programme in mammals, excluding bats.

Table 2: Number of tests performed per country (NRL and regional laboratories data) in 2017 in the frame of rabies diagnosis (mammals excluding bats and passive surveillance only) (Green box: number of test; red box: number of positives cases; [i]= imported case; ND= No data)

	Reference Techniques			Molecular Biology Techniques			
Country	**FAT**	**RTCIT**	**MIT**	**RT-PCR**	**RealTime**	**Typing**	**n cases**
Austria	253	41	0	0	35	0	0
Belgium	345	0	0	0	1	0	0
Bulgaria	3288	0	7	0	0	0	0
Croatia	687	0	0	62	0	0	0
Cyprus	1	0	0	0	0	0	0
Czech Republic	3359	0	111	0	0	0	0
Denmark	8	2	0	0	8	0	0
Estonia	1094	29	0	0	61	0	0
Finland	110	38	0	1	0	0	0
France	1341	15	0	0	0	0	0
Germany	4054	259	0	0	0	0	0
G. D.of Luxembourg	177	0	0	0	0	0	0
Greece	817	0	0	1	192	0	0
Hungary	1226	2	512	0	112	2	3
Italy	3526	456	83	346	53	0	0
Latvia	931	898	0	163	0	0	0
Lithuania	587	99	0	0	0	0	0
Montenegro	12	0	0	0	0	0	0
Norway	2	0	0	5	6	0	0
Poland	3547	1654	0	8	0	1	2
Republic of Kosovo	2	0	0	0	0	0	0
FYROM	72	0	0	0	0	0	0
Romania	1018	6	419	2	0	2	2
Serbia	207	2	48	1	1	1	1
Slovakia	379	0	0	0	201	0	0
Slovenia	1768	55	0	1	1	0	0
Spain	73	0	0	71	70	3	3[i]
Sweden	6	0	0	0	26	0	0
The Netherlands	12	0	0	0	12	0	0
United Kingdom	22	5	0	0	5	0	0
Total (n analysis)	**28924**	**3561**	**1180**	**661**	**784**	**9**	**11**
Total (% analysis)	**82%**	**10%**	**3%**	**2%**	**2%**	**0%**	
Total (n laboratorie	**30**	**15**	**6**	**11**	**15**	**5**	**5**
Total (% laboratorie	**100%**	**50%**	**20%**	**37%**	**50%**	**17%**	**17%**

Sampling effort in the frame of rabies surveillance has been estimated by dividing the number of FAT tests (excepted bats) by the total area (km^2) of the country multiplied by 100. This provided a surveillance indicator of the number of samples analysed for 100 km^2 in each country. 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 3).

The groups are the following:

Group A: Countries with at least one positive case in the year n^{-1} (2016) and conducting ORV in 2017.

Group B: Countries excluded from group A with at least one positive case in a bordering country in the year n^{-1} (2016) and conducting ORV in 2017.

Group C: Countries excluded from group A with at least one positive case in a bordering country in the year n^{-1} (2016) and not conducting ORV in 2017.

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

Table 3: Number of FAT tests performed in the frame of rabies surveillance programmes (mammals excluding bats and passive surveillance only) per country for 100 km^2. Countries are classified in groups according to their rabies situation or implementation of oral vaccination programmes or not.

A	Hungary	1.3
	Poland	1.1
	Romania	0.4
	Serbia*	0.2
B	Slovenia	8.7
	Bulgaria	3.0
	Estonia	2.4
	Latvia	1.4
	Croatia	1.2
	Lithuania	0.9
	Slovakia	0.8
	Greece	0.6
	FYROM	0.3
	Montenegro	0.1
	Finland	<0.1
C	Czech Republic	4.3
	Italy	1.2
	Germany	1.1
	Austria	0.3
	Spain**	<0.1
	Kosovo	<0.1
D	Luxembourg	6.8
	Belgium	1.1
	France	0.2
	Netherlands	<0.1
	Denmark	<0.1
	Cyprus	<0.1
	United Kingdom	<0.1
	Sweden	<0.1
	Norway	<0.1

*results based on the data of one out of two laboratories involved in the surveillance.

**country experiencing imported cases regularly.

5 RABIES CASES IN MAMMALS EXCLUDING BATS

In 2017, 5 of the 30 (17%) participating laboratories identified a positive case corresponding to a total of 11 detected cases (Figure 4).

The highest numbers of rabies cases identified by NRLs and regional laboratories in 2017 were observed in Hungary (3). Romania as well as Poland recorded a reduced amount of positive cases compared to previous years (Romania: 462 in 2013, 166 in 2014, 28 in 2015, 16 in 2016 and 2 in 2017; Poland: 93 in 2015, 16 in 2016, 2 in 2017).

Rabies imported cases in domestic animals were recorded in Spain as regularly observed in previous years (2016: 5 cases in Spain, 2013: 1 case in France and 5 cases in Spain, 2012: 5 cases in Spain).

The number of detected cases within the European Union reached the lowest reported annual figures never observed.

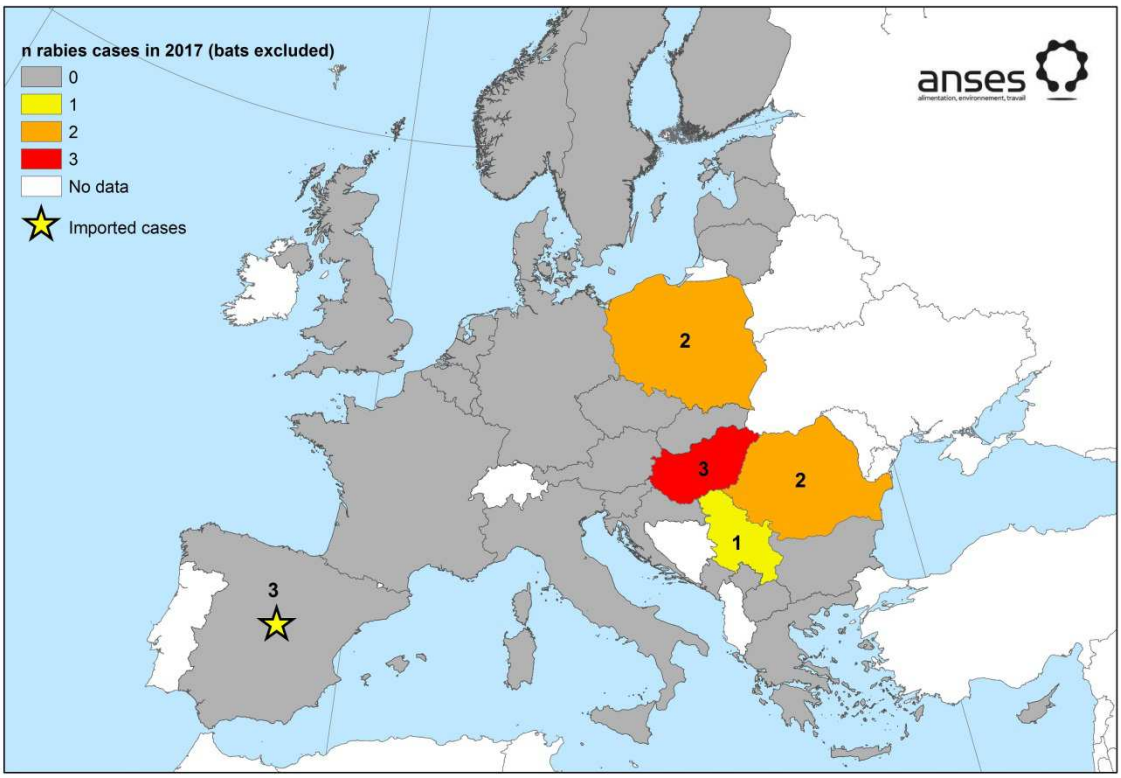

Figure 4: Number of reported rabies cases per country in mammals excluding bats in 2017.

6 RABIES CASES IN BATS

Nineteen countries performed rabies diagnosis on bats routinely (Figure 5 and Table 4). The number of samples tested by FAT throughout 2017 varied from 1 (Croatia) to 608 (Germany) tests within the year according to the country. The most implicated countries in rabies surveillance in bats are principally located in Western Europe (France, United Kingdom, Germany, the Netherlands, Poland and Spain).

Rabies diagnosis technique commonly used to identify a positive case is commonly the FAT even if in some countries molecular biology techniques are principally used instead of reference techniques (Italy, the Netherlands).

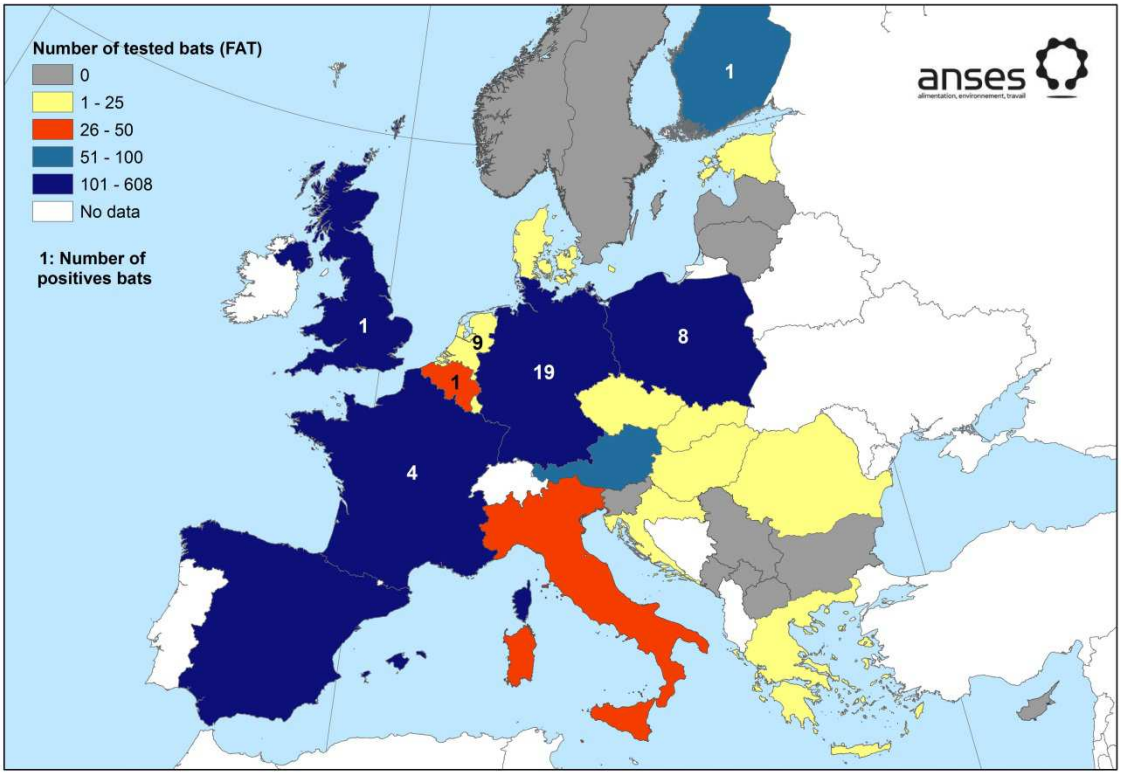

Figure 5: Number of bats tested by FAT per country in 2017 in the frame of passive surveillance programme and number of associated positive cases.

Table 4: Number of tests performed per country (NRL and regional laboratories data) in 2017 in the frame of passive surveillance on bats (Green box: number of tests; red box: number of positives cases)

	Reference Techniques			Molecular Biology Techniques		
Country	**FAT**	**RTCIT**	**MIT**	**RT-PCR**	**RealTime**	**Typing**
Austria	99	0	0	11	11	0
Belgium	42	0	0	0	3	1
Bulgaria	0	0	0	0	0	0
Croatia	1	0	0	0	0	0
Cyprus	0	0	0	0	0	0
Czech Republic	16	0	0	0	0	0
Denmark	12	2	0	0	12	0
Estonia	2	0	0	0	0	0
Finland	78	13	0	50	0	1
France	434	140	1	4	4	4
Germany	608	48	1	6	30	0
G.D. of Luxembourg	14	0	0	0	0	0
Greece	25	0	0	2	6	0
Hungary	22	0	1	0	21	0
Italy	38	38	0	103	0	0
Latvia	0	0	0	0	0	0
Lithuania	0	0	0	0	0	0
Montenegro	0	0	0	0	0	0
Norway	0	0	0	0	0	0
Poland	234	65	0	18	0	8
Republic of Kosovo	0	0	0	0	0	0
FYROM	0	0	0	0	0	0
Romania	2	2	0	0	0	0
Serbia	0	0	0	0	0	0
Slovakia	2	0	0	0	1	0
Slovenia	0	0	0	0	0	0
Spain	232	0	0	232	231	0
Sweden	0	0	0	0	0	0
The Netherlands	24	0	0	0	96	0
United Kingdom	433	279	0	2	61	1
Total	**2318**	**587**	**3**	**428**	**476**	**15**

7 ORAL VACCINATION MONITORING

7.1 Oral Vaccination

Fifteen countries implemented oral vaccination campaigns in 2017 (Table 5 and Figure 6). All countries performed two ORV campaigns within the year (one in spring and one in autumn) except Finland and Serbia (one campaign) and Poland (3 campaigns). In 2017, a total of 32 559 728 baits were distributed over 1 473 769 km^2. Bait titration of vaccine batches before release in the field was carried out by all countries excepted one and all the titres of batches were found satisfactory.

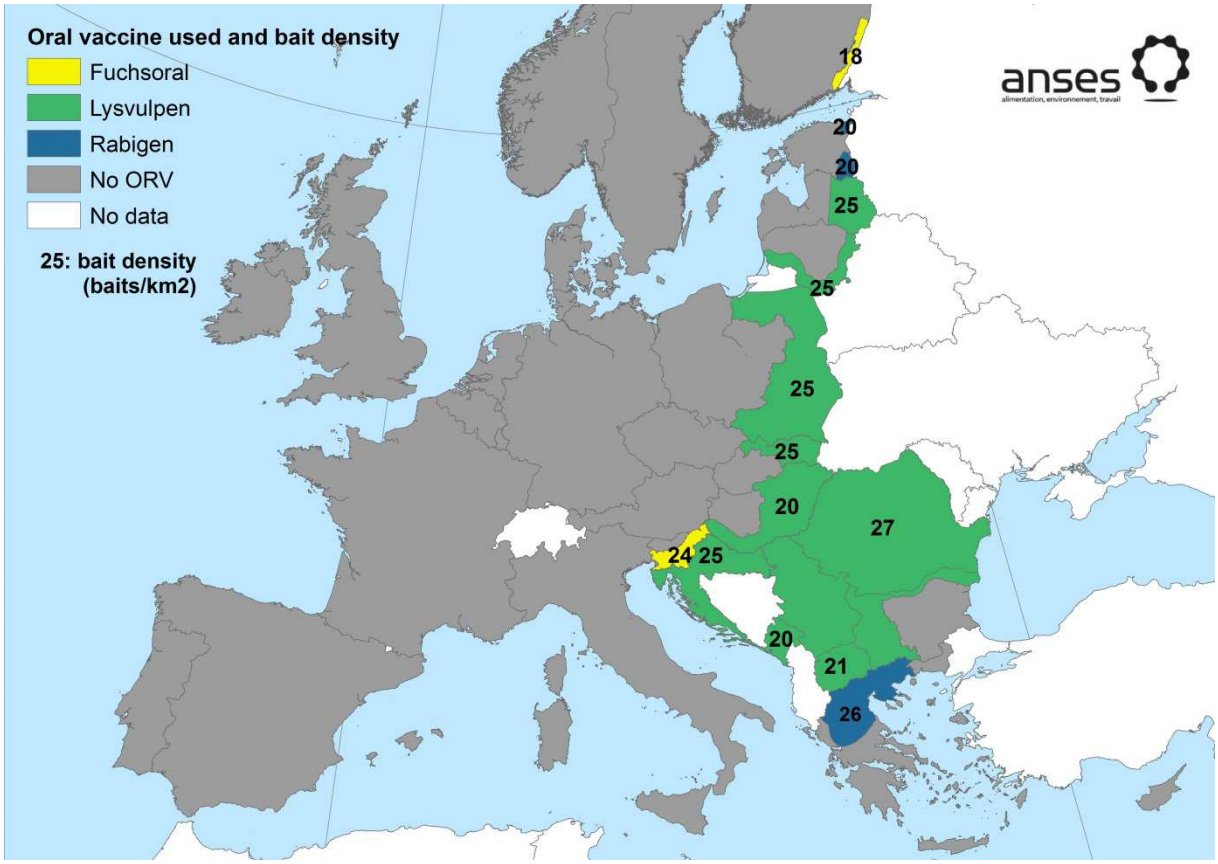

Figure 6: Oral vaccines used in the oral rabies vaccination and corresponding overall bait density per country. *ORV area limitation kindly provided by the European Commission.*

Table 5: Oral vaccination campaigns performed in European countries and number of oral vaccine batches analysed for titration in NRLs

Country	Number of campaign	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						
Bulgaria	2	Lysvulpen	100,946	ND	ND	10
Croatia	2	Lysvulpen	113,084	2,827,100	25	6
Cyprus						
Czech Republic						
Denmark						
Estonia	2	Rabigen	18,650	373,000	20	4
Finland	1	Fuchsoral	10,000	180,000	18	1
France						
Germany						
Grand Duchy of Luxembourg						
Greece	2	Rabigen	115,010	2,978,420	26	62
Hungary	2	Lysvulpen	133,768	2,675,360	20	21
Italy						
Latvia	2	Lysvulpen	38,490	961,800	25	3
Lithuania	2	Lysvulpen	68,600	1,715,000	25	6
Montenegro	2	Lysvulpen	27,000	550,000	20	2
Norway						
Poland	3	Lysvulpen	251,352	6,161,622	25	16
Republic of Kosovo						
FYROM	2	Lysvulpen	47,138	995,206	21	2
Romania	2	Lysvulpen	431,519	11,761,920	27	26
Serbia	1	Lysvulpen	60,996	ND	ND	5
Slovakia	2	Lysvulpen	24,916	620,300	25	2
Slovenia	2	Fuchsoral	32,300	760,000	24	5
Spain						
Sweden						
The Netherlands						
United Kingdom						
Total			1,473,769	32,559,728	23	171

7.2 Sampling pressure in Monitoring

The sampling pressure was calculated using the maximum number of animals collected in the frame of ORV monitoring (hunting bag origin from vaccinated areas) analysed for TTC (tetracycline) or serology. A ratio of animals analysed per 100 km^2 of the area vaccinated during the year was computed (sample size index: total number of animals tested in TTC or Serological analysis / (Maximum ORV area of the year) x100).

Sampling pressure index was found highly variable depending on the country from 0.1 to 6.4 as compared to the previously recommended sample size for ORV monitoring of 4 individuals per 100 km^2 per year (EFSA, 2015). Only 3 countries (Estonia, Slovakia and Slovenia) reached this target (Figure 7).

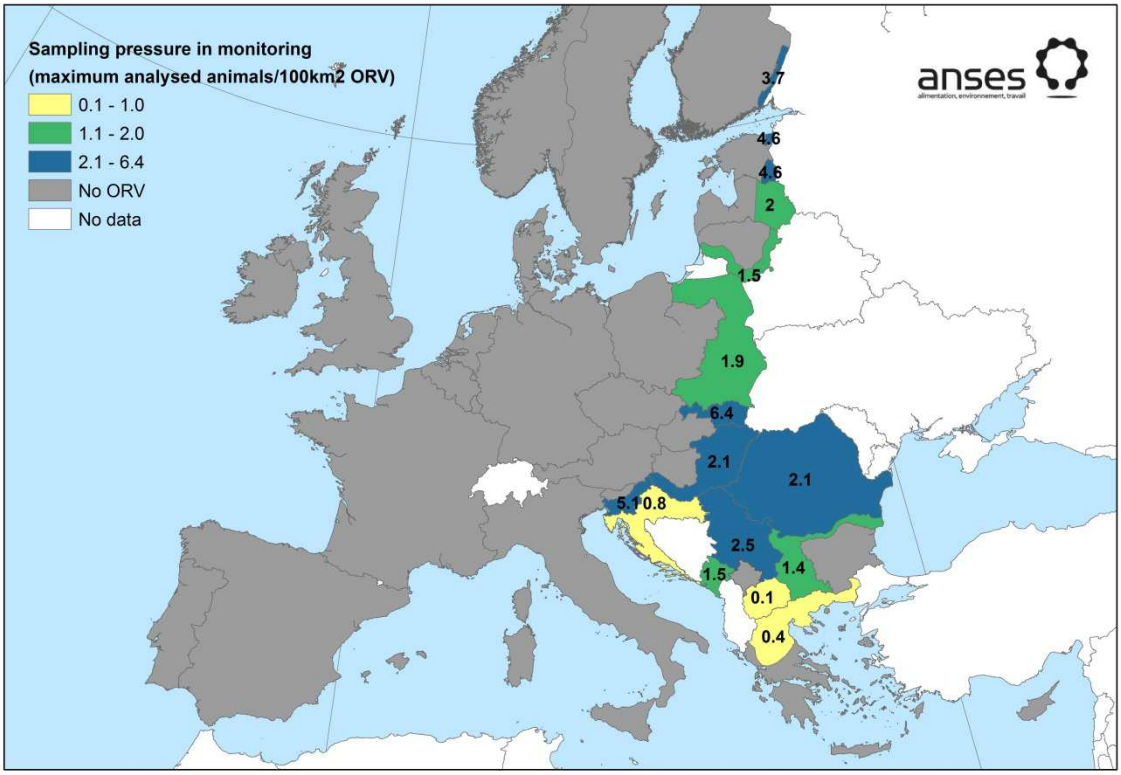

Figure 7: Number of animals analysed in the frame of ORV monitoring (TTC or Serology) per 100 km^2 of vaccinated area in 2017. *ORV area limitation kindly provided by the European Commission.*

7.3 Percentage of Seroconversion in the target population

Percentages of seroconversion were found highly variable and ranged from 12% to 66% (Figure 8). As in 2016, such data suggest that none of the countries seems to reach the minimum 70% recommended vaccination coverage of the WHO (2018).

A variety of tests is used for the serological analysis within Europe: 13/16 laboratories (81%) used an ELISA kit (9 laboratories used BioPro and 4 used Biorad). In laboratory group not using ELISA tests, Croatia used an mFAVN test, and Slovakia used a “home made” ELISA test. As in previous years, the variety of serological tests used within Europe and their sensitivity and specificity variations make the comparison of serological level among countries difficult to interpret.

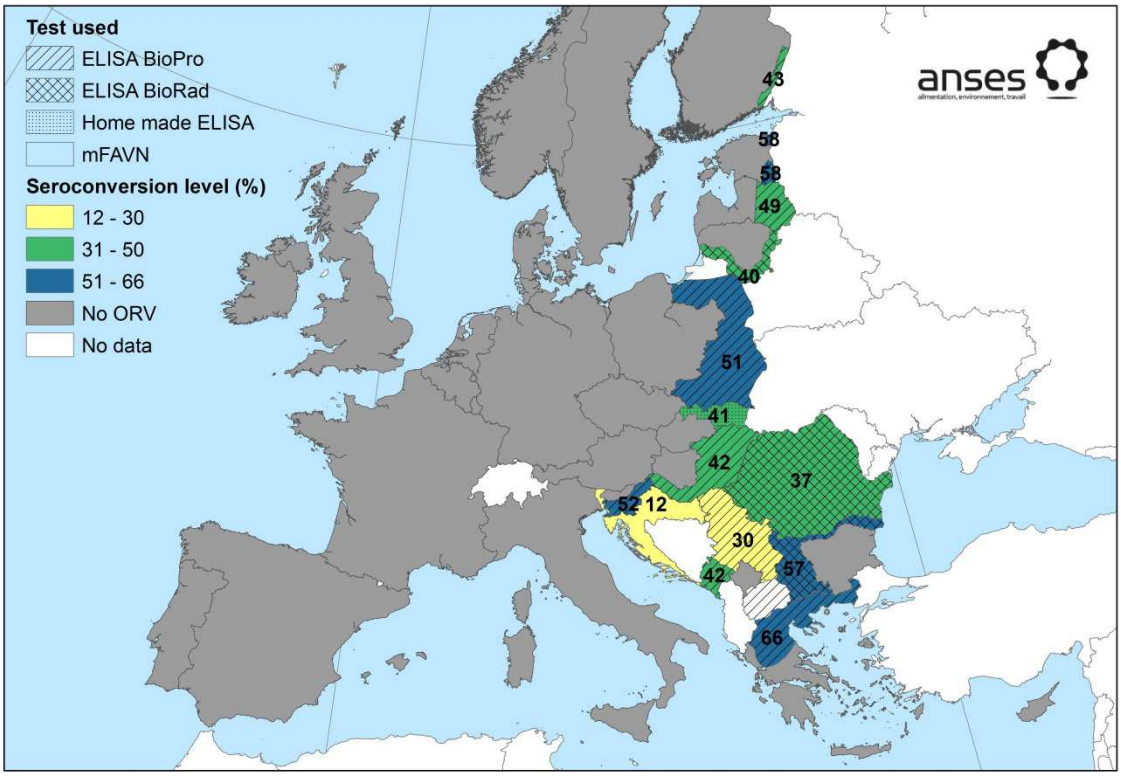

Figure 8: Proportion of sero-conversion in the target population and type of test used in 2017. *ORV area limitation kindly provided by the European Commission.*

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 66% to 88% (Figure 9). All the countries except five (Finland, Greece, Latvia, Romania, and Slovakia) reported a bait uptake that exceeds 70%, which is in accordance with the minimum 70% recommended vaccination coverage of the WHO (2018). Countries vaccinating a buffer zone only and with particularly thin area could present a low TTC level due to the "edge effect". The areas being small, the perimeter-to-surface ratio is higher and the probability of sampling an unvaccinated animal in bordering areas is indeed higher than for large ORV areas.

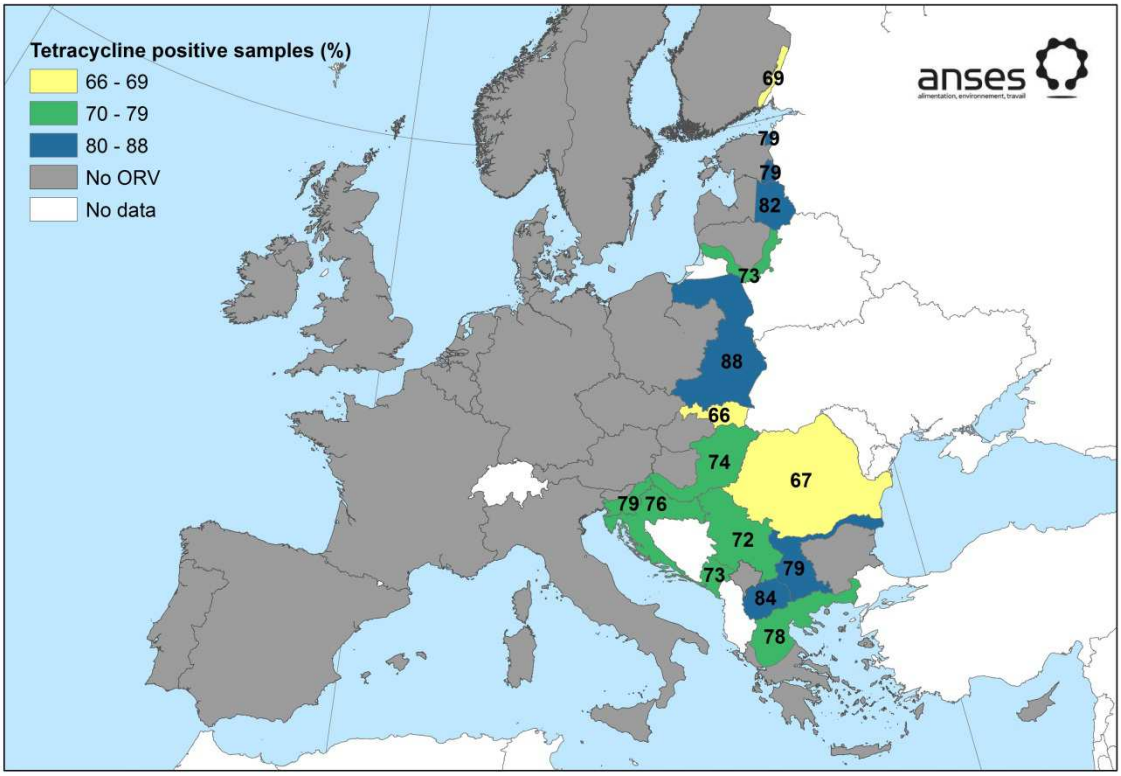

Figure 9: Proportion of positive samples for Tetracycline presence in the target population in 2017. *ORV area limitation kindly provided by the European Commission*

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