REVIEW OF THE ANALYSIS RELATED TO RABIES DIAGNOSIS AND FOLLOW-UP OF ORAL VACCINATION PERFORMED IN NRLS IN 2015

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TABLE OF CONTENTS

An annual activity online questionnaire was submitted to all National Reference Laboratories (NRLs) on last February 2015 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 2015 analysis performed in 27 NRLs from the European Union and in 7 NRLs from third countries involved in a rabies control programme.

1 GENERAL DATA

In 2015, the European National Reference Laboratories network for Rabies included 28 laboratories from the European Union. Twenty seven 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. Seven laboratories (from Kosovo, Former Yugoslav Republic of Macedonia, Montenegro, Serbia, Switzerland, Turkey and Norway) were added in the dataset. At the end, a total of 34 countries were included in the survey (Figure 1), which is the highest number of participants ever recorded. The 2015 survey is consequently the most exhaustive produced review.

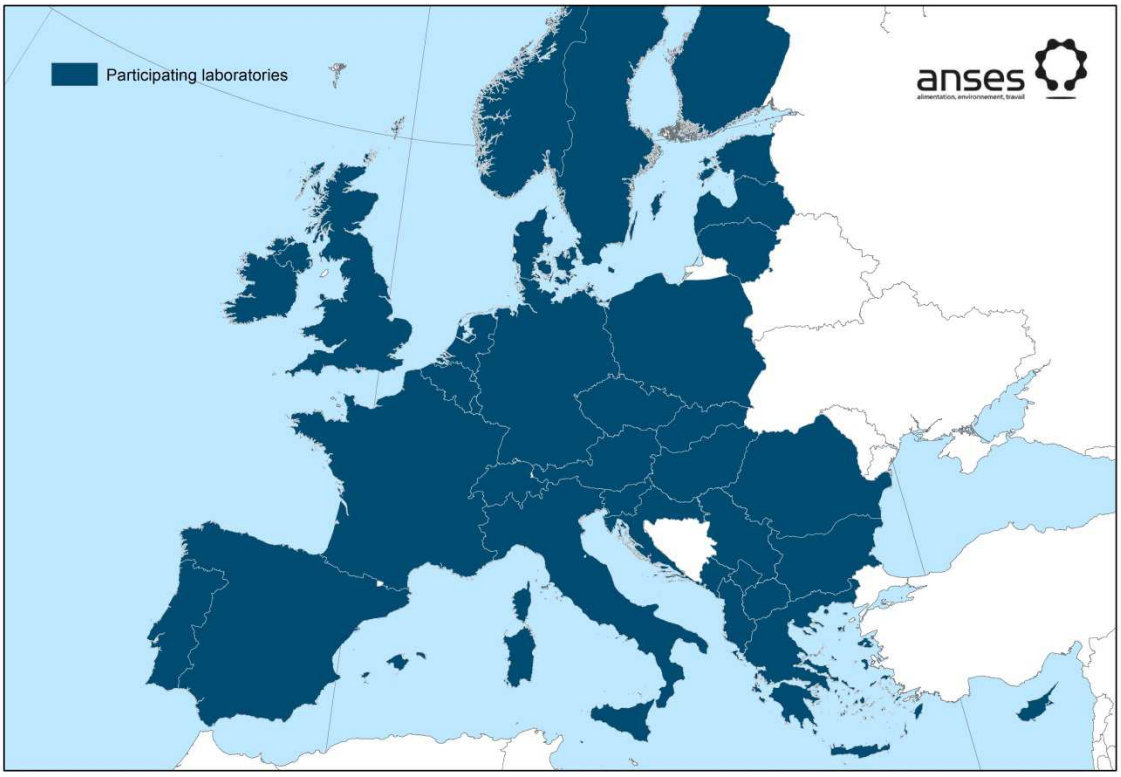

Figure 1: Map of the participating countries in the 2015 review

2 QUALITY ASSURANCE

In 2015, 27 on 34 laboratories (79%) 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 (65% of laboratories accredited) and the FAVN test (50%) (Figure 2). The proportions did not vary compared to 2014.

Fourteen on 34 (41%) participating national laboratories are working in BSL3 facilities.

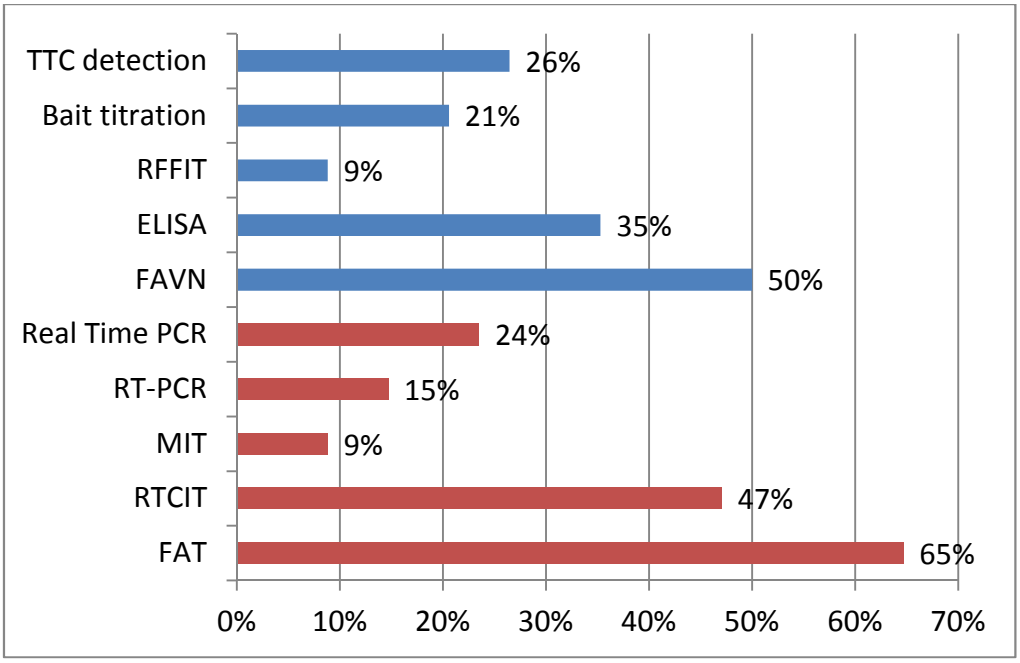

Figure 2: Percentage of laboratories accredited for the different techniques related to rabies field (diagnosis techniques in red and monitoring techniques in blue).

3 RABIES DIAGNOSIS IN THE FRAME OF RABIES SURVEILLANCE IN NON FLYING ANIMALS

As expected, the FAT gold standard technique (OIE, 2013; WHO; 1996) is the mostly used technique (representing 84% of the total amount of diagnosis tests performed during the year and used by 97% of laboratories) (Table 1).

The RTCIT is the second most widely used technique (48% of laboratories and 8% of the total amount of diagnosis tests performed during the year) and is often used as confirmatory test (Table 1). Real-Time and RT-PCR techniques are used by 48% and 36% of laboratories respectively. In consequences, proportion of laboratories using Real Time and RTCIT techniques are equivalent.
Still 27% of laboratories (n=9) are using the MIT techniques in their rabies diagnosis process although 5 of them are also using the virus isolation on cells (RTCIT) (Table 1).

Number of animals analysed in the frame of rabies surveillance programme (bats excluded) varied from 0 to 4958 samples per country (Figure 3). Globally, 139 positive cases were identified for a total of 30 508 FAT (0.005%).

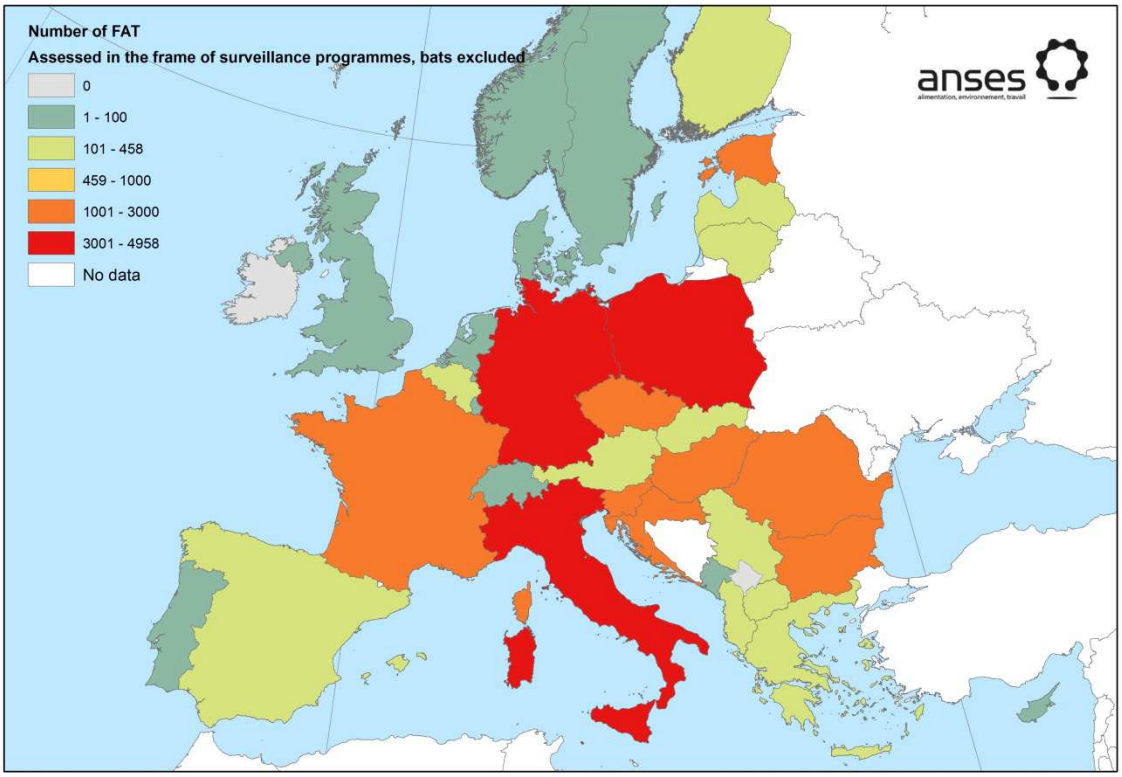

Figure 3: Number of FAT performed per country under rabies surveillance programme in non flying species.

Table 1: Number of tests performed per country (NRL and regional laboratories data) in 2015 in the frame of rabies diagnosis (non flying animals rabies surveillance only)
(Green box: number of test; red box: number of positives cases; [i]= imported case; [v]= vaccinal strain; ND= No data)

	Reference Techniques			Molecular Biology Techniques			
Country	**FAT**	**RTCIT**	**MIT**	**RT-PCR**	**RealTime**	**Typing**	**n cases**
Albania	439	12	9	0	0	0	0
Austria	390	64	0	4	3	0	0
Belgium	347	0	0	0	1	0	0
Bulgaria	2497	0	7	0	0	0	0
Croatia	1050	0	0	77	0	0	0
Cyprus	2	0	0	0	0	0	0
Czech Republic	2520	0	186	0	0	0	0
Denmark	3	0	0	0	3	0	0
Estonia	1574	39	0	0	40	0	0
Finland	135	29	0	1	0	0	0
France*	1459	0	0	0	0	0	1[i]
Germany	4958	224	0	0	2	2	0
Greece	251	0	0	4	85	0	0
Hungary	1110	0	487	0	40	0	1[v]
Ireland	0	0	0	0	0	0	0
Italy	3496	361	74	302	44	0	0
Kosovo	0	0	0	0	0	0	0
Latvia	206	197	0	10	0	0	0
Lithuania	132	177	0	2	0	2	2
Luxembourg	40	0	0	0	0	0	0
FYROM	241	1	0	0	1	0	0
Montenegro	13	0	0	0	0	0	0
Netherlands	19	0	0	0	0	0	0
Norway	5	0	0	0	13	0	0
Poland	3903	1514	162	177	67	97	93
Portugal	5	5	0	5	0	0	0
Romania	2852	9	129	56	0	56	28
Serbia**	199	3	66	0	0	0	3
Spain	117	0	0	117	117	6	6[i]
Slovakia	458	0	11	0	201	1	5
Slovenia	1963	89	0	2	2	0	0
Sweden	8	0	0	0	23	0	0
Switzerland	99	89	0	0	0	0	0
United Kingdom	17	2	0	0	2	0	0
Total (n analysis)	**30508**	**2815**	**1131**	**757**	**644**	**164**	**140**
Total (% analysis)	**84%**	**8%**	**3%**	**2%**	**2%**	**0.5%**	
Total (n laboratories)	**32**	**16**	**9**	**12**	**16**	**6**	**8**
Total (% laboratories)	**97%**	**48%**	**27%**	**36%**	**48%**	**18%**	**24%**

** data of RTCIT, MIT, PCR from one of the two laboratories performing diagnosis is missing*
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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 2).

The groups are the following:

Group A: Countries with at least one positive case in the year n^{-1} (2014) and conducting ORV in 2015.

Group B: Countries excluded from group A with at least one positive case in a bordering country in the year n^{-1} (2014) and conducting ORV in 2015.

Group C: Countries excluded from group A with at least one positive case in a bordering countries in the year n^{-1} (2014) and not conducting ORV in 2015.

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

As expected, countries without notified rabies case in 2014 nor in their country, nor in their bordering countries, and without ORV programmes present globally a lower surveillance pressure compared to others.

Table 2: Number of FAT tests performed in the frame of rabies surveillance programmes (excluding bats) per country for 100 km^2. Countries are classified in groups according to their rabies situation or occurrence of oral vaccination programmes or not. * *data from one of the two laboratories performing diagnosis is missing*

A	Bulgaria	2.2	B	Slovenia	9.7	C	Czech Republic	3.2	D	Luxembourg	1.5
	Croatia	1.9		Estonia	3.5		Germany	1.4		Belgium	1.1
	Poland	1.2		Albania	1.5		Austria	0.5		Switzerland	0.2
	Romania	1.2		Italy	1.2					France	0.2
	Hungary	1.2		FYROM	0.9					Netherlands	0.0
	Slovakia	0.9		Latvia	0.3					Spain	0.0
	Serbia*	0.2		Lithuania	0.2					Cyprus	0.0
	Greece	0.2		Montenegro	0.1					Denmark	0.0
				Finland	0.0					United Kingdom	0.0
				Kosovo	0.0					Portugal	0.0
										Sweden	0.0
										Norway	0.0
										Ireland	0.0

4 RABIES CASES IN NON FLYING ANIMALS

In 2015, 8 of the 34 (24%) participating laboratories identified a positive case corresponding to a total of 139 detected cases (Figure 4).

The highest numbers of rabies cases identified by NRLs and regional laboratories in 2015 were observed in Romania (28) and in Poland (93). Romania recorded a reduced amount of positive cases compared to 2014 and 2013 (462 in 2013, 166 in 2014 and 28 in 2015). Number of detected cases in Hungary decreased from 23 in 2014 to one vaccine induced case in 2015 and not a single rabies case was identified in Greece in 2015 while 10 cases were recorded in 2014.

Reoccurrence of rabies (two cases) was reported in Lithuania previously declared free of rabies in early 2015. Rabies imported cases in domestic animals were recorded in both France and Spain as regularly observed in previous years (2014: 5 cases in Spain, 2013: 1 case in France and 5 cases in Spain, 2012: 5 cases in Spain). Four countries finally harboured a low incidence of endemic cases below 5 cases. It should be notified that on French case is not present on the map because detected in French Overseas (One infected dog in French Guinea).

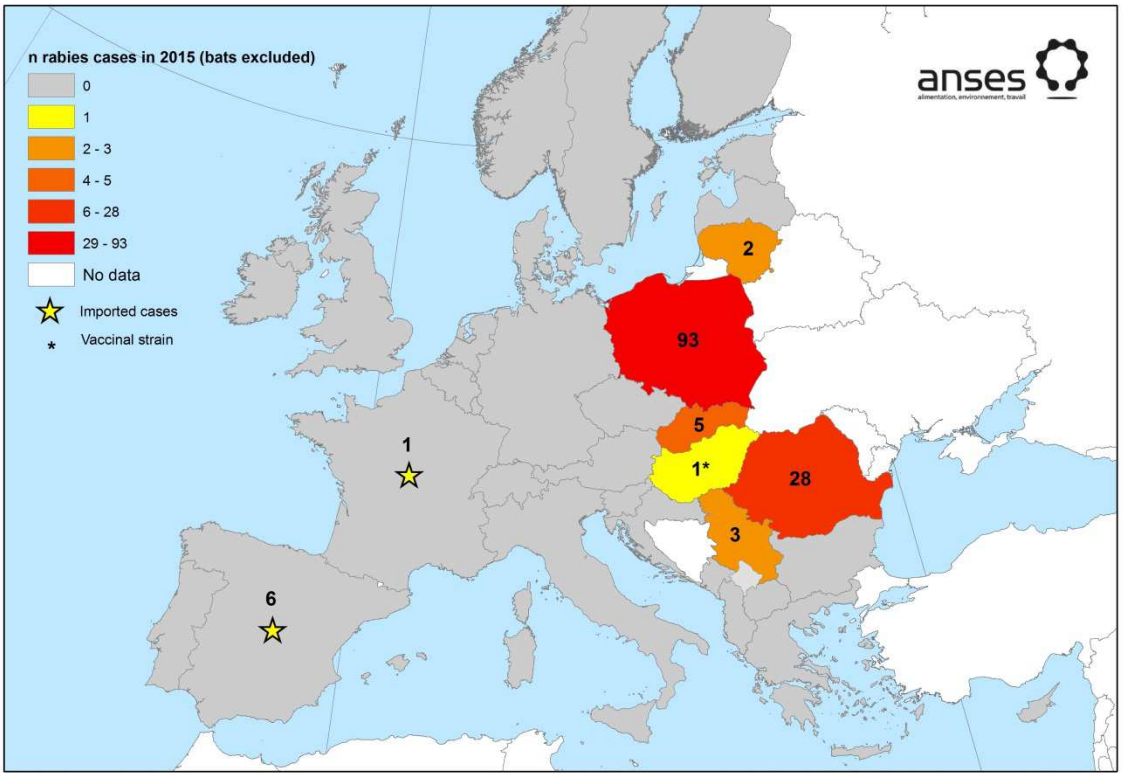

Figure 4: Number of reported rabies cases per country in non flying animals in 2015.

5 RABIES CASES IN BATS

Twenty two countries performed rabies diagnosis on bats routinely (Figure 5). The number of samples tested by FAT throughout 2015 varied from 1 (Slovakia) to 370 (France) tests within the year according to the country. The countries the most implicated in rabies surveillance in bats are principally located in Western Europe (France, United Kingdom, Germany, Netherlands, Poland, Spain and Portugal).

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 (Belgium, Italy, Netherlands, Portugal, Sweden).

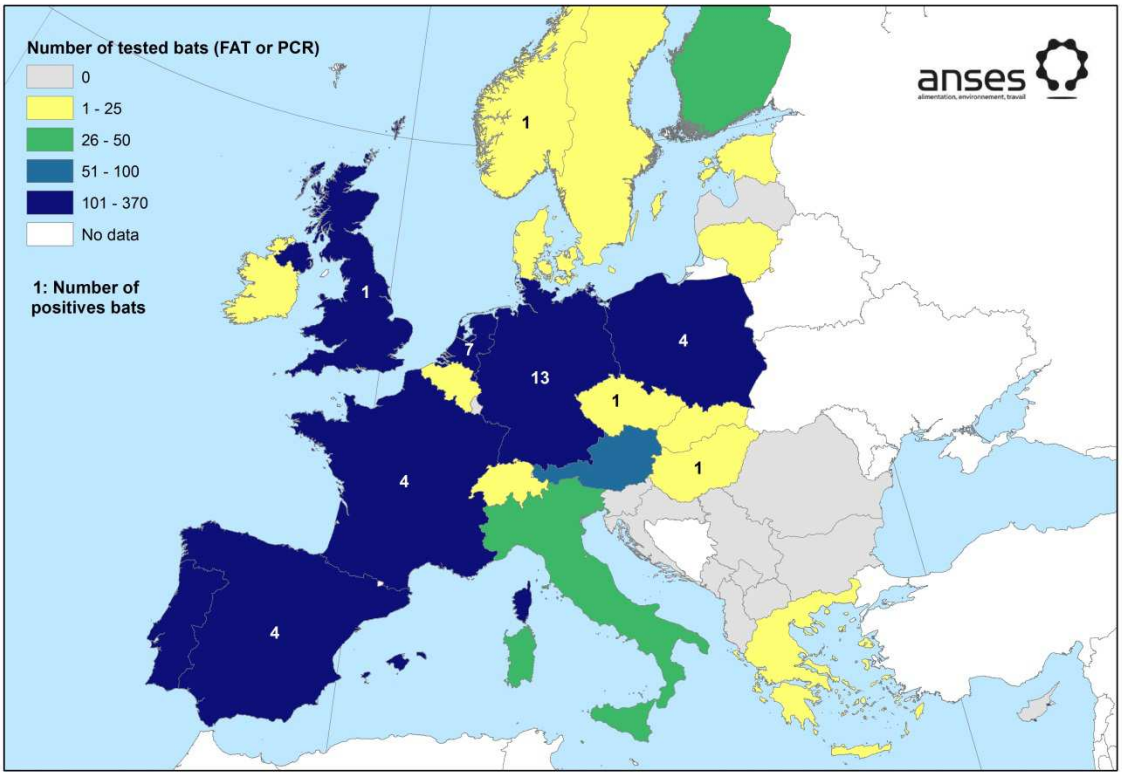

Figure 5: Number of bats tested by FAT per country in 2015 and number of positive cases.

6 ORAL VACCINATION MONITORING

6.1 Oral Vaccination

Eighteen countries implemented oral vaccination campaigns in 2015 (Table 3 and Figure 6). All countries except Finland, Greece, Italy, Kosovo and FYROM performed two ORV campaigns within the year (one in spring and one in autumn). In 2015, a total of 32 592 749 baits were distributed over 1 635 901 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.

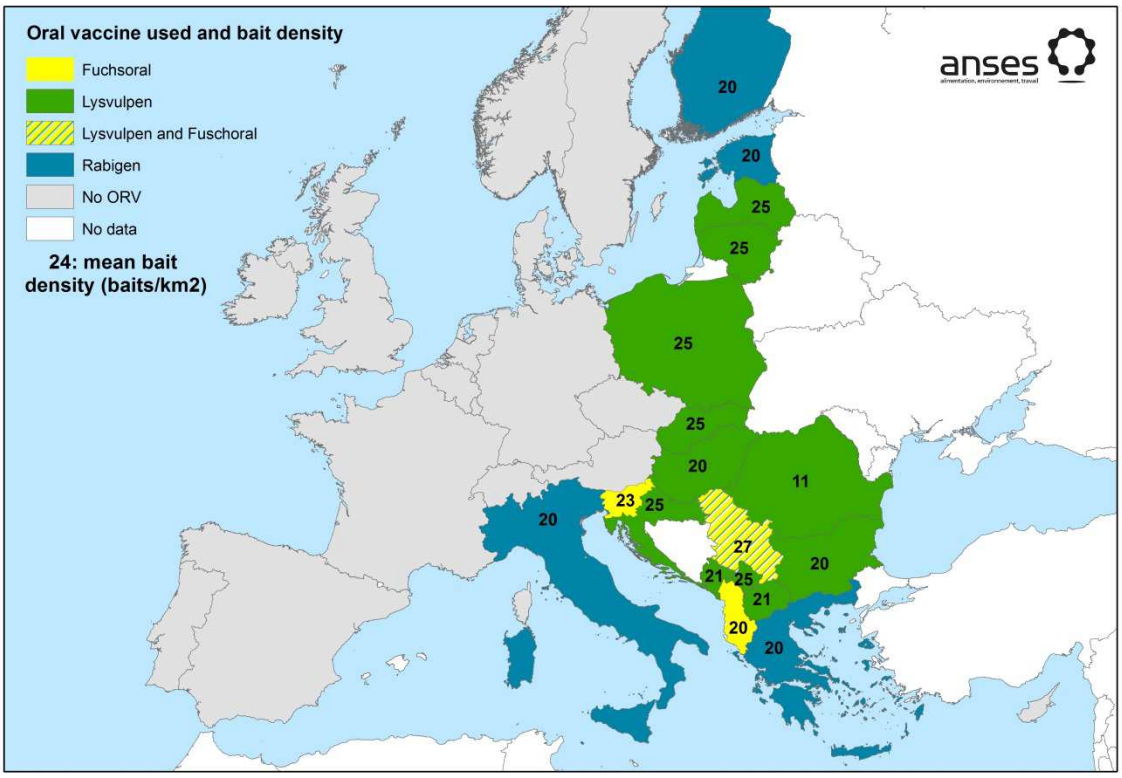

Figure 6: Oral vaccines used in the oral rabies vaccination and corresponding overall bait density per country.

Table 3: 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
Albania	2	Fuschoral	55,798	1,120,000	20	6
Austria	No					
Belgium	No					
Bulgaria	2	Lysvulpen	100,946	2,042,920	20	3
Croatia	2	Lysvulpen	113,084	2,827,100	25	7
Cyprus	No					
Czech Republic	No					
Denmark	No					
Estonia	2	Rabigen	18,650	373,000	20	5
Finland	1	Rabigen	9,000	180,000	20	3
France	No					
Germany	No					
Greece	1	Rabigen	54,584	1,317,983	24	10
Hungary	2	Lysvulpen	133,768	2,660,700	20	9
Ireland	No					
Italy	1	Rabigen	2,385	49,312	21	0
Kosovo	1	Lysvulpen	10,000	250,000	25	1
Latvia	2	Lysvulpen	51,200	1,280,000	25	5
Lithuania	2	Lysvulpen	68,600	1,715,000	25	4
Luxembourg	No					
FYROM	1	Lysvulpen	23,235	497,900	21	2
Montenegro	2	Lysvulpen	25,684	545,274	21	2
Netherlands	No					
Norway	No					
Poland	2	Lysvulpen	310,562	7,616,060	25	8 Fuchsoral; 20 Lysvulpen
Portugal	No					
Romania	2	Lysvulpen	474,000	5,325,900	11	29
Serbia	2	Lysvulpen; Fuschoral	121,992	3,310,000	27	8 Fuchosral; 4 Lysvulpen
Slovakia	2	Lysvulpen	24,413	607,600	25	3
Slovenia	2	Fuchsoral	38,000	874,000	23	3
Spain	No					
Sweden	No					
Switzerland	No					
United Kingdom	No					
Total			**1,635,901**	**32,592,749**	**20**	**132**

6.2 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 0.2 to 4.2 as compared to the previously recommended sample size for ORV monitoring of 4 individuals per 100 km^2 per year (WHO, 2005; EFSA, 2015). Nearly all the countries did not reach this target (Figure 7).

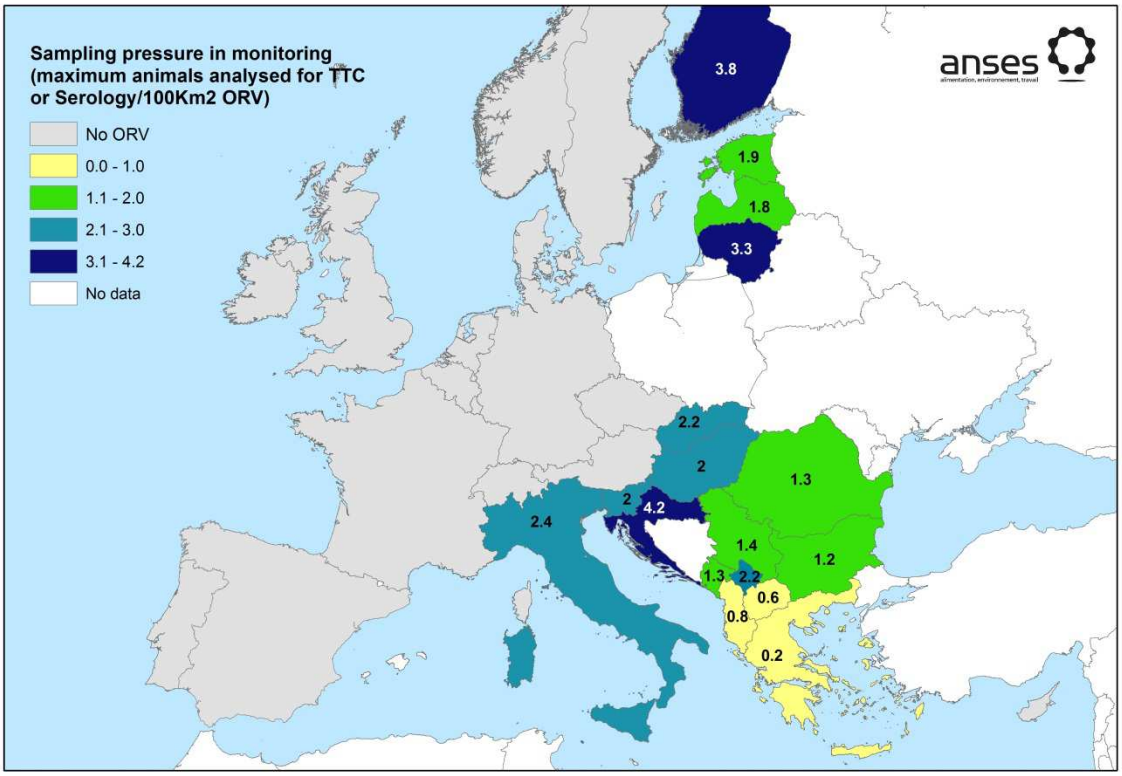

Figure 7: Number of animals analysed in the frame of ORV monitoring (TTC or Serology) per 100 km^2 of vaccinated area in 2015.

6.3 Percentage of Seroconversion in the target population

Percentages of seroconversion were found highly variable and ranged from 26% to 73% (Figure 8). While all the South-Eastern European countries had a seroconversion level below 30% in 2012 and while half of the South-Eastern European countries had a seroconversion level below 30% in 2013, all except one presented a seroconversion level up to 30% in 2014 and 2015 suggesting a constant increase since few years. A variety of tests is used for the serological analysis within Europe: 14/18 laboratories (78%) used an ELISA kit (10 laboratories used BioPro and 4 used Biorad). In laboratory group not using ELISA tests, three laboratories (Croatia, Italy, Slovenia) used the FAVN test, and one laboratory (Slovakia) used a "home made" 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 hazardous.

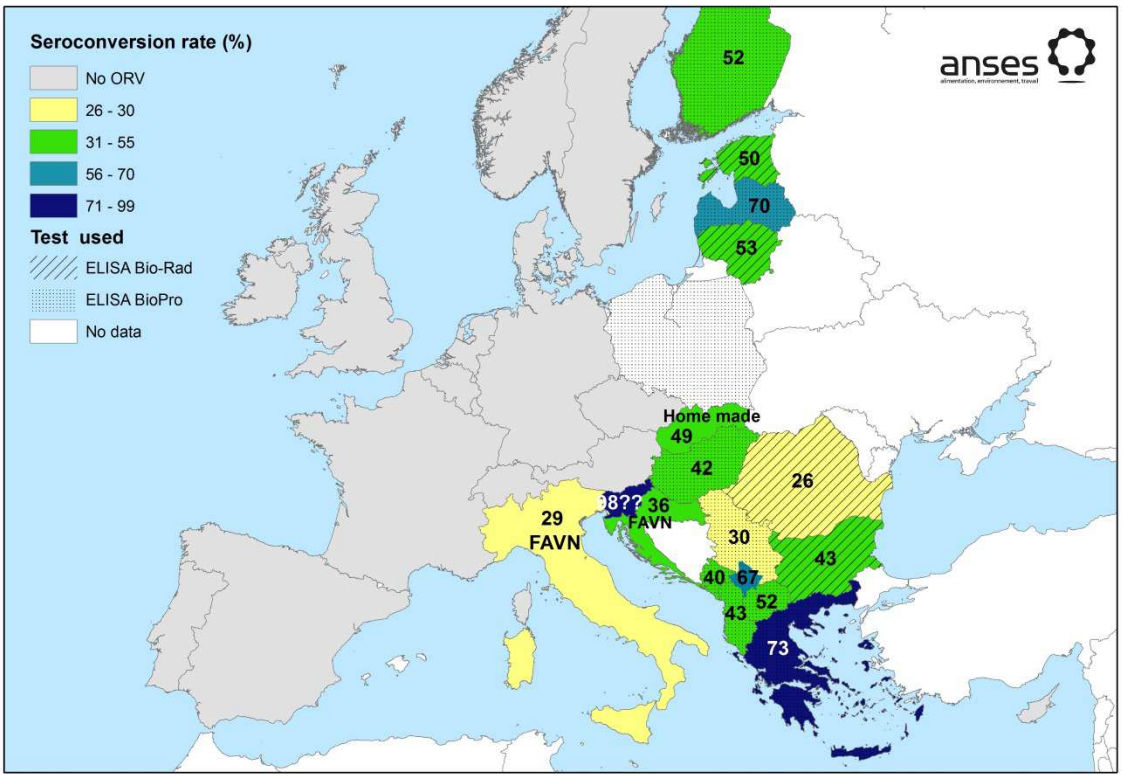

Figure 8: Proportion of sero-conversion in the target population and type of test used in 2015.

6.4 Percentage of tetracycline marking presence in target population

The proportion of animals identified positive for the presence of tetracycline in teeth (bait uptake level) ranged from 46% to 98% (Figure 9). While it was noticed in 2012 that most of the south-western countries harboured a bait uptake level ranged between 30 and 60%, as for 2013 and 2014, in 2015 all of them evaluated their bait uptake level up to 60% (excepted for Montenegro with 46% of TTC positive samples). This trend could be explained by the fact that most of these countries started ORV in 2011 and that two consecutive years of vaccination are generally necessary to reach the overall target population. Nevertheless, 5 countries found their bait uptake level below the requested target of 70% of vaccination coverage required to achieve control and eventual elimination of rabies (WHO). Among the five countries, one (Finland) is vaccinating a buffer zone in the border only with the objective to build a rabies immune belt. Its low TTC level could be explained by an "edge effect" due to the small size of the vaccinated areas (9000 km^2). The areas being small, the perimeter-to-surface ratio is higher and the probability of sampling an unvaccinated animal in bordering areas is higher than for large ORV areas. Moreover, this country is vaccinating once per year. Albania presenting a bait uptake level of 61% is vaccinating since 2014 only.

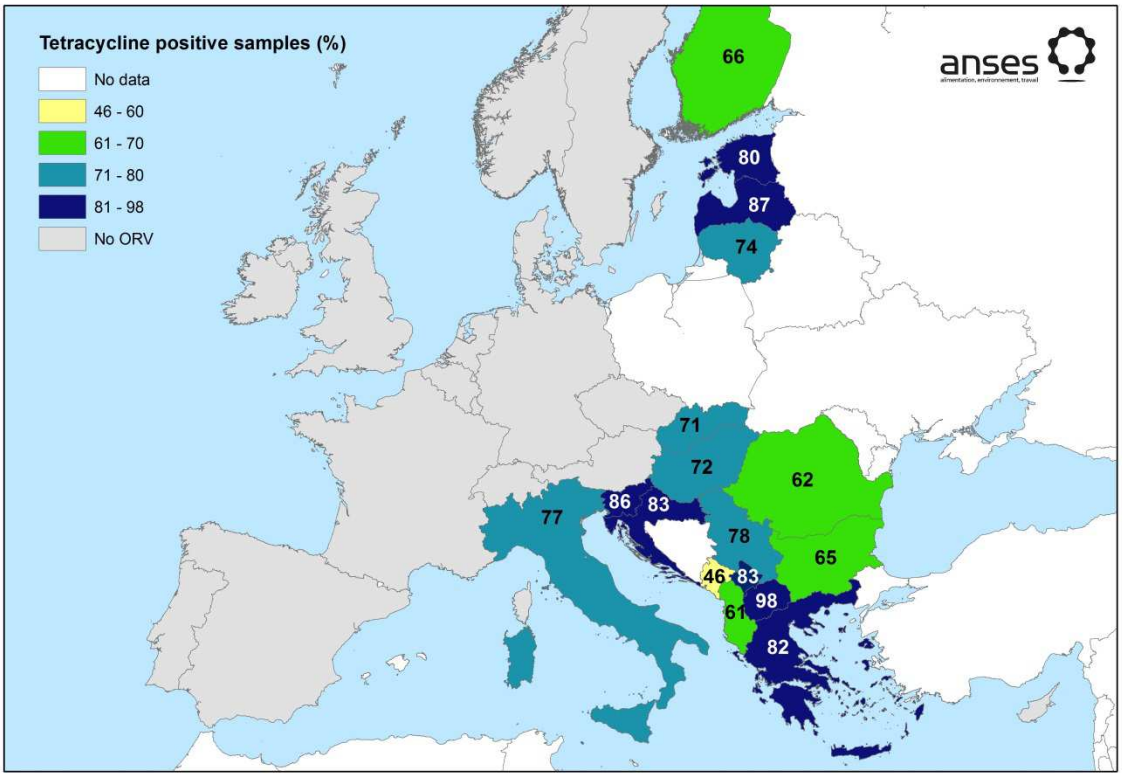

Figure 9: Proportion of samples positive for Tetracycline presence in jaws of the target population in 2015.

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