TECHNICAL REPORT of EURL for

RABIES

PERIOD: 2021-2022

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CONTACT DETAILS

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SUMMARY

INTRODUCTION

The ANSES Nancy Laboratory for Rabies and Wildlife has been nominated as European Union Reference Laboratory (EURL) for rabies on 1st July 2008.

The functions and duties of the European Union Reference laboratory (EURL) for rabies are described in the Commission Regulation (EU) No 415/2013 of 6 May 2013. This regulation amends the Regulation (EC) No 737/2008 designating the EURL for crustacean diseases, rabies and bovine tuberculosis as well as the Commission Regulation (EU) No 737/2008 Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council.

The main objective of the EURL for rabies is to coordinate the network of National Reference Laboratories (NRLs) for Rabies to obtain high quality results by the provision of reference methods, evaluation of new techniques or reagents, proficiency testing schemes and training to laboratory staff. These activities support the maintenance of a well-performing disease surveillance network throughout the European Union.

Regulation (EU) 2017/625, Art 94(2) and taking into account Art 147:
European Union reference laboratories designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories' annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.

Please provide activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.a* ***Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.***
- *Art. 94.2.b* ***Providing reference materials to national reference laboratories***
- *Art. 94.2.c* ***Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.***
- *Art. 94.2.l* ***Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.***

Sub-activity 1.1 Proficiency test on rabies diagnosis

Objectives:
Comparability of surveillance data and, as a result, comparability of laboratory diagnosis capabilities is a critical element for a precise estimation of the epidemiological situation over large areas. Proficiency tests (PTs) on rabies diagnosis will evaluate the individual performance of NRLs from the EU and certain bordering third countries on the current rabies diagnosis reference techniques and will monitor

laboratories' continuing performance. Participants will have to mimic their routine diagnosis process as much as possible.

Description:
Performance of NRLs will be assessed by conducting PTs on rabies diagnosis capabilities by using (i) the fluorescent antibody test (FAT), (ii) the rabies tissue culture inoculation test (RTCIT) and (iii) the conventional RT-PCR and (iiii) the real-time RT-PCR. Such a study will provide an estimation of the diagnostic specificity and sensitivity at the network level. The different steps of the trials are the followings:

- Contacting all NRLs (and possibly some from third countries after consultation and agreement of the EC) to establish a list of laboratories which are required to take part to the PT;
- Producing positive and negative reference materials. Eight new batches will be produced for the need of the trial (Four batch per year as the PT for rabies diagnosis is organised every two years). A minimum of one month is necessary to produce and validate one batch of rabies virus in vivo.
- Testing validity, stability, homogeneity of the constituted panel;
- Distributing a panel of characterised samples for inter-laboratory comparison;
- Interpreting all results of participating laboratories, then writing and dispatching a synthesis report.

- 2021: PT for rabies diagnosis
- 2022: production of four virus batches for the 2023 session of PT for rabies diagnosis

Expected Output:
Performance evaluation of NRLs from EU and some of the EU's neighbouring countries to maintain an adequate quality level of rabies diagnosis capacity within the network. In case of discrepant results, follow up will be intiated. Technical recommendations will be established based on discussion with the NRLs and on-site training will be proposed if need is identified.

Duration: continuous programme

Work carried out:

- Batch production of reference virus

Based on the virus bank collection of the EURL, lyophilised batches of viruses are regularly produced in vivo in the laboratory. Each batch is characterised by FAT, RTCIT, RTPCR and qRT-PCR and validated for homogeneity and stability under +4°C. These batches can be supplied as rabies diagnosis positive controls to NRLs on request and also used in the context of inter-laboratory testing. Nine batches of lyophilised virus were produced in 2021 and 2022 (Table 1).

Table 1:Lyophilised batch virus produced in 2021 and 2022:

Strain name	Lyssavirus species	Virus production protocol number	Date of Batch production	Batch number	N sample product
CVS27	RABV	2021-03	01/10/2021	01-21	121
NEGATIVE	/	/	05/10/2021	02-21	150
EBLV1 Espagne	EBLV-1	2021-04	26/10/2021	03-21	135
DUVV	DUVV	2021-28	16/11/2021	04-21	91
BBLV	BBLV	2021-42	18/11/2021	08-21	137
GS5	RABV	2022_Atton	13/12/2022	06-22	112
EBLV-2	EBLV-2	2022_12	02/06/2022	02-22	78
DUVV	DUVV	2022_19	26/10/2022	03-22	145
EBLV-1a	EBLV-1	2022_23	16/11/2022	04-22	156

- Rabies diagnosis proficiency test

The call for participation in the proficiency test for rabies diagnosis was sent to laboratories by e-mail in December 2020 and the proficiency test itself started on May 31st with the shipment of the samples. This test was based on the NRLs analyses performed on 10 submitted samples. Each batch of virus used for proficiency test purposes was produced in vivo and was validated by testing the panel for homogeneity (by FAT, RTCIT, RT-PCR and qPCR) and under various conditions for stability (by FAT, RTCIT and qPCR) prior to sending. The panel to be tested was sent to NRLs on the same day and NRLs were asked to provide a rabies diagnosis conclusion using FAT and/or RTCIT and/or Real Time and/or RT-PCR, depending of the techniques used in routine in their laboratory, the objective being to mimic as much as possible standard rabies diagnosis conditions.

Twenty-five NRLs from EU and 11 laboratories from third countries registered in this trial under EURL budget. The eleven laboratories from third countries granted to participate in this session under EURL budget were previously agreed with DG-SANTE.

For the 2021 session, on the 44 evaluated laboratories, only two laboratories (5% of evaluated laboratories) provided at least one discrepant result: two false positive diagnosis conclusion on a negative sample and a false negative conclusion on a CVS 27 positive sample.

Results of laboratories were also provided per technique (FAT, RTCIT, RT-PCR and qRT-PCR) in a complementary report.

Proficiency report entitled “Proficiency test for Rabies diagnosis session 2021” and complementary report “Proficiency test for rabies diagnosis - Complementary report, session 2021” were sent to NRLs by email in August 2021 and are available in the part under restrictive access of the EURL website (https://sitesv2.anses.fr/en/minisite/rabies/inter-laboratory-trials).

Sub-activity 1.2 Intra-laboratory evaluation of rapid diagnostic kits (RIDT)

Objectives:

RIDT, a rapid diagnostic test, is a medical diagnostic test easy and quick to perform. The principal advantages of such tests are their suitability for preliminary or emergency medical screening and for use in facilities with limited resources while they provide results within only few minutes. EURL intends to organise an intra-laboratory study to evaluate the repeatability and the reproducibility of RIDT that has been extensively described and evaluated in the recent years by individual laboratories.

Description:

The adaptation of Lateral Flow Assays (LFA) for rabies was first described in the mid-2000s. During the 10 past years, more than 11 publications relating the evaluation of LFA for rabies diagnosis have been published, demonstrating the growing interest for these immunochromatographic technologies. One of these LFA, the Rapid ImmunoDiagnostic Test (RIDT), has been extensively evaluated by individual laboratories and has provided promising results when compared to the gold standard Fluorescent Antibody Test (FAT). Currently new rapid diagnosis kits from different manufacturers regularly appear available on the market and can be purchased on the internet. Several different rapid immunochromatographic kits available on the market will then be purchased and evaluated by EURL. To allow proper evaluation of diagnosis sensitivity and specificity of kits, import of naturally infected samples from the field will be organised by EURL with the help of several laboratories.

Expected Output:

Although the Lateral Flow Assays (LFA) are not intended (for the time being) to be a reference test for rabies diagnosis, nor to replace the current FAT and Molecular Biology techniques, this intra-laboratory study will contribute to determine whether or not such new rapid immunochromatographic tests

provides similar performances. The information obtained on the sensitivity and specificity will contribute to have a clearer picture of the performance of new rapid kits and make it potentially suitable as an additional test for rabies diagnosis.
Rapid diagnostic kits do not require any special equipment (apart from usual protective equipment such as blouses, gloves, etc.) and are therefore interesting techniques for easy animal disease screening. They are booming techniques developed by many manufacturers and evaluating their quality is thus an important issue.

Duration: 2021

Work carried out in 2021:
Lateral flow assays (LFAs), also called immunochromatographic tests, or Lateral Flow Device (LFDs) are rapid tests able to detect an analyte at the point of care. They do not require cold chain to store the devices, making them particularly appropriate for field studies. They are quite rapid, low cost and one-step tests. Test results and validation can be easily visualized by the naked-eye. LFAs are generally based on colloidal gold conjugated monoclonal antibodies that capture the antigen contained in a sample. The antigen–antibody complex migrates on a nitrocellulose membrane and binds to a detection antibody fixed in the test zone "T" revealing a coloured line for a positive sample.
In the rabies context, this technology could have the potential to contribute to rabies prevention and surveillance more especially in countries with limited resources. Giving that they are simple, rapid and that they do not request any special equipment, LFAs have a strong potential for rabies diagnosis carried-out in field conditions. Unfortunately, depending on the brand, batch, etc., they have provided very unstable performances leading to consider them as inappropriate for rabies diagnosis and detrimental for a surveillance system. The objective of the present study was to assess the technical performance of two commercially available rapid immunochromatographic tests for rabies diagnosis newly found on the market, and to compare them to the Fluorescent Antibody Test.

The result obtained in this study demonstrated once again that the quality of rabies rapid immunochromatographic tests is highly heterogeneous. Previous studies indeed reported that the sensitivity may vary from 0 to 100% according to LFDs. So far, 8 different LFAs have been evaluated. Considering the present study, a total of 5 kits (62.5%) presented a sensitivity of less than 20%, a figure that gives a clear idea on the quality of the rabies diagnostics LFAs that are commercially available. To date, the Anigen rapid test remains the most extensively studied rapid kit that demonstrated the highest sensitivity indexes and the broadest range of reactivity (RABV and non-RABV lyssaviruses).

As conclusion, the uneven performance of rabies diagnostic rapid kits should prompt caution. Even if commercially available and open to any laboratory, LFAs are still not recommended tests to carry-out rabies surveillance and official controls. As demonstrated here, many of them still suffer from a lack of reliability and need to be better standardized and quality controlled. Therefore, validation of these LFAs are of upmost importance before any use in laboratories and cannot substitute for currently recommended reference techniques.
The report entitled "Technical evaluation of two rapid kits for rabies diagnosis" was sent to NRLs by email in December 2021 and is available in the part under restrictive access of the EURL website (https://sitesv2.anses.fr/en/minisite/rabies/eurl-technical-evaluation-0).

Sub-activity 1.3 Inter-laboratory evaluation of real-time PCR techniques

Objectives:

The main objective of this inter-laboratory study is to evaluate the inter-laboratory sensitivity of real-time reverse transcriptase polymerase chain reaction (RT-PCR) systems (SYBR Green and TaqMan) for the detection of different Lyssavirus RNA species.

Description:
Real-time RT-PCR presents the advantage to be rapid, specific and sensitive and is increasingly used by many laboratories in clinical virology, in replacement of classical methods. Diagnosis of rabies is routinely conducted post-mortem on brain tissue. The primary tests prescribed in 2018 by the World Organisation for Animal Health (WOAH, former OIE) for rabies diagnosis are: the direct fluorescent antibody test, the direct rapid immunohistochemistry test, or pan-lyssavirus PCR assays.
The EURL for rabies will organise in early 2022 an inter-laboratory study (ILS) to evaluate the real-time RT-PCR using a panel of twenty viral RNAs. RNAs will be extracted from mouse brain samples (as prepared for the current PTs for rabies diagnosis), validated and stored at -80°C before sending to the laboratories participating in the ILS. The panel of RNAs will include blind samples with different rabies RNA levels (highly positive, moderately positive and weak positive) as well as blind negative samples. Negative and positive control samples will be included in the panel for the validation of the test as well as a positive control (standard RNA) for determining the efficiency of PCR as well as the sensitivity of the qPCR method. All batches of RNAs will be validated for their homogeneity and stability at -80°C prior to sending. 2021 will be dedicated to panel preparation and validation.
RNA samples being highly susceptible to degradation, each panel will be shipped on dry ice (UN1845) to ensure that they remain frozen until the test is performed (sending planned in 2022).

Expected Output:
This inter-laboratory study on real-time RT-PCR will determine and evaluate the sensitivity of the molecular method, which is increasingly used in routine by NRLs in replacement of reference techniques, such as the cell-isolation test.
This ILS will contribute to determine whether or not the real-time RT-PCR provides similar performances when used by different laboratories.

Duration: 2021-2022

Work carried out:
The laboratory prepared about 30 panels for real-time RT-PCR evaluation in NRLs, each consisting of 21 RNA samples (20 blind samples and 1 sample with a known status, identified as CVS-27 1.10^7 copies/µL). The panel constituted of blind positive and negative RNA samples and mock sample was also included in each panel to avoid the risk of collusion between the participating laboratories.
Positive RNA samples were prepared with different Lyssavirus strains: two RABVs (a fixed RABV challenge strain CVS-27 and a field RABV from Greece), three European bat Lyssaviruses (EBLV-1, EBLV-2 and BBLV) with three different RNA levels (highly positive (16<Ct≤21), moderately positive (21<Ct≤26) and weak positive (26<Ct≤31)). An additional positive sample (CVS-27 at a RNA concentration of 10^7 copies/µL) was included in each panel to determine the PCR efficiency as well as the inter-laboratory sensitivity of the qPCR method.

Panel homogeneity was tested and validated for all panel samples. The impact of the transport on the assigned values -positive/negative- was evaluated by storing the panels at a temperature < -18°C for 4 days and 8 days. The results validate a 72-hour transport. The number of thawing and refreezing cycles of the sample that are allowed was also evaluated by testing four successive thawing and refreezing cycles. The results showed no impact on the assigned values –positive/negative- with a loss of at least an average Ct value of 2 (i.e. < 1 log of RNA level) for each positive sample.

Of all NRLs that were re-contacted in 2021 to participate in the inter-laboratory test, 25 confirmed their participation for 2022. The ILT was achieved from April to August 2022 and laboratories were asked to use their own technique real-time RT-PCR used in routine.
To summarize the results, the pan-Lyssavirus RT-PCR that was able to detect all lyssavirus species was the technique the mostly carried out in this inter-laboratory test. Indeed, 66% of the assays were pan-Lyssavirus RT-PCR (among which 63% was the SYBR Green RT-PCR and 37% was the pan-Lyssavirus Probe based RT-PCR, respectively) and 34% were TaqMan specific Probe RT-PCR. The lowest proportion of discrepancies on positive samples was shown for the pan-Lyssavirus TaqMan RT-PCR (4%) compared to the SYBR Green (10%) and the Lyssavirus specific Probe RT-PCR (5%). The three methods gave a false positive result (in different laboratories) on the negative sample, with respectively a proportion of 8.3% discrepant results for the SYBR Green RT-PCR (1 out of 12), 10% discrepant results for the TaqMan specific Probe RT-PCR (1 out of 10), and 14.3% discrepant results for the pan-Lyssavirus TaqMan RT-PCR (1 out of 7). The three methods showed a good sensitivity with 100% of detection for the strongly positive samples RABV and EBLV-1. Regardless of the method used, the ranged limit of detection of the PCR techniques was:

- LD PCR ranges from 1 copy/µL to 1000 copies/µL for SYBR Green RT-PCR assays-
- LD PCR ranges from 1 copy/µL to 100 copies/µL for Lyssavirus specific Probe RT-PCR assays-
- LD PCR ranges from 1 copy/µL to 100 copies/µL for pan-Lyssavirus Probe RT-PCR assays-

The full report entitled “Inter-laboratory assay for performance characteristics evaluation of lyssavirus real time RT-PCR techniques” was sent to NRLs by email in January 2023 and is available in the part under restrictive access of the EURL website.

Sub-activity 1.4. Epi-Vo project

Objectives:
Monitoring programmes of oral vaccination campaigns held in the European Union have produced since several years a lower seroconversion rate than initially expected and targeted, as well as lower than the bait uptake rate, which seems apparently relatively stable.
Thus, DG SANTE (D4-Food Safety Program, Emergency Funding) has mandated the EURL for Rabies to coordinate an original study aiming to identify possible explanations of such situation.

Description: (based on the letter and proposal amended work programme from DG SANTE on 02/02/2021)
Data of EU Member States (MSs) having carried out oral vaccination campaigns in the last 10 years will be collected. Data from vaccination campaigns, but also biological and environmental data of interest will be gathered. Bringing data together will indeed certainly increase the statistical power, necessary in such situation, given the large number of variables that may have impacted the monitoring results.

To achieve this goal, a comprehensive collection and review of data will be done:

- The data of oral vaccination implementation and monitoring thereof in EU MS over the past years, including any other parameter that may have an effect in the results of oral vaccination monitoring (e.g. lab methods, type of vaccines, vaccine distribution methods, sampling methods, concurrent passive surveillance etc.).
- Available data on other variables that may affect the effectiveness of oral vaccination and monitoring thereof (e.g. temperature or other climate parameters, information of wildlife distribution etc) over the same time period.
- Epidemiological data on Rabies during the same period (e.g. outbreaks).

All data used in the study will be requested and provided directly from the Competent Authorities / MSs' NRLs, since:

- they have the ownership of this information and they are the ones that can authorise its use in the framework of the proposed study,
- they are the only ones in possession of the original (raw) data with all available accompanying details/information.

Expected Output: (based on the letter and proposal amended work programme received from DG SANTE on 02/02/2021)

Analysis and evaluation of the above data with a view :

- To assess whether the variations observed over the years, in the levels of vaccine uptake and seroconversion :
  - are statistically significant,
  - are random or linked to specific factors.
- To assess the above fluctuations, in the results of the oral vaccination monitoring, correlate them or not with actual changes in the levels of immunity and vaccine uptake among wildlife (e.g. instead of changes in the levels of immunity / vaccine uptake, do they reflect changes in the composition or the habitats of the wildlife population, temporary migrations of wildlife, changes in sampling /monitoring methodology or effectiveness etc. ?).
- To propose (if possible) specific / pragmatic levels of vaccine uptake and seroconversion, as detected by oral vaccination monitoring, that may indicate satisfactory immunisation of the wildlife population.
- To propose likely interpretation(s) of the oral vaccination monitoring paradox (one or more, whatever more appropriate).

Duration: 2021-2022

Work carried out:

Member States having carried out oral rabies vaccination campaigns in the last 10 years were brougth together to gather the data from vaccination campaigns, but also biological and environmental data of interest.

Ten countries (CA and NRLs) registered in 2021 to participate in this study and an online meeting was organised the 10th June 2021 to present more extensively the study and to fix the factors to be taken into account in the analysis. Data collection was organised from September to December 2021 through the sending of online Sphinx questionnaires. Eight countries finally fulfilled the data submission.
2022 was dedicated to the data cleaning and to the statistical analysis of the factors highlighted as potentially influencing the decrease of seroconversion level.

The generalised linear model analysis revealed a relationship between the serological test used and the seroconversion level, which is in accordance with the literature and performance results discrepancies observed during previous ILT. To allow merged and easy use of oral vaccination efficacy surveillance data at community level, a single serological test with a common threshold value should be recommended for the evaluation of seroconversion levels within the community.

This study also highlighted a link between the maximum temperature reached during ORV campaigns and bait uptake rates. While these variables were statistically associated during our study, the causality cannot be established as no published studies are available to confirm these observations. It may indeed also be the result of random chance, the variables only appearing to be related without a true underlying relationship, or there may be a confounding variable that makes the relationship appear stronger than it actually is. Experimental studies and laboratory investigations should be conducted to

elucidate the link between bait uptake and a potential melting scenario leading to a decrease in uptake rates. Such studies would determine whether such a causality really exists.

Moreover, a limited number of participating countries and a subsequent significant number of missing data may have caused a lack of statistical power leading to difficulties in assessing some correlations. Nevertheless, the simultaneous collection and monitoring of the parameters mentioned in this study (relative population indices of foxes, wild boars, maximum temperatures reached during the oral vaccination phases) with the collection of vaccination campaign data should be encourage at the community level in order to ensure a comprehensive database support for further analyses. These data would undoubtedly constitute a valuable source of information that could be exploited to better understand ORV drivers.
The report was sent to DG SANTE on 13 December 2022.

Sub-activity 1.5. Annual Review of NRLs Rabies data

Objectives:
The objective will be to supply the NRLs with an annual review on tests and analyses performed in the frame of rabies diagnosis carried out for surveillance and rabies control within the network. Assessment of the number of tests performed in EU Member States and in some bordering countries for diagnosis, typing, virus titration, serology, tetracycline detection and age determination and to report their results and their trend at the European level will provide a better knowledge of the network, the techniques used and the needs for the future.

Description:
An online-questionnaire survey will be sent each year in February to each EU NRL and certain bordering countries to collate the data of the previous year. The data will be then analysed, maps and tables will be built and the report will be sent to all NRLs in June of the same year.

Expected Output:
Production of an annual report of tests and analyses performed in the frame of rabies surveillance and control within the EU and some bordering countries. Better information-sharing within the network. This annual survey and report allows the centralisation of rabies laboratories data and to share and compare results of rabies programmes between NRLs. All laboratory results (diagnosis, serology, tetracycline detection, etc.) are reviewed at national level, providing tables and maps that give a good picture of the overall situation at EU level.

Duration: annual activity

Work carried out in 2021 and in 2022:
An online questionnaire was set up using Sphinx software and sent to all NRLs in February 2021 for 2020 data collection, and sent in February 2022 for 2021 data collection. The objective was to collect and collate data on methods used and results of tests carried out in the EU and certain bordering countries.

The survey collated rabies surveillance data (rabies cases, number of diagnoses and techniques performed within the year for passive and active surveillance), but also data related to analysis performed in the frame of oral vaccination programme's control (tetracycline detection on teeth used as biomarker of oral vaccine bait-uptake, serological tests performed in the frame of monitoring of oral vaccination campaign, typing to exclude vaccine induced cases, etc..).

Data were analysed, mapped and compiled in the report entitled "Review of the analyses related to rabies diagnosis and follow-up of oral vaccination performed in NRLs in 2020" sent to the network in May 2021 and in the report entitled "Review of the analyses related to rabies diagnosis and follow-up of oral vaccination performed in NRLs in 2021" sent to the network in June 2022.
These reports are available in the part under restrictive access of the EURL website (https://eurl-rabies.anses.fr/en/minisite/rabies/inter-laboratory-trials).

Sub-activity 1.6. Inter-laboratory comparison to evaluate tetracycline and age determination techniques

Objectives:
To evaluate the performance of EU NRLs for rabies, and laboratories involved in rabies oral vaccination programmes co-funded by EC, on oral vaccine bait uptake biomarker detection.

Description:
The technique of tetracycline (TTC) and age determination is widely used within the EU in the frame of oral vaccination follow-up. Oral vaccine baits used in the EU include tetracycline to provide a life-long marking of bones and teeth of the bait consumers. When applying ORV, international institutions (WHO, OIE, EC) recommend controlling the vaccination effectiveness by notably analysing the presence of oral vaccination biomarker in fox and raccoon dog teeth.
The different steps of the PTs are the following:

- Contacting all EU NRLs (and possibly some from third countries after consultation and agreement of DG SANTE desk officer) to establish a list of laboratories requested to participate;
- Collecting positive and negative reference materials (red fox jaws issued from vaccinated areas);
- Testing half jaws to characterize the sample (positive, negative for TTC, age determination);
- Constituting a panel with the remaining half jaws;
- Distributing a panel of characterised samples for PT;
- Interpreting all results of participating laboratories, writing and dispatching a synthesis report.

Expected Output:
Performance evaluation of NRLs from EU and laboratories involved in rabies oral vaccination programmes co-funded by EC on their capacity to detect tetracycline biomarker in teeth and to distinguish juvenile from adult foxes. In case of discrepant results, follow-up will be initiated. Technical recommendations will be established based on discussion with the NRLs and on-site training will be proposed if need is identified.

Duration: 2022

Work carried out in 2022:
The inter-laboratory comparison started on 04 October 2022. At this date, the samples and result forms were dispatched by an international specialised courier. Six half jaws were sent to participants to evaluate results on tetracycline detection and age determination and 16 laboratories participated in the test. As for previous years, online technical questionnaires were used to collect information on the procedure used by participants for both tetracycline detection and age determination. The survey and statistics software "Sphinx iQ" was used and answer of laboratories were used to estimate the origin of laboratory discrepancies.

The fifth inter-laboratory session has revealed that 13/16 participating laboratories (81%) presented 100% concordant results in the tetracycline detection test. This is a stable proportion compared to previous sessions (2017, 2014, and 2012) but with a higher result compare to the first session of 2010. Considering the age determination of the samples, 12/16 laboratories (75%) estimated a correct age class on the whole panel. This is a comparable result with the 2017 session, with a higher performance compare to 2012 session.

These results demonstrate a constant satisfactory level of performance of the laboratories in both detection of tetracycline and age determination since the 2017 session. They are encouraging and demonstrate the laboratories capacity and the satisfactory results comparability for bait uptake estimations performed at EU level in the frame of oral vaccination campaigns.

The few misinterpretations of age estimation were observed between juveniles samples (0- 1 year) and adult samples of 1-2 years only. It has been reminded that, considering the birth period of cubs in Europe in March- April, for animals sampled in October-November, after the summer season, harbouring one pale line should have been aged >1-2 years and not 1-2 years old. This observation highlights the importance of taking into account the age of death of the animal for proper age estimation.

The report entitled "Inter-laboratory comparison: tetracycline biomarker detection and age class determination in red fox teeth." was sent to NRLs by email in December 2022 and is available in the part under restrictive access of the EURL website (https://sitesv2.anses.fr/en/minisite/rabies/inter-laboratory-trials).

Sub-activity 1.7 Digital PCR

Objectives:
To evaluate the digital PCR as a new molecular biology tool for routine rabies diagnostic method.

Description:
Rabies diagnosis in animals relies on postmortem laboratory tests, as recommended by WOAH. The WOAH recommended diagnosis tests for clinical rabies case confimation in animals are the Fluorescent Antibody Test, the Direct Rapid Immunohistochemical Test, the Rabies Tissue Culture Infection Test, the Pan-Lyssavirus real–time and/or conventional PCR assays.
The detection of all known Lyssavirus species can be achieved by two types of molecular approaches, that are the SYBR Green RT-qPCR based on universal pan-Lyssavirus primers, described by Hayman et al., in 2011, and/or the pan-Lyssavirus TaqMan RT-qPCR (LN34 assay) described by Wadwha et al. in 2017.
Both approaches present advantages and limits. The EURL for rabies evaluated deeply the SYBR Green RT-qPCR according to the French Standard NF U47-600on validation of PCR methods. We observed a PCR detection limit of 50 copies/µL, 100% for the diagnosis sensitivity and 100% for the diagnosis specificity. Although pan-Lyssavirus SYBR Green RT-qPCR is sensitive, specific and allows the detection of all known Lyssavirus species, a dissociation curve analysis must be undertaken at the end of the real-time PCR to confirm the specificity of the test result. The dissociation curve analysis can sometimes be challenging, especially when samples are autolyzed or in the presence of PCR interferences. Similarly,

in the case of degraded or mummified sample, non-specific amplification can take place and yield a non-specific positive result that is very difficult to interpret. Compared with the SYBR Green method, the TaqMan multiplex probe RT-qPCR (LN34 assay) is based on the combination of degenerated primers and two probes to achieve high sensitivity and specificity. The TaqMan RT-PCR LN34 assay has been shown by Wadwha et al. (2017) to detect a wide range of classical rabies virus (RABV) variants and other lyssaviruses as well as a limit of detection > 1 copy/µL. Moreover, the LN34 assay is described as able to eliminate the non specific amplifications from the non specific binding of the degenerated primers and/or primer dimers, making the implementation/adaptation of the LN34 assay in the laboratories equipped with real-time PCR for the detection of rabies virus RNA, easier to perform than the SYBR Green RT-PCR.

PCR-based techniques have been developed and extensively used over the last two decades for research. Since the 2000s, numerous RT-qPCR methods have been developed and published for the detection of lyssavirus RNA, either by conventional RT-PCR and/or by real-time RT-qPCR. Digital PCR is a new generation of traditional quantitative PCR which can be used for the absolute quantification of target nucleic acids. The digital PCR (dPCR) provides several advantages over real-time PCR, including more precise measurements and absolute quantification without the need for a standard curve. Since the 2010s, dPCR has been performed for the detection of a variety of viruses, including human immunodeficiency virus, cytomegalovirus, human herpes virus and recently the virus SARS COV-2. The dPCR that relies on the detection of end-point PCR products is less susceptible than qPCR to inefficient amplification, which can occur due to primer and/or probe mismatches or inhibitors in samples. The dPCR is known as more reliable than qPCR for inhibition-prone samples. A good correlation has also been shown by Pavšič et al. (2016) between the dPCR and accurate viral load in clinical samples.

The EURL for rabies laboratory should be equipped with a digital PCR at the end of 2021, in the frame of collaborative research studies on SARS-COV-2 pandemy (call for proposal in progress).Based on the validation study undertaken on the LN34 assay and provided that the laboratory will be equipped with the digital PCR equipement, we intend to develop and evaluate the dPCR real-time cycler by using the LN34 assay for a sensitive and rapid detection of RABV variants in EU, in addition to bat lyssaviruses.

In a first step, we will develop the LN34 assay on the dPCR real-time cycler and determine the limit of detection of PCR using a synthetic RABV RNA (synthesis in progress). Other PCR parameters will be evaluated according to the French Standard NF U47-600, including the limit of detection of the whole method, the inclusivity, the diagnosis sensitivity and the diagnosis specificity.
Expected Output: The overall objective will be to evaluate this new high quality/state of art analytical method in a European rabies context for a possible future use for routine rabies diagnosis.

Duration: 2021-2022

Work carried out :
Due to delay in funding provision, the recruitment of the staff (one full time technician) dedicated to this activity was not feasible, this activity was consequently deleted from the program.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please provide activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.d* ***Coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing national reference laboratories of advances in this field.***

- *Art. 94.2.e* ***Conducting training courses for staff from national reference laboratories and, if needed, from other official laboratories, as well as of experts from third countries.***

- *Art. 94.2.g* ***Providing information on relevant national, Union and international research activities to national reference laboratories.***

Sub-activity 2.1 Training activities and scientific consulting

Objectives:
To provide full technical and scientific assistance to NRLs from EU MSs and bordering countries on laboratory analyses related to rabies control and surveillance. Training and consulting will be provided on request, and depending on the outcomes of proficiency tests.

Description:
The Laboratory Lyssavirus Unit staff includes 15 people. The Unit is composed of four teams, each headed by an experienced scientist who can provide expertise, scientific and technical support under the rabies EURL mandate. The areas of expertise are diagnosis, molecular biology, virology, virus titration, biomarker determination, vaccinology and epidemiology.

Upon NRL requests, the EURL will organise training sessions on:
- rabies diagnosis,
- molecular biology, typing,
- rabies virus production (in vivo and in vitro), rabies virus titration,
- biomarker detection in teeth,
- antibody detection in wildlife (ELISA).

The training will take place at the EURL (column “training”) or in the facilities of the trained laboratories (column “mission” for the EURL staff).

Expected Output:
To maintain a harmonised and high-level laboratory analysis capacity within the EU.
Duration: annual activity

Work carried out:

One two days training was performed on the Fluorescent Antibody Technique in the Swedish NRL (National Veterinary Institute - Microbiology division) from 13 to 14 december 2022. The objective was to train new staff of the laboratory and to review, at the demand of the NRL, the rabies diagnosis system in place for potential improvement proposals.

Sub-activity 2.2 Website and management and newsletter

Objectives:
To facilitate and accelerate the dissemination of information.

Description:
The EURL for Rabies has established an internet website dedicated to the NRLs network. The website is hosted at https://eurl-rabies.anses.fr and allows consultation of news and events dealing with rabies in the EU, the NRL network presentation, the EURL activities and reports, workshop presentations, as well as work programmes and technical reports. Each NRL has received a login and a password giving an access to the documentation, training list, reagent catalogue, etc...
The website will be regularly updated with the news of the network and a newsletter will be prepared every two months and sent to the NRL network to facilitate the dissemination of these updates.
In 2021, EURL will also upload in its website, in free access part and according to the request of the Animal Health Law, guidance on the collection of samples, the techniques, validation and interpretation of the diagnostic methods for rabies diagnosis.

Expected Output:
Improvement of rabies knowledge dissemination. EURL procedures available for NRLs in the website. The list of NRLs (in public access) and the list of reference reagents and their supplier will be updated.

Duration: continuous

Work carried out in 2021 and in 2022:
The website of the EURL for rabies is regularly updated by including the news of the network, as well as agenda of activities and reports. In 2021, guidance on the collection of samples, the techniques, validation and interpretation of the diagnostic methods for rabies diagnosis have been upload in free access part and according to the request of the Animal Health Law.
Continuous updating by including the news of the network, as well as agenda of activities and reports is performed monthly. A EURL newsletter, linked with these activities and other related Rabies information (news from international institutions, news from NRLs, cases notification, publication, etc.) was also produced and sent to the network every two months.

Sub-activity 2.3 Organising annual Workshop for Rabies

Objectives:
To facilitate networking as well as sharing information within the NRLs network.

Description:
On an annual basis, the EURL for rabies will organise a workshop for gathering all EU National Reference Laboratories for rabies and several laboratories from some third countries after consultation and agreement of the DG SANTE desk officer. The workshop gives the opportunity to share information on rabies news and on the work that has been carried out during the past year.

Participants might be invited to deliver a presentation, especially for participants from countries where rabies still occurs.
Depending on the COVID-19 epidemic situation, the workshop will be organised at EURL premises or through online meeting.

Expected Output:
High number of NRLs attending the meeting.

Duration:
annual activity

Work carried out in 2021:
Due to the COVID-19 pandemic, the 12th Workshop for rabies was organised online, leading to a large under-consumption of credits. This EU NRLs meeting was joined to a TAIEX partnership to include the participation of EU bordering countries. In this context, and as in previous Workshops, national laboratories from Bosnia and Herzegovina; the former Yugoslav Republic of Macedonia; Kosovo; Morocco; Montenegro; Moldova; Serbia; Tunisia; Turkey and Ukraine took part in this event. In total, sixty-six persons took part in the Workshop that held on 20 April 2021.
EC, WOAH, FAO and EFSA representatives also took part in this event by providing updates on the rabies activities of the international institutions. EC DG SANTE (Pedro Rosado Martin) presented rabies related aspects of the Animal Health Law while WOAH (Patricia Pozzeti and Rachel Tidman) presented Endorsement of dog-mediated rabies control programmes and the United Against Rabies Forum (Phase 2 of Zero by 30). Angelique Angot from FAO presented some case examples of FAO contribution to rabies control and Frank Boelaert from EFSA provided update on rabies in EU, based on EU One Health 2019 Zoonoses report.
A second session was dedicated to the update on rabies situation within EU and bordering countries. The 2020 NRL survey was presented by the EURL while update on rabies surveillance and control program in Poland, Ukraine, Romania and Moldavia was presented by the respective NRLs.
Regarding bat rabies emergence, first spillover of West Caucasian Bat Lyssavirus in a domestic cat was presented by the Italian NRL while emergence of EBLV-1 in UK was presented by the APHA.

A third session was dedicated to laboratory techniques advanced. Three studies were presented by the EURL members:
- 2019 Proficiency test for rabies diagnosis – Emmanuelle Robardet
- 2020 Inter-laboratory test for rabies diagnostic on the Direct Rapid Immunohistochemical Test – Alexandre Servat
- 2019 Inter-laboratory study on rabies antibody detection in wildlife samples – Marine Wasniewski

During a last session the multi-annual EURL tasks were reminded and the future specific activities (Proficiency test for rabies diagnosis, evaluation of rapid diagnosis kit, inter-laboratory evaluation of real-time PCR techniques and the study of oral vaccination monitoring data (EPI-VO project)) were presented.

Grouped picture of the 12th Workshop for rabies

Work carried out in 2022:

In 2022, the 13th edition of Workshop for rabies took place in Warsaw, Poland on June 15-16. For the first time in its history, the event was simultaneously held online and in real life, allowing for some joyful reunion. As the workshop was initially planned on site, the “hybrid” format led to a large underconsumption of allocated credits.

In Total 62 participants took part into this event. Among those participants were representatives from European Union Reference laboratory as well as National Reference Laboratories from EU bordering countries including Algeria, Bosnia and Herzegovina Macedonia, Moldova, Morocco, Montenegro, Serbia, Tunisia and Turkey.

Divided in two half days, the workshop started with some welcoming words from Polish veterinary authorities. Those first words were followed by a presentation of EFSA activities on Rabies by Sotiria Antoniou. The following session was then dedicated to the situation of Rabies within EU. The third session concluded the first half day with presentations about the study of an imported case in Germany and Current challenge of pet importation in EU.


Grouped picture of the 13th Workshop for rabies

The Second Half day conference was opened with a presentation from Angelique Angot, representative of the FAO, followed by a presentation from Rachel Tidman from WOAH about the United Against Rabies Forum. The next session was focused on Bat Lyssaviruses with presentations from Conrad Freuling (Germany) and Stefania Leopardi (Italy). The last session was the prime time of this event with Laurent Dacheux from the Pasteur institute of Paris who gave a presentation about the challenge of diagnosis in human rabies. The session ended with presentations from Emmanuelle Robardet about results of Proficiency test on rabies diagnosis in 2021 and from Alexandre Servat about the technical evaluation of two rapid kits.

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please provide activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.f* ***Providing scientific and technical assistance to the Commission within the scope of their mission.***

- *Art. 94.2.h* ***Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).***

- *Art. 94.2.i* ***Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.***

Sub-activity 3.1 Scientific consulting

Objectives:
To offer full scientific assistance to the Commission and to other EU institutions (EFSA, EMA, ECDC).

Description:
At request, the EURL staff composed of five scientists of whom each has its own expertise in a defined rabies area (Diagnosis, vaccinology, virology, serology, molecular biology, epidemiology and oral vaccination control) will provide assistance to the EU institutions.

Expected Output:
Providing skilled scientific and technical advices.

Duration: annual activity

Work carried out in 2021 and in 2022:
The EURL staff responded regularly to technical requests sent by the commission and other institution on diagnosis, vaccinology, virology, serology, molecular biology, epidemiology and oral vaccination control and relative quality assurance system.
The European Union One Health Zoonoses Report, of the EFSA and the European Centre for Disease Prevention and Control, presents the results of zoonoses monitoring activities carried out in 27 EU Member States (MS) and nine non-MS. Key statistics on zoonoses and zoonotic agents in humans, food,

animals and feed are provided and interpreted historically. In 2021 and 2022, a member of the EURL staff took part to the consortium for data analysis, interpretation and chapter drafting of the rabies chapter.

Sub-activity 3.2 Diagnosis confirmation and /or typing

Objectives:
To provide rabies diagnosis confirmation, virus isolation and typing at request.

Description:
In case of unexpected outbreaks, of questionable results, and of any unexpected results within a NRL, the EURL will provide case confirmation. Once sent by the NRL, the EURL will examine the submitted sample(s) using FAT, RTCIT and molecular biology techniques. In case of positive diagnosis, sequencing of the partial N genome will be performed. In the situation of the detection of a vaccine-induced case, or a newly identified species, a full genome analysis (NGS) will have to be undertaken for an accurate genetic characterisation. FTA® papers, for the isolation, purification and storage of nucleic acids, will be offered to NRLs to simplify and reduce the cost of sample shipment (only if partial gene sequencing is needed as NGS techniques need to obtain organic tissue, which requires a specialised transport in dry ice).

Expected Output:
Actively assisting NRLs in the diagnosis of outbreaks or investigation of unexpected or unexplained diagnosis results.

Duration: 2021 (annual activity)

Work carried out in 2021 and 2022:
Four samples from Luxembourg have been submitted for rabies diagnosis confirmation to the EURL for Rabies (Table 2). Romanian samples were also sent in November 2022 for sequencing. The analysis of the Romanian samples are still under progress.

Table 2:Sample received in the EURL for rabies confirmation

N sample	Reception date	Country	Species	FAT result	RTCIT result	Real time result	Sequencing	Conclusion	Report sent on
1	20/05/2021	Luxembourg	*Felis silvestris catus*	negative	negative	negative	NA	negative	01/06/2021
1	02/09/2021	Luxembourg	*Felis silvestris catus*	negative	negative	negative	NA	negative	06/09/2021
1	12/11/2021	Luxembourg	*Vulpes vulpes*	negative	negative	negative	NA	negative	18/11/2021
1	05/01/2022	Luxembourg	*Felis silvestris catus*	negative	negative	negative	NA	negative	10/01/2022
18	23/11/2022	Romania	Multiple	/	/	/	In progress		

Exchange of RNA sequences was also realised between Hungary, Slovakia and the EURL following the last rabies re-emergence in the border with Ukraine.

Sub-activity 3.3 Participation in meeting or congress

Objectives:
Keeping abreast of development in surveillance, epidemiology and prevention of rabies throughout the world.

Description:
One expert of the EURL will attend annually the international rabies conference (RITA Rabies In the Americas) to be informed on rabies scientific advances on epidemiology, vaccination and laboratory techniques. Such conference participation will also be the opportunity for the EURL staff to share its experience and to present results on the ongoing projects to the worldwide rabies scientific community.
One expert will also attend annually the PARACON (Pan-African Rabies Control Network) or ARACON (Asian Rabies Control Network network) or MERACON (Middle East, Eastern Europe, Central Asia and North Africa Rabies Control Network) meeting (according to agendas, dates not available up to now). These networks were born in 2015 (PARACON) and 2018 (ARACON and MERACON) and are coordinated by the Global Alliance for Rabies Control (GARC). Each network provides unique opportunities for member countries and laboratories to share lessons learnt and challenges faced. The European Union being the only part of the world where rabies elimination in wildlife and domestic animals has been almost achieved, the participation of the EURL and sharing of the EU experience in such meeting could potentially be helpful for the rabies community. Also as specified in the EURL for rabies responsibilities, keeping abreast of development in surveillance, epidemiology and prevention of rabies throughout the world is of utmost importance and such meeting is a unique opportunity to update such information.

Expected Output:
Maintaining strong skills of the EURL scientists.

Duration: annual activity

Congress or meetings attended in 2021:
Unfortunately, following the emergence of the COVID-19 epidemic most of the meetings and congresses organised in 2021 were cancelled or moved to a virtual event. Virtual rabies events when possible were attended.
The virtual rabies meetings attended in 2021 were the followings:

- The Pasteur Institute of Tunis (Rabies Laboratory) and the French WOAH national reference laboratory (ANSES-Nancy) are involved in a WOAH twinning for Rabies since September 2020 for a period of three years. As part of this twinning, a Workshop dealing with Rabies and more particularly epidemiological surveillance and anti-rabies vaccination of dogs in Africa was organized. WOAH experts and international scientific personalities in the field of Rabies were present. The Rabies EURL director was invited to participate. This Workshop held online on February 16 and 17, 2021.

- FAO/WOAH/WHO United Against Rabies Forum - Effective use of vaccines, medicines, tools, and technologies towards Zero by 30 - Monday, October 4, 12h00-13h30 UTC. A series of webinars discussed how rabies control and prevention fit into a global One Health approach and its potential to deliver tangible health benefits for hundreds of millions of people, especially in the 150 countries where rabies remains a serious public health problem.

Congress or meetings attended in 2022:

The RITA meeting is an annual event that has been held since 1990. It has been hosted in many countries across the Americas. For many years, RITA has grown in popularity and prominence with delegates now coming from more than 20 countries across five continents. The meeting provides a unique opportunity for rabies researchers in advancing knowledge of surveillance, prevention and control, to meet each other, to share their experiences and also to discuss the challenges to be met.

In 2022, the Rabies in the Americas (RITA) conference held in Querétaro, Mexico, from October 23-27, 2022. Two scientists involved in EURL activities took part in this event and two oral presentation were presented. One mission was firstly intended in the workprogramme, but due to available budget and after agreement of the desk officer, two scientist took part. The presentations were the followings:

- Results on 21 years of enhanced bat rabies surveillance in France. Servat Alexandre, Cliquet Florence and Picard-Meyer Evelyne.
- Filter Papers to Collect Blood Samples from Dogs: An Easier Way to Monitor the Mass Vaccination Campaigns against Rabies? Marine Wasniewski, Jacques Barrat, Samia Ben Maiez, Habib Kharmachi, Mariem Handous and Florence Cliquet.

4 REAGENTS AND REFERENCE COLLECTIONS

Please provide activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.*j ***Coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants.***

- *Art. 94.2.k* ***Where relevant for their area of competence, establishing and maintaining:***
  - ***i. reference collections of pests of plants and/or reference strains of pathogenic agents;***
  - ***ii. reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to national reference laboratories;***
  - ***iii. up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents.***

Sub-activity 4.1 *(Development and maintenance of the EU rabies virus collection)*

Objectives:
To maintain and develop the EU rabies virus bank collection.

Description:
The choice of new rabies virus strains to be included in the virus collection will depend on outbreaks and collaboration opportunities. New strains will be collected, produced in vivo on mice and stored in liquid nitrogen. The batch will be validated using molecular biology techniques and each new strain will also be sequenced for its genetic characterisation.

Expected Output:
Variability enlargement of strains available for the benefits of the NRL network.

Duration: annual activity

Work carried out in 2021 and in 2022:
Since the EURL designation, newly acquired rabies strains are regularly collected, produced on mice, validated by molecular techniques and typing before storage. Each new batch is classically composed of 10-40 vials of 800 µl of virus suspension.

Seven batches were produced over 2021-2022 (Table 3).

Table 3: Newly batches virus produced for the rabies bank

Strain characteristic	Protocol Number	Date of production	Batch number	N sample
Ukrainia 1, fox, kiev, 2021	2021-30	01/10/2021	lot 05-21	0
Ukrainia 10, dog, kiev, 2020	2021-31	05/10/2021	lot 06-21	23
Hungary 2, fox, 2013	2021-33	26/10/2021	lot 07-21	2
Serbia 1, fox, Belgrade, 2011	2021-38	16/11/2021	lot 09-21	14
Serbia 6, fox, Mačvanski, 2018	2021-40	18/11/2021	lot 10-21	15
Hungary 3, fox, 2013	2021-41	19/11/2021	lot 11-21	18
Ukrainia 1, fox, kiev, 2021	2022-08	24/02/2022	lot 01-22	24

To date 63 batches of field rabies viruses are stored in the EURL and maintained at -150°C for the conservation of the viral biodiversity.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation:
(Number of Sub-activity boxes can be adjusted)

Sub-activity 5.1 *(name of Sub-activity)*

Objectives: Description: Output: Duration:

Sub-activity 5.2 *(name of Sub-activity)*

Objectives: Description: Output: Duration:

Sub-activity 5.3 *(name of Sub-activity)*

Objectives: Description: Output: Duration:

Sub-activity 5.x *(name of Sub-activity)*

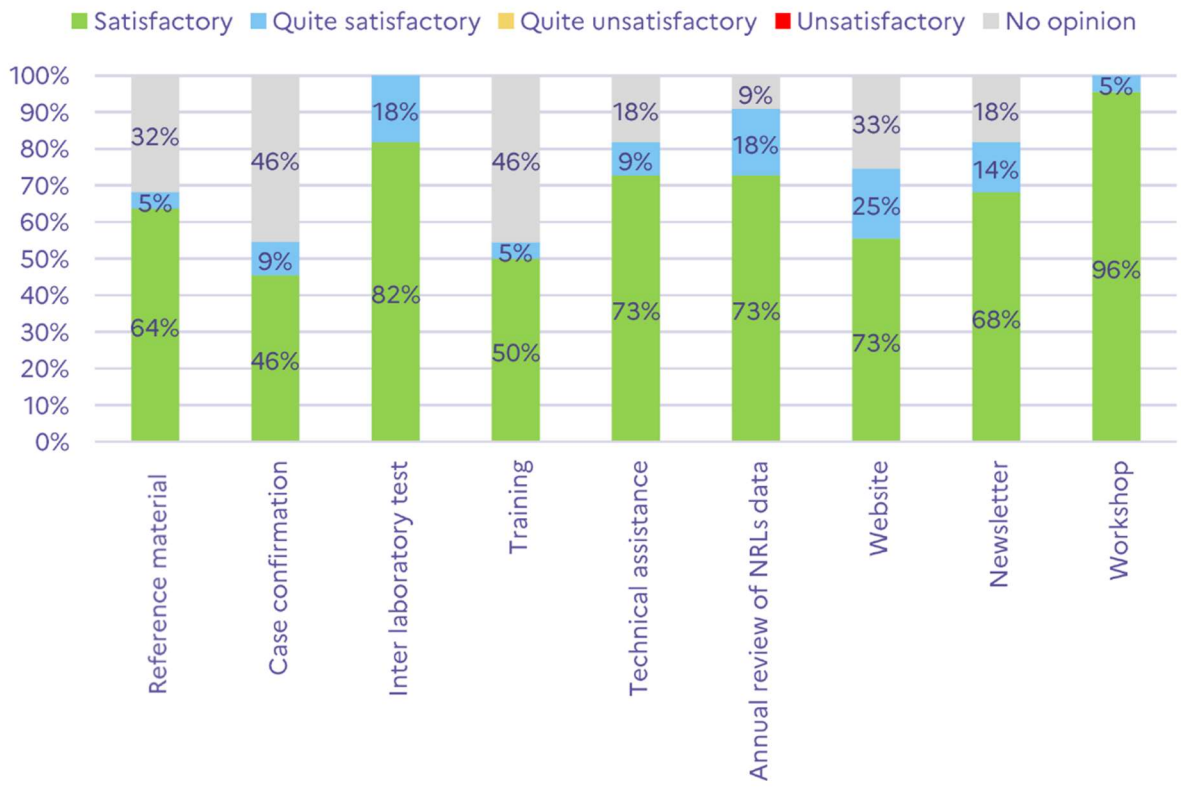
Objectives: Description: Output: Duration:

REMARKS

Staff cost for the 2021-2022 activities:
Following several changes within the Lyssavirus unit (departure of a senior scientist, departure of a senior technician replaced by a junior technician) and the presence of a vacant post (administrative secretary) which remained vacant for more than a year (difficulty in recruiting), plus the recruitment of the staff on the PCR digital activity not feasible because of delay in Budgetary commitment (leading impossible a new recruitment), the staff cost of the period 2021-2022 was unexpectedly under-consumed.

EURL activities evaluation by EU NRLs:

Every year, following the EURL for Rabies workshop, the EURL submits a satisfaction evaluation related to EURL activities to the EU NRL network.
The 2022 evaluation result (EU participants only) is presented below:



Results indicated a positive feedback from the EU National Reference Laboratories. Out of 'no opinion' all the answers indicated "satisfactory" and "quite satisfactory" evaluations regarding the EURL activities.

Quality assurance:

The laboratory is accredited according to NF EN ISO/IEC 17025 by the French national accreditation body (COFRAC) for the following tests:

- Seroneutralisation test for rabies antibodies (FAVN test) since February 2008.
- Rabies diagnosis (FAT and RTCIT) since October 2012.
- Rabies diagnosis by molecular biology since March 2020.

The laboratory is also recognised as an OMCL by EDQM through the Mutual Joint Audit system (according to ISO/IEC 17025) for the following tests:

- Potency test of rabies inactivated vaccines for veterinary and human use (challenge test and serological test) since March 2008.
- Potency test of rabies vaccines (live oral) for foxes and raccoon dogs since January 2012.

In addition, the laboratory is accredited since May 2017 according to NF EN ISO/CEI 17043 by the French national accreditation body (COFRAC) as a proficiency test provider for rabies serology.

International publications on rabies published in 2021 and 2022 by the staff involved in EURL activities :

Alvarez, J., Nielsen, S.S., Robardet, E., Stegeman, A., Van Gucht, S., Vuta, V., Antoniou, S.-E., Aznar, I., Papanikolaou, A., Roberts, H.C.
Risks related to a possible reduction of the waiting period for dogs after rabies antibody titration to 30 days compared with 90 days of the current EU legislative regime
(2022) EFSA Journal, 20 (6).

Wasniewski, M., Barrat, J., Maiez, S.B., Kharmachi, H., Handous, M., Cliquet, F.
Filter Papers to Collect Blood Samples from Dogs: An Easier Way to Monitor the Mass Vaccination Campaigns against Rabies?
(2022) Viruses, 14 (4).

Crozet, G., Lacoste, M.-L., Rivière, J., Robardet, E., Cliquet, F., Dufour, B.
Management practices of dog and cat owners in France (pet traveling, animal contact rates and medical monitoring): Impacts on the introduction and the spread of directly transmitted infectious pet diseases (2022) Transboundary and Emerging Diseases, 69 (3), pp. 1256-1273.

Crozet, G., Rivière, J., Rapenne, E., Cliquet, F., Robardet, E., Dufour, B.
Quantitative risk assessment of rabies being introduced into mainland France through worldwide noncommercial dog and cat movements (2022) Risk Analysis.

Arnaout, Y., Djelouadji, Z., Robardet, E., Cappelle, J., Cliquet, F., Touzalin, F., Jimenez, G., Hurstel, S., Borel, C., Picard-Meyer, E.
Genetic identification of bat species for pathogen surveillance across France
(2022) PLoS ONE, 17 (1 January), art. no. e0261344.

Crozet, G., Charmet, T., Cliquet, F., Robardet, E., Dufour, B., Rivière, J.
Benefit–risk assessment of the french surveillance protocol of apparently healthy biting dogs and cats for human rabies prevention (2021) Veterinary Sciences, 8 (7).

Robardet, E., Servat, A., Rieder, J., Picard-Meyer, E., Cliquet, F.
Multi-annual performance evaluation of laboratories in post-mortem diagnosis of animal rabies: Which techniques lead to the most reliable results in practice?
(2021) PLoS Neglected Tropical Diseases, 15 (2), pp. 1-18.