anses
Nancy Laboratory
for rabies and wildlife

European Union Reference Laboratory for Rabies

European Union Reference Institute for Rabies Serology

WHO Collaborating Centre for Research and Management in Zoonoses Control

OIE Reference Laboratory for Rabies

EUROPEAN UNION REFERENCE LABORATORY
RABIES

European Union Reference Laboratory For Rabies

Technical Report for 2019-2020

Nancy Laboratory for Rabies and Wildlife - February 2021

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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. It therefore contributes to the maintenance of an efficient disease surveillance network throughout the European Union.

Regulation (EU) 625/2017 Art 94(2):

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:

(taking into account Art 147 of (EU) 625/2017)

A. PROJECTS FUNDED BY THE EC UNDER THE EURL FOR RABIES MANDATE

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

Activities related to Regulation (EU) 2017/625:

- *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: Evaluation of antigen detection using DRIT

Objectives:

The EURL will organise an inter-laboratory trial (ILT) to evaluate laboratories in the handling of the Direct Rapid Immuno-histochemical Test (DRIT) that has been recognised in 2018 as a new OIE reference method for rabies diagnosis. Prior to this ILT, the EURL will assess intra-laboratory evaluation of the technique and candidates antibodies.

Description:

The DRIT was developed at the CDC (USA) in the 2000's. Like the Fluorescent Antibody Test (FAT) test, the DRIT detects viral antigens in the central nervous system of mammals (RABV and other lyssaviruses). This method is a simplified version of the standard avidin-biotin complex immuno-histochemical diagnostic assay. It is already in routine use in North America for support of oral wildlife rabies vaccination programmes. This technique is as specific and sensitive as the FAT. Because of its recent inclusion in the OIE Terrestrial Manual chapter on rabies (2018), the EURL for rabies aims at organising an inter-laboratory assay to evaluate the performance of laboratories with this new method. Before the implementation of the proficiency test (planned in 2020), a several-month phase will be required:

- to purchase antibodies which are a key element of the DRIT,
- to mark them with biotin,
- to evaluate them (optimal working doses determination) for a proper detection of the different lyssaviruses to be included in the ILT panel.

Two antibody preparations have been previously identified: One cocktail of monoclonal antibodies from the Wistar Institute (USA) and one preparation of polyclonal antibodies from the GARC (South Africa). These different steps are planned for the year 2019.

Expected Output:

To obtain two different candidate-antibody preparations (monoclonal and polyclonal) that could be used in DRIT by laboratories participating in the ILT organised in 2020. The ready-to-use marked antibodies should be in a sufficient amount for an upstream distribution to laboratories willing to perform the DRIT before the official ILT.

Duration: 2019

Work carried out in 2019:

In 2019, the EURL for rabies performed the intra-laboratory evaluation of DRIT by achieving different steps:

- Purchase of antibodies which are a key element of the DRIT,
- Evaluation of candidate antibody.

Whereas two candidate antibody preparations were initially identified, only one was purchased (a preparation of polyclonal antibodies from the GARC, South Africa). The cocktail of monoclonal antibodies from the Wistar Institute (USA) was finally out of stock in 2019. Once purchased, the GARC preparation has been evaluated internally to determine the optimal working dose (1/300) for a proper detection of various RABV and lyssaviruses maintained in the laboratory. It has been tested on about 30 samples obtained from experimentally infected animals. A list of biological reagents to be distributed to all participants (together with the panel of samples in 2020) have also been set up. Reagents and appropriate bottles/flasks/vials were also purchased to repackage the reagents and supply ready-to-use products.

Sub-activity 1.2: 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 testing 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 on rabies diagnosis will be assessed by conducting an inter-laboratory test on (*i*) the fluorescent antibody test (FAT), (*ii*) the rabies tissue culture inoculation test (RTCIT) and (*iii*) on the molecular biology techniques. 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 EU laboratories (and possibly some from third countries after consultation and agreement of the EC to establish a list of interested laboratories;
- Producing positive and negative reference materials. Eight new batches will be produced for the need of the trial. 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 and validation;
- Interpreting all results of participating laboratories, then writing and dispatching a synthesis report.

Expected Output:

Performance evaluation of NRLs from EU and some of the EU neighbouring countries to maintain an adequate quality level of rabies diagnosis capacity within the network. In case of discrepant results, technical recommendations based on the technical questionnaire analysis will be established.

Duration: 2019 (Proficiency test for rabies diagnosis is organised every two years)

Work carried out in 2019:

The proficiency test for rabies diagnosis started on June 3, 2019. 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* (Table 1) and was validated by testing the panel ten times in duplicate for homogeneity and five times under various conditions for stability (by FAT, RTCIT, RT-PCR and qPCR) prior to sending. The panel to be tested was sent to NRLs on the same day and NRLs were asked to perform rabies diagnosis on these samples using FAT and/or RTCIT and/or Real Time and/or RT-PCR.

Table 1: Composition of the panel test of the inter-laboratory test on rabies diagnosis techniques.

ID	Batch name	Passaged on	Strain Origin	Species	Country	Year of isolation	Infected species
1	CVS 27 04-17	Mouse	CVS 27 11-14	RABV	/	/	*Fixed strain*
2	RABV dog 12-17	Mouse	124155	RABV	France	2004	*Canis lupus familiaris*
3	RABV fox 02-17	Mouse	VB1071	RABV	Slovakia	2015	*Vulpe vulpes*
4	RABV fox GS5 15-17	Fox	GS5	RABV	France	1982	*Vulpe vulpes*
5	EBLV-1b 19-18	Mouse	123008	EBLV-1	France	2002	*Eptesicus serotinus*
6	DUVV 18-15	Mouse	DUVV 05-11	DUVV	South Africa	1971	*Homo sapiens sapiens*
7	ABLV 06-17	Mouse	ABL1	ABLV	Australia	1997	*Pteropus alecto*
8	Negative 11-18	Fox	/	/	France	2018	*Vulpes vulpes*
9	Negative 16-18	Pig	/	/	France	2018	*Sus scrofa domesticus*
10	Sample to avoid collusion	Mix	mix	mix	mix	mix	*mix*

Twenty-six NRLs from the EU and 23 laboratories from third countries participated in this trial. Third countries participation was out of the scope of the EURL and performed without EURL budget. They included 10 laboratories from Europe, four from America, four from Africa, four from Asia, and one from Oceania. The number of participating EU laboratories remained stable compared to previous years (Figure 1).

The report entitled “Inter-laboratory test for rabies diagnosis: Session 10-2019” was sent to NRLs by email in August 2019 and is available in the part under restrictive access of the EURL website (https://eurl-rabies.anses.fr/en/minisite/rabies/inter-laboratory-trials).

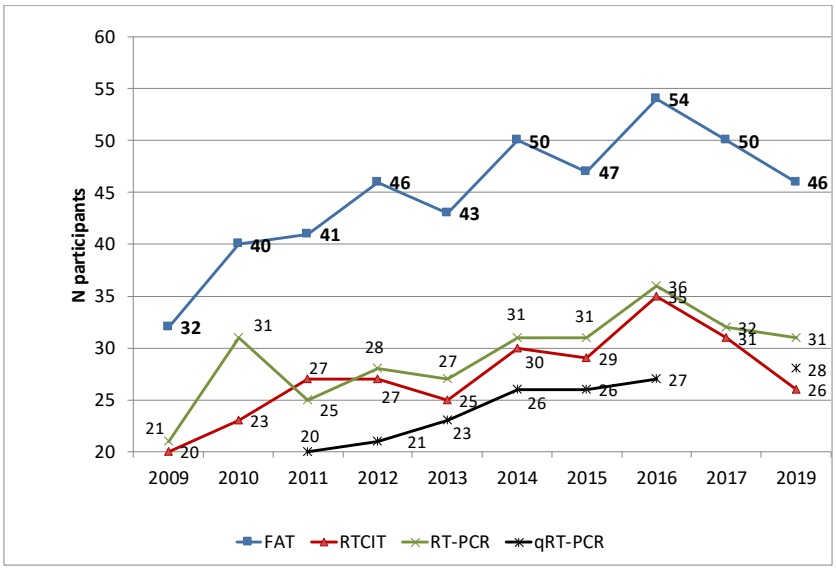

Figure 1: Evolution of the number of EU participating laboratories in the rabies diagnosis inter-laboratory test

For the 2019 sessions, only two laboratories provided wrong overall conclusion on sample status. For each laboratory, only one sample was concerned, a negative one (Table 2). One of this two laboratories performed molecular biology techniques only, that could have increased probability of false positive results because of their very high sensitivity.

Overall, the lowest proportion of laboratories producing discrepant results was found in the FAT (4%), followed by the RT-PCR (7%), then the RT-qPCR (11%) closely followed by the RTCIT (12%).

For the fourth year, not a single false negative result was detected using the RT-PCR. RT-PCR assay demonstrated the best diagnosis sensitivity. Not a single false positive result was found using the RTCIT, demonstrated the best diagnosis specificity.

Table 2: Rabies diagnosis laboratory discrepancies in 2019 session (percentage are given in brackets).

n discrepant/N (%)	FAT	RTCIT	RT-PCR	RT-qPCR	Overall Diagnosis conclusion
Number of laboratories	2/46 (4.4)	3/25 (12.0)	2/29 (6.9)	3/27 (11.1)	2/45 (4.4)
Negative samples (false positives)	1/92 (1.1)	**0/50**	2/58 (3.5)	2/54 (3.7)	2/90 (2.2)
Positive samples (false negatives)	1/322 (0.3)	4/175 (2.3)	**0/203**	1/189 (0.5)	**0/315**
RABV	1/184 (0.5)	4/100 (4.0)	**0/116**	1/108 (0.9)	**0/180**
CVS 27	**0/46**	**0/25**	**0/29**	**0/27**	**0/45**
RABV fox GS5	**0/46**	**0/25**	**0/29**	**0/27**	**0/45**
RABV fox SK	**0/46**	1/25 (4.0)	**0/29**	**0/27**	**0/45**
RABV dog Spain	1/46	3/25 (12.0)	**0/29**	1/27 (3.7)	**0/45**
ABLV	**0/46**	**0/25**	**0/29**	**0/27**	**0/45**
EBLV-1	**0/46**	**0/25**	**0/29**	**0/27**	**0/45**
DUVV	**0/46**	**0/25**	**0/29**	**0/27**	**0/45**

Sub-activity 1.3: Inter-laboratory test on antibody detection in wildlife samples

Objectives:

In 2016-2017, the EURL organised an inter-laboratory study on serological techniques performed by the NRLs by using wildlife samples collected in the field. The main objective of this first study was to get a global overview of the performances of the techniques and protocols undertaken by the NRLs to titrate the rabies antibodies in wildlife samples. However, as a first approach for assessing methods, this study could introduce a bias as only naive, strong and mean positive samples were tested. Therefore, and as the first results obtained by NRLs were satisfactory, the EURL will organise a second inter-laboratory study, in 2019, on serological techniques performed by the NRLs by using wildlife samples having titres around the threshold of 0.5 IU/mL.

Description:

The panel sent to participating laboratories will be composed of items including negative and positive samples with titres around the threshold of 0.5 IU/mL. The samples will be obtained from field and caged foxes and from caged raccoon dogs.

Expected Output:

This new study will allow collecting more information on the performance of the methods performed by NRLs for rabies serology with wildlife samples having titres around the threshold of 0.5 IU/mL. Considering the number of different ELISA kits in use in the EU, an ultimate objective for next years would be to try to get a better harmonisation of results obtained by the NRLs, hence a better harmonisation of methods used.

Duration: 2019

Work carried out in 2019:

A first study carried out in 2016 demonstrated that two commercial ELISA tests (for testing wildlife samples) were mainly used by NRLs and that satisfactory results were obtained with both kits whatever the origin and quality of the tested samples. However, only strong and mean positive samples as well as negative samples were tested in this study. In 2019, the EURL has organised a second inter-laboratory study on serological techniques performed by the NRLs by using wildlife samples having titres around the threshold of positivity. This second study, dedicated to the ELISAs only, allowed to collect additional information on the performance of these tests. Sixteen laboratories among the 28 invited NRLs took part in this study. The panels, containing 24 samples, were sent to the participants in November 2019. Every participating laboratory was invited to titrate the panel 3 times in three independent manipulations. All participants sent back their results. The last results were received in December 2019 and the report was sent to the network in June 2020. This study demonstrated that the limit of detection obtained using the BioPro ELISA kit was lower than the one obtained using the Bio-Rad ELISA kit as specified by manufacturers and that the limit of detection varied for the same ELISA

kit between participating laboratories. Seven among 16 participating laboratories provided at least one false negative result on submitted samples while six among 16 provided at least one false positive result on submitted samples. Although all laboratories used the same reagents, followed the same SOP (the one available with the kit) to test the same panel of samples, the different obtained results showed that the use of ELISA tests is not quite standardised through the European NRLs when samples are closed to the limit of detection, potentially resulting in an over- or an underestimation of the serological titres according to the testing laboratory when samples are closed to the threshold of 0.5 IU/mL.

Sub-activity 1.4: Inter-laboratory test on antigen detection using DRIT

Objectives:

The EURL wishes to organise an inter-laboratory (ILT) assay to evaluate laboratories in the handling of the Direct Rapid Immuno-histochemical Test (DRIT) that has been recognised in 2018 as a new OIE reference method for rabies diagnosis.

Description:

The DRIT was developed at the CDC in the 2000's. Like the Fluorescent Antibody Test (FAT) test, the DRIT detects viral antigens in the central nervous system of mammals (RABV and other lyssaviruses). This method is a simplified version of the standard avidin-biotin complex immuno-histochemical diagnostic assay. It is already in routine use in North America for support of oral wildlife rabies vaccination programmes. This technique is as specific and sensitive as the FAT. Because of its inclusion in the OIE Terrestrial Manual chapter on rabies (2018), the EURL for rabies aims at organising an inter-laboratory assay to evaluate the performance of the DRIT in different laboratories. To that end, a panel of around 10 lyophilised brain samples will be prepared and distributed to all participants. Performance of DRIT will be compared to the performance of the fluorescent antibody test (FAT) that will be performed in parallel. The different steps of the trial are the followings:

- Contacting all European laboratories (and possibly some from third countries after consultation and agreement of the EC) to establish a list of interested laboratories;
- Producing positive and negative reference materials. 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 and validation;
- Interpreting all results of participating laboratories, then writing and dispatching a synthesis report.

Expected Output:

Although the DRIT is more particularly indicated for laboratories with limited resources (in Africa and Asia), this inter-laboratory test will contribute to determine whether or not the DRIT provides similar performances when used by different NRLs for rabies and to evaluate the performance of both the monoclonal-antibody cocktail and the polyclonal preparation that will be selected for this study. The

results should contribute to have a clearer picture of the overall performance of the DRIT and determine the ease of use of the DRIT by unexperienced laboratories.

Duration: 2020

Work carried out in 2020:

An inter-laboratory trial (ILT) dedicated to the evaluation of an immuno-histochemical test, which can now be used as an alternative to the Fluorescent Antibody Test (FAT) in routine rabies diagnosis, was organised by the EURL for Rabies. The call for participation in the ILT was sent to laboratories by e-mail in October 2019. Due to the Covid-19 pandemic, the organisation of the ILT, initially planned during the first half of 2020, had to be postponed to the last quarter of 2020.

For this trial, two different shipments were scheduled. The first one contained a package of the most critical reagents required to carry out the DRIT. These reagents were sent beforehand so that laboratories could train themselves with the DRIT, before testing the official panel. The second shipment contained the panel of samples to be tested with both FAT and DRIT. The panel was composed of 10 coded samples containing lyophilised brain homogenates (mouse or fox origin) susceptible to be infected by a RABV, EBLV-1, EBLV-2 or BBLV strain.

Twenty-two NRLs from the EU, randomly coded from L1 to L22, registered and participated in the inter-laboratory test for rabies diagnosis rapid techniques.

The preliminary results tend to support a very good agreement between FAT and DRIT on this collection of experimentally infected samples. A report should be available for early March 2021 after complete analysis of results.

Sub-activity 1.5: Inter-laboratory test on RNA detection using molecular biology techniques

Objectives:

The main objective of this inter-laboratory assay is to evaluate the performance in NRLs and to evaluate the sensitivity of a real-time RT-PCR (SYBR Green and TaqMan) for the detection of different species of Lyssavirus RNA.

Description:

Real-time RT-PCR that presents the advantage to be rapid, specific and sensitive 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. Since 2018, the primary tests prescribed for rabies diagnosis by OIE have been the direct fluorescent antibody test, the direct rapid immuno-histochemistry test, or pan-lyssavirus polymerase chain reaction (PCR) assays.

The EURL for rabies will organise in early 2020 an inter-laboratory assay specific 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 proficiency tests for rabies diagnosis), validated and stored at - 80°C before

sending to the laboratories participating in the ILT. The panel of RNAs will include blind samples with different rabies RNA levels (strongly positive, moderately positive and weakly 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 RT-PCR method. All batches of RNAs will be validated for their homogeneity and stability at - 80°C prior to sending.
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.

Expected Output:
This inter-laboratory test specific to the real-time RT-PCR will determine the performance in NRLs and evaluate the sensitivity of the molecular methods, which is increasingly used in NRLs in replacement of reference techniques, such as the cell-isolation test. This inter-laboratory test will also contribute to determine whether the real-time PCR provides similar performances when used by different laboratories.

Duration: 2020

Work carried out in 2020:
Stock of RNA samples specific to the real-time RT-PCR have been prepared for the inter-laboratory test. The different samples consisted of isolates of the following species: RABV including the fixed laboratory CVS27 strain and two field samples as well as three other bat lyssaviruses currently isolated in bats in Europe. Synthetic standard RNA targeting the whole rabies nucleoprotein gene and negative bird brain samples have been made available for the ring test. Each batch of positive samples were prepared based on 10 inoculated mice. All stocks of viral RNA were stabilised in a saline buffer and stored at - 20°C. NRLs were contacted to participate in the interlaboratory test: 18 confirmed their willing to participate. Due to the sanitary situation of COVID19, RNA extraction, production of panel with range dilutions and the sending of panels have been postponed to 2021-2022.

Sub-activity 1.6: Update of EURL procedures

Objectives:
To provide the NRL network with the most up-to-date detailed techniques of laboratory analysis.

Description:
The EURL reference procedures will be revised and updated according to the international standards (OIE guidelines). All the documentation will be available on the EURL website for consultation and download.

Expected Output:

To ensure the dissemination of up-to-date versions of reference procedures and consequently helping NRLs in maintaining a high-quality level of harmonised techniques within the network.

Duration: 2019-2020 (annual activity)

Work carried out in 2019:

All the technical procedures of the EURL have been updated in 2019. Standard Operating Procedures for rabies diagnosis (FAT (Fluorescent Antibody Test), RTCIT (Rabies Tissue Culture Infection Test), RT-PCR, qRT-PCR, Sequencing) are online in the private access part of the EURL for Rabies website (https://eurl-rabies.anses.fr/en/minisite/rabies/protocols) and available on request.

Sub-activity 1.7: Annual review of the NRLs 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. Collection 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 reporting 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 NRL from the EU 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, as well as sharing and comparing 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: 2019-2020 (annual activity)

Work carried out in 2019 and in 2020:

In 2019: An online questionnaire was set up and sent to all NRLs in February 2019 to collect and collate data on methods used and results of tests carried out in the EU and certain bordering countries in the frame of rabies control programmes in 2018 (rabies cases, number of diagnoses and techniques performed within the year for passive and active surveillance, tetracycline detection tests on teeth, serological tests performed in the frame of monitoring of oral vaccination campaign, typing, etc..). Data were collected, analysed and compiled in the report entitled “Review of the analyses related to rabies diagnosis and follow-up of oral vaccination performed in NRLs in 2018” sent to the network in May 2019.

In 2020: As in 2019, an online questionnaire was set up and sent to all NRLs in February 2020 to collect and collate data on methods used and results of tests carried out in the EU and certain bordering countries in the frame of rabies control programmes in 2019. Data were collected, analysed and compiled in the report entitled “Review of the analyses related to rabies diagnosis and follow-up of oral vaccination performed in NRLs in 2019” sent to the network in August 2020.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided 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: 2019-2020 (annual activity)

Work carried out in 2019:

A training session for two participants was organised in the frame of the EURL mandate (column "training" of the budget report):

Country	Number of participants	Laboratory	Training title	Training place	Starting date	Ending date
Greece	2	Ministry of Rural Development and Food, Athens Veterinary Centre / Department of Molecular Diagnostics, FMD, Virological, Rickettsial and Exotic Diseases, Virology Laboratory	Molecular Biology: synthesis of synthetic RNA for 3 Lyssavirus strains	Anses Nancy	15/09/2019	18/09/2019

The training in rabies molecular biology was conducted from 15 to 18 September in the EURL, Nancy, France. The objective of the training was to support the Greek NRL in producing synthetic RNA for Lyssavirus strains, to be used as control in qPCR tests.

In 2019, as no need for on-site support was identified, no mission was carried out by the ANSES Nancy laboratory using the EURL for rabies EU budget (column "mission" of the budget report).

Work carried out in 2020:

A training session for two participants of the Portuguese NRL was planned in Anses Nancy in summer 2020 on FAT and RTCIT. Unfortunately, following the emergence of the COVID 19 epidemic, leading to disruption of air traffic and fluctuations in regulations for border crossing, and for sanitary reasons, all trainings and travels have been cancelled. The training is postponed and will be realised when the situation will be stabilised.

Sub-activity 2.2: Website management

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, including the 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.
The newsletter will include the news of the EURL activities, the news of the NRL network, the agenda of forthcoming events linked to rabies, the rabies case notifications and a selection of recent publications on rabies laboratory techniques and epidemiology in Europe.

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: 2019-2020 (annual activity)

Work carried out in 2019 and 2020:
In 2019, the website of the EURL for rabies undergone a major reshuffle (change of sites and provider at national level for all EURL websites of ANSES). The year 2019 was therefore a year of transition with a large amount of time allocated to the validation of transfer content from the old to the new website version. Some redesigns of the new version of the website have also been required. The new version has been online since October 2019. Since this period, the website has been regularly updated by including the news of the network, as well as agenda of activities and reports (Figure 2).

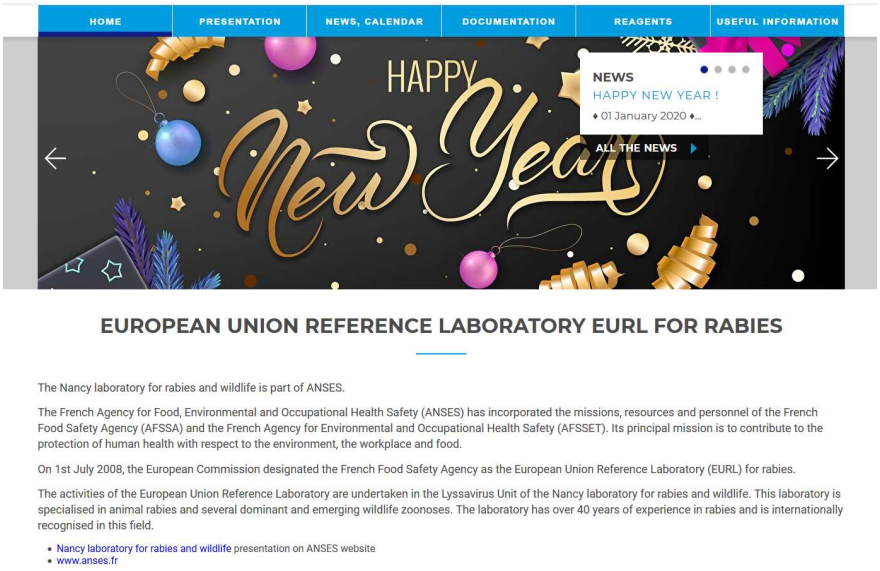

Figure 2: The new EURL for Rabies website

Sub-activity 2.3: Organising the annual Workshop for Rabies

Objectives:
To facilitate the networking as well as the sharing of 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 EC. The workshop gives the opportunity to share information on rabies actualities 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.

Expected Output:

Satisfaction of the participants (assessed through a survey questionnaire) and high number of NRLs attending the meeting.

Duration: 2019-2020 (annual activity)

Work carried out in 2019:

The 11th annual meeting organised by the EURL for rabies was held on June 12 and 13, 2019 in Bucharest, Romania. The event brought together 65 participants, among which many scientists from EU Member States and neighbouring countries, as well as personalities from international institutions who all met to share and exchange on rabies (Figure 3).

Twenty-five NRLs from EU Member States attended the workshop. Delegates from Albania, Belarus, Bosnia and Herzegovina, Republic of North Macedonia, Republic of Kosovo, Moldova, Serbia, Ukraine as well as Algeria, Morocco, Tunisia and Turkey could also take part in the meeting, thanks to TAIEX contribution. Finally, representatives from Norway and Switzerland attended the meeting too.

Presentations and discussions focused mainly on epidemiology of rabies in Europe and in neighbouring countries, including North African countries, as well as on techniques on rabies diagnosis with a specific presentation analysing the results and performances of the laboratories during the inter-laboratory tests organised the previous year.

During the meeting, Dr M. Vigilato, from PAHO (Pan American Health Organization), invited as a guest of honour, shared his valuable experience with a presentation pointing out the lessons learned on dog-mediated human rabies elimination in Latin America and in the Caribbean. This presentation fitted perfectly with the recommendations and objectives of the official institutions attending the meeting (namely Dr Pedro Rosado-Martin for the EC, Dr Patricia Pozzetti for OIE, and Dr Inma Aznar for EFSA), which aim at the elimination of human rabies in the world by 2030 and the elimination of the disease in wildlife by 2020.

Rabies surveillance systems, epidemiological situations, and control and monitoring programmes were presented by NRLs from Algeria, Croatia, Lithuania, Poland, Romania, and Turkey. The UK reported an increased detection of bat lyssaviruses in 2018, and confirmed an imported human case of rabies in Great Britain on the same year.

The EURL team, for its part, respectively with Drs E. Robardet and A. Servat made the last interventions presenting a review of oral vaccination programmes performed in NRLs in 2018, and a technical evaluation of two rapid kits carried out during an inter-laboratory test for rabies diagnosis.
Dr F. Cliquet, Director of the EURL, has acted as master of ceremonies, guiding questions, discussions and arguments raised after each presentation.

Figure 3: Group picture of the 11th Workshop for Rabies

A few days after the meeting, an online satisfaction survey was sent to all participants in order to inquire about their level of satisfaction not only about the meeting organisation but also about the various EURL activities. In 2019, hardly 45% of the participants filled in the survey (29 fulfilled questionnaires, 19 from EU Member States NRLs and 10 from other institutions). Globally, 93% were satisfied or quite satisfied regarding workshop organisation, place, conference centre choice and timing (70% of satisfactory, 23% of quite satisfactory).
The figure 4 records more details of the satisfaction survey: one question per item; suggested answers: “Satisfactory”, “Quite satisfactory”, “Quite unsatisfactory”, “Unsatisfactory” and “No opinion”.

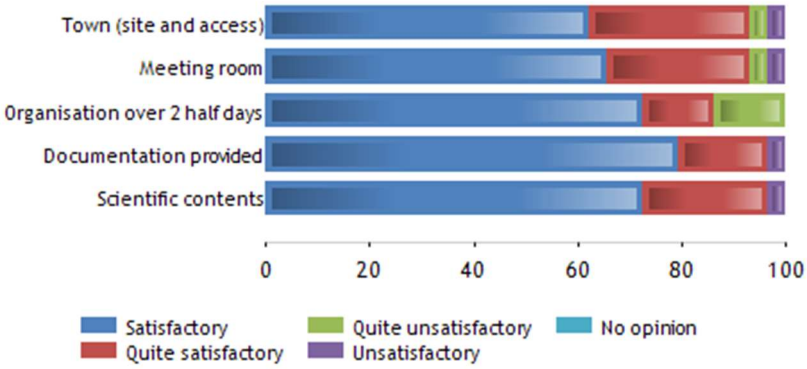

Figure 4: Results of the rabies EURL satisfaction survey on the 11th workshop for rabies (Workshop participants)

As far as the EURL activities are concerned, the rate of global satisfaction of NRLs reached 74.3% (59,1% of “Satisfactory”, and 15,2% of “Quite satisfactory”) when considering the rate of 24% of “No opinion”, and amounts to 98% when the item “No opinion” was not taken into account (Figure 5 for more details: one question per item; suggested answers: “Satisfactory”, “Quite satisfactory”, “Quite unsatisfactory”, “Unsatisfactory”, and “No opinion”).

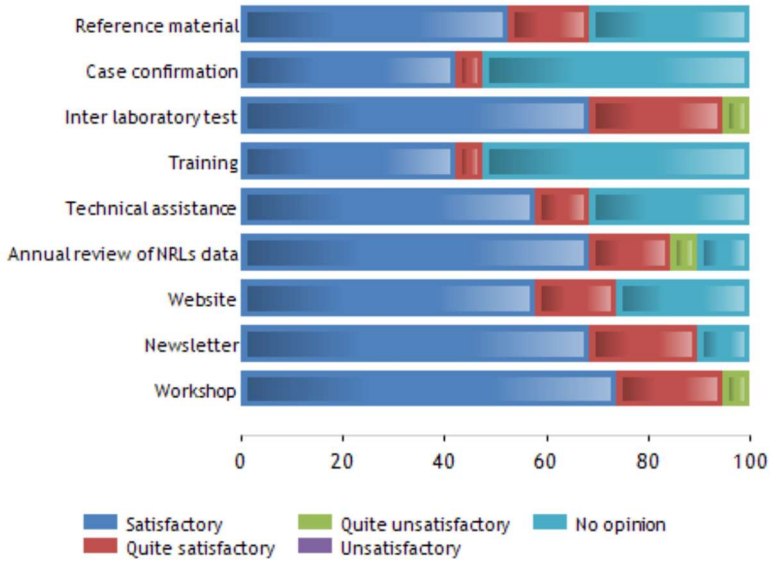

Figure 5: Results of the 2019 rabies EURL satisfaction survey (EU Member States only)

A booklet including the agenda and abstracts of the presentations was provided to each participant at the opening of the workshop. Workshop presentations are available online in the part under restrictive access of the EURL website: (https://eurl-rabies.anses.fr/en/minisite/rabies/former-workshops).

Work carried out in 2020:

The 12th annual meeting organised by the EURL for rabies was initially planned on June 23 and 24, 2020 in Warsaw, Poland. Following the emergence of the Covid-19 and the global sanitary situation, the rabies EURLs meeting was cancelled for sanitary reason.

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

- *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: 2019-2020 (annual activity)

Work carried out in 2019 and in 2020:

The EURL staff responded regularly to technical requests sent by the NRLs (diagnosis, vaccinology, virology, serology, molecular biology, epidemiology and oral vaccination control and relative quality assurance system). The EURL was also enrolled in the annual evaluation of EU co-financed programme on eradication and control of Rabies in wildlife submitted by Member States.

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: 2019-2020 (annual activity)

Work carried out in 2019: In 2019, six samples have been submitted for rabies diagnosis confirmation to the EURL for Rabies:

Anses ID	Reception date	Country	Species	FAT result	RTCIT result	RT-PCR result	Real time result	Conclusion	Report sent on
DR-1804	20/02/2019	Luxembourg	*Capreolus capreolus*	negative	negative	nd	negative	negative	04/03/2019
DR-1849	28/03/2019	Luxembourg	*Felis silvestris catus*	negative	negative	nd	negative	negative	05/04/2019
DR-1879	30/04/2019	Luxembourg	*Felis silvestris catus*	negative	negative	nd	negative	negative	03/05/2019
DR-1937	21/06/2019	Luxembourg	*Vulpes vulpes*	negative	negative	nd	negative	negative	27/06/2019
DR-1947	14/09/2019	Luxembourg	*Martes foina*	negative	negative	nd	negative	negative	19/09/2019
DR-1968	20/11/2019	Luxembourg	Bat	nd	nd	nd	negative	negative	28/11/2019

nd: not done

All the samples were from Luxembourg and resulted as negative.

Work carried out in 2020: In 2020, 4 samples have been submitted for rabies diagnosis confirmation to the EURL for Rabies:

Anses ID	Reception date	Country	Species	FAT result	RTCIT result	RT-PCR result	Real time result	Conclusion	Report sent on
DR-1991	16/01/2020	Ireland	*Martes spp.*	negative	negative	positive	positive	positive	23/01/2020
DR-2010	29/01/2020	Ireland	*Canis lupus familiaris*	negative	negative	negative	negative	negative	03/02/2020
DR-2046	05/06/2020	Luxembourg	*Sciurus vulgaris*	negative	negative	nd	negative	negative	12/06/2020
DR-2051	23/06/2020	Luxembourg	Bat	negative	negative	nd	negative	negative	02/07/2020

nd: not done

A molecular biology analysis was requested by the Croatian NRL in May 2020 on a dog sample with doubtful PCR results. RNA sample was sent to the EURL, the real time PCR was positive showing presence of rabies virus RNA. Despite several attempts, the RT-PCR was unfortunately negative leading the sequencing impossible.

Sub-activity 3.3: Participation in meetings or congresses

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: 2019-2020 (annual activity)

Work carried out in 2019: An expert of the EURL for rabies participated in the "30th Rabies In the Americas meeting (RITA)" that was held in Kansas City, USA, from 27 October to 01 November 2019 (http://www.rabiesintheamericas.org). The RITA congress is an annual event that has been held since 1990, hosted in different countries across the Americas. To date, this is the only worldwide rabies expert meeting. The congress provides the opportunity for researchers, health professionals, international, national and local managers of rabies programmes, wildlife biologists, laboratory personnel and other people interested in advanced knowledge of rabies surveillance, prevention and control, to meet each other, to share their experiences and also to discuss the challenges to be met. Following abstract submission of a work previously completed in the frame of the EURL activities, the EURL was invited to present the communication: "Inter-laboratory comparison to evaluate the technical performance of rabies diagnosis Lateral Flow Assays, A. Servat, E. Robardet and F. Cliquet." No PARACON, ARACON, MERACON meeting were organised in 2019.

Work carried out in 2020:
Unfortunately, following the emergence of the COVID 19 epidemic all meetings and congresses where EURL staff had been registered were cancelled for sanitary reasons.

Sub-activity 3.4: Phylogeographic study of rabies in Romania

Objectives: To analyse the spreading pattern of rabies in Romania.

Description: Phylogeography is the study of the processes responsible for the geographic spread of a disease pathogen. In the case of rabies, despite the fact that the EU managed to quasi-eliminate the disease from its area, no recent tools have been used to characterise the dispersal movement of the virus over time. Using population genetic diversity analysis could provide more insights in the virus historical/biogeographic processes. The study will include around 100 samples, sampled in the laboratory archive, from different periods and different representative areas. Virus samples will be firstly tested in the NRL by standard diagnosis techniques and then analysed by molecular biology methods in the EURL before their propagation by MIT (mouse inoculation test). Extracted rabies RNA will be amplified by NGS at the ANSES-Ploufragan National Genomics Platform (France). Full-genome

sequences will be analysed for determining the rabies variant in the country as well as included in the phylogeography analysis for studying the dispersal of strains throughout the studied zone.
As stipulated in the rabies EURL mandate (Regulation No 415/2013 Annex 1, point 1.g) the EURL will thus “characterise the rabies virus by the most modern methods to allow a better understanding of the epidemiology of this disease". This project is therefore part of this context as it involves analysing the strains of Romania with the latest molecular tools and then using the latest techniques in bioinformatics. This will make it possible to trace the route of the different rabies strains in an EU country, study that, to our knowledge, has not yet been done.

Expected Output: Acquisition of further knowledge on the geographical spread of the rabies virus in Romania, to forecast its responses to biogeographic barriers and to gain unique insights of its interactions with the environment.

Duration: 2018-2019-2020

Work carried out in 2019:

The genetic diversity study of rabies virus in animals in Romania has started. Seventy-eight blind samples stabilised in FTA cards were received in the laboratory in June 2019 and processed by using molecular biology techniques and phylogeny tools. The 78 samples were firstly controlled by hnRT-PCR. Of the 78 samples tested, 77 were shown positive for the presence of the rabies nucleoprotein (N) gene RNA (-606bp). In a first step, all generated PCR products (-606-bp) were sequenced and analysed.
The complete nucleoprotein gene (-1353 bp) was amplified in a second step from the 77 positive samples. Of the 77 samples tested, 64 were shown positive for the presence of the 1353-bp nucleoprotein gene. The genetic diversity study has been undertaken on the entire N gene sequences (-1353bp, 59 sequences) using a pipeline of phylogeny tools. First results demonstrated the presence of two phylogroups in the sample population analysed: the NEE group with 53 sequences and the D group with seven sequences, respectively. Four samples were not usable for phylogeny. In a next step, the samples will be analysed in depth with molecular-dated phylogeny coupled with the spatial distribution. The phylogenetic analysis will serve to select the strains of interest to be propagated in mice then analysed by NGS.

Work carried out in 2020:

Phylogenetic analysis was performed on the entire nucleoprotein gene sequence of 60 Romanian samples sequenced at the start of 2020. Out of 6 phylogenetic groups detected named: NEE B5a; NEE B5b; NEE B2; NEE B3; NEE B4; D), 4 strains were selected per phylogroup (24 samples + one sample which inferred the others) for full genome (NGS) analysis. All the 25 selected strains were amplified on mice in the Romanian NRL to allow NGS analysis and further in-depth molecular-dated phylogeny. Mouse brain samples were received in November in Anses Nancy. Extraction of viral RNA was thus performed following by the purification of the extracted RNAs for the 25 amplified samples. Ten RNA

isolates (each phylogroup is represented with 1-2 samples/group) were selected based on the DNA/RNA quantification results and sent to the NGS Anses platform for full genome sequencing where they are currently under analysis. Results are expected for the first quarter of 2021 that will allow the analysis of evolution in time and space of these different variants.

4 REAGENTS AND REFERENCE COLLECTIONS

- *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 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 FAT (Fluorescent Antibody test), RTCIT (Rabies Tissue Culture Infection test) and molecular biology techniques (Real-time PCR test). 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: 2019-2020 (annual activity)

Work carried out in 2019 and in 2020:

- Since the EURL designation, newly acquired rabies strains are regularly collected, produced on mice, then validated by FAT, RTCIT, RT-PCR, qPCR and typing before storage. Each new batch is composed of 10-40 vials of 800 µl of virus suspension. In 2019, a Lithuanian strain of virus

isolated in 2018 has been collected, amplified and validated. To date 56 batches of field rabies viruses are stored in the EURL and maintained in liquid nitrogen for the conservation of the viral biodiversity.

- Based on the strain collection of the EURL, lyophilised batches of viruses are produced *in vivo* in the laboratory (batch characterised by FAT, RTCIT, RTPCR and qRT-PCR, 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. Six batches of lyophilised virus were produced in 2019 and four in 2020. Two negative batches (fox and pig origin) were also produced and validated as rabies diagnosis negative controls to supply NRLs on request and also to be used in the context of inter-laboratory testing.

- In addition, as part of the improvement of animal experimentation protocols on mice related to virus batches production, three refinement tests were carried out. They were set up to compare the diagnostic results on stage 3 or stage 4 of the disease. The analyses did not show any differences of diagnosis results between stages 3 and 4 for the CVS27, GS7, EBLV-1 and EBLV-2 strains. In future protocols of production of mentioned strains, euthanasia will therefore take place at an earlier stage than previously, which will reduce the risk of animal suffering.

REMARKS

No remarks

B. PROJECTS NOT FUNDED BY THE EURL GRANT BUT CONSIDERED OF INTEREST FOR THE EURL RESPONSABILITIES

1. Quality assurance activities performed in 2019 and in 2020

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.

In 2019:

- A surveillance audit was performed by COFRAC for the 17043 accreditation.
- A reassessment audit was conducted by EDQM for the OMCL network.
- Several internal audits were also performed.

In 2020:

- A surveillance audit was performed by COFRAC for the 17043 accreditation.
- A surveillance and extension audit was conducted by COFRAC for the 17025 accreditation.
- Several internal audits were also performed.

2. Research programmes and cooperation projects ongoing in 2019 and in 2020

The laboratory took part in different research and cooperation projects on rabies epidemiology in countries infected with canine and wildlife rabies and in a collaborative project with OIE reference laboratories for evaluating some lateral flow device kits for rabies diagnosis.

An OIE Twinning project between the laboratory and a research institute in the Republic of Taiwan was validated by OIE and initiated in October 2018.

The laboratory has hosted a researcher (for obtaining a PhD diploma) from the University of Agriculture, Makurdi, Nigeria, from 17 September to 06 January 2019 for a project on rabies epidemiology in Nigeria on dogs and bats.

3. Training of third or EU country laboratories not under EURL mandate grant performed in 2019 and in 2020

For the period 2019-2020, six training courses (gathering six trainees) were organised in the ANSES Nancy laboratory in the frame of the rabies activities (rabies diagnosis, virus production and titration, cell culture maintenance, serology, vaccine potency test):

Number of participants	Country	Subject of the training	Dates
1	Italy	Development of an ELISA for testing the potency of rabies inactivated vaccines	From 20 to 24 May 2019
2	Sri Lanka	ELISA for rabies serology in dog samples	From 11 to 14 June 2019
3	Tunisia	CVS 11 production, cell culture and FAVN test	From 07 to 25 October 2019
4	Sri Lanka	Potency test on mice for rabies inactivated vaccines	From 14 to 25 October 2019
5	Republic of Taiwan	Production of rabies strains for the diagnostic proficiency tests and FAT	From 09 to 13 December 2019
6	Uganda	Cell culture and ELISA test for rabies serology in dog serum samples	From 17 to 21 February and From 26 to 28 February 2020

4. Participation and presentation in international and national congresses and meetings in 2019-2020

1. Hsu, A.P., S.Y. Lee, C.H. Tseng, Y.T Lu, Y.H. Shih, E. Robardet, J. Barrat and F. Cliquet. 2019. "Pathogenicity investigation of Taiwan ferret badger rabies virus on Gem-faced civets." 30th International conference on rabies in the Americas (RITA), Kansas City (USA), 27 October – 01 November 2019.
2. Hsu A.P., S.Y. Lee, C.H. Tseng, Y.T. Lu, Y.H. Shih, E. Robardet, J. Barrat, M. Wasniewski, A. Servat and F. Cliquet. 2019. "Implementation summary of OIE Twinning Project for Rabies." 30th International conference on rabies in the Americas (RITA), Kansas City (USA), 27 October – 01 November 2019.
3. Wasniewski, M. Presentations of the FAVN test part 1, the FAVN test part 2, the cell culture, the virus production and the titre calculation and validation of the FAVN test - Man's best friend: A crossborder transdisciplinary One Health approach to rabies control in dogs in Southeast. Workshop on rabies serology diagnosis, Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 21st-25th January 2019
4. Wasniewski, M., Robardet, E., Cliquet, F. Experience from an EU-Ref Lab: mandate, responsibilities, challenges in disease diagnostic and networking. Veterinary Diagnostic Laboratory (VETLAB) Network Coordination Meeting with Directors of African and Asian Veterinary Laboratories, Vienna, Austria, 19-23 August 2019
5. Wasniewski, M., Laurentie, M., Rizzo, F., Servat, A., Aubert, M., Cliquet, F. Proficiency tests for rabies serology: a design complying with international standards for a reliable assessment of participating laboratories - Round table: rabies serology and vaccination. 30th International conference on rabies in the Americas (RITA), Kansas City (USA), 27 October-01 November 2019
6. Wasniewski, M., Rizzo, F., Cliquet, F. Presentation of the ISO/IEC 17025: 2017 and its implementation on rabies serological testing- 2019. Asian Symposium on Quality Improvements for Rabies Serological Testing, Taipei, Taiwan, 27 November 2019
7. Wasniewski, M. Presentations of the FAVN test part 1, the FAVN test part 2, the cell culture, the virus production and the titre calculation and validation of the FAVN test. Workshop on Quality Improvements for Rabies Serological Testing, Taipei, Taiwan, 28 November 2019

8. Servat, A., Robardet, E., Cliquet, F. Inter-laboratory comparison to evaluate the technical performance of rabies diagnosis Lateral Flow Assays. 11th workshop for rabies, Bucharest, Romania, 12-13 June 2019
9. Servat, A, Robardet, E., Cliquet, F. Inter-laboratory comparison to evaluate the technical performance of rabies diagnosis Lateral Flow Assays. 30th International conference on rabies in the Americas (RITA), Kansas City (USA), 27 October – 01 November 2019
10. Robardet, E., Cliquet, F. Review of the analysis related to rabies diagnosis and follow-up of oral vaccination programmes performed in NRLS in 2018. 11th workshop for rabies, Bucharest, Romania, 12-13 June 2019
11. Cliquet, F. La surveillance de la rage : le rôle clé du diagnostic de laboratoire. Workshop SARE sur l'élimination de la rage en Algérie, Alger, Algeria, 12-17 October 2019
12. Cliquet, F. Dog rabies control - Vaccination of dogs. Pasteur Institute International Workshop on Surveillance and Control of Rabies, Casablanca, Morocco, 17-28 September 2019
13. Robardet, E., Cliquet, F. KAP studies for rabies. Pasteur Institute International Workshop on Surveillance and Control of Rabies, Casablanca, Morocco, 17-28 September 2019
14. Cliquet, F., Robardet, E. Programmes of Rabies Surveillance and Control In Europe. IX International Veterinary Congress, Svetlogorsk, Kaliningrad, Russia, 17-20 April 2019
15. Picard-Meyer, E., Servat, A., Cliquet, F. Surveillance de la rage des chauves-souris en France. Réunion du Groupe de Travail Stratégie Sanitaire, Pérols, France, 18 March 2019
16. Picard-Meyer, E., Stroucken, N., Servat, A., Robardet, E., Cliquet, F. Surveillance de la rage des chauves-souris en France métropolitaine et actualités. IX Rencontres Chiroptères Très Grand Est, La Bresse, France, 20 October 2019
17. Cliquet, F., Binot A. Gestion intégrée de la rage dans le cadre d'une approche One health. Colloque du réseau francophone sur les maladies tropicales négligées – Approche One health dans la lutte contre les MTN, Marseille, France, 01-02 October 2019

5. International publications related to rabies published in 2019-2020

1. Wallace, R.M., Cliquet, F., Fehlner-Gardiner, C., Fooks, A.R., Sabeta, C.T., Setién, A.A., Tu, C., Vuta, V., Yakobson, B., Yang, D.-K., Brückner, G., Freuling, C.M., Knopf, L., Metlin, A., Pozzetti, P., Suseno, P.P., Shadomy, S.V., Torres, G., Vigilato, M.A.N., Abela-Ridder, B., Müller, T. Role of oral rabies vaccines in the elimination of dog-mediated human rabies deaths (2020) Emerging Infectious Diseases, 26 (12), pp. E1-E9.
2. Šimić, I., Zorec, T.M., Lojkić, I., Krešić, N., Poljak, M., Cliquet, F., Picard-Meyer, E., Wasniewski, M., Zrnčić, V., Ćukušić, A., Bedeković, T. Viral metagenomic profiling of croatian bat population reveals sample and habitat dependent diversity (2020) Viruses, 12 (8), art. no. v12080891
3. Šimić, I., Zorec, T.M., Lojkić, I., Krešić, N., Poljak, M., Cliquet, F., Picard-Meyer, E., Wasniewski, M., Zrnčić, V., Ćukušić, A., Bedeković, T. Viral metagenomic profiling of croatian bat population reveals sample and habitat dependent diversity (2020) Viruses, 12 (8), art. no. v12080891
4. Klein, A., Fahrion, A., Finke, S., Eyngor, M., Novak, S., Yakobson, B., Ngoepe, E., Phahladira, B., Sabeta, C., de Benedictis, P., Gourlaouen, M., Orciari, L.A., Yager, P.A., Gigante, C.M., Kimberly Knowles, M., Fehlner-Gardiner, C., Servat, A., Cliquet, F., Marston, D., McElhinney, L.M., Johnson, T., Fooks, A.R., Müller, T., Freuling, C.M. Further evidence of inadequate quality in lateral flow devices commercially offered for the diagnosis of rabies(2020) Tropical Medicine and Infectious Disease, 5 (1), art. no. 13
5. Dascalu, M.A., Wasniewski, M., Picard-Meyer, E., Servat, A., Daraban Bocaneti, F., Tanase, O.I., Velescu, E., Cliquet, F. Detection of rabies antibodies in wild boars in north-east Romania by a rabies ELISA test (2019) BMC Veterinary Research, 15 (1), art. no. 466, DOI: 10.1186/s12917-019-2209-x
6. Wasniewski, M., Laurentie, M., Rizzo, F., Servat, A., Aubert, M., Cliquet, F. Proficiency test for rabies serology: A design complying with international standards for a reliable assessment of participating laboratories (2019) PLoS neglected tropical diseases, 13 (12), p. e0007824. DOI: 10.1371/journal.pntd.0007824
7. Servat, A., Wasniewski, M., Cliquet, F. Cross-protection of inactivated rabies vaccines for veterinary use against bat lyssaviruses occurring in Europe (2019) Viruses, 11 (10), art. no. 936, DOI: 10.3390/v11100936
8. Servat, A., Robardet, E., Cliquet, F. An inter-laboratory comparison to evaluate the technical performance of rabies diagnosis lateral flow assays (2019) Journal of Virological Methods, 272, art. no. 113702, DOI: 10.1016/j.jviromet.2019.113702
9. Pfaff, F., Müller, T., Freuling, C.M., Fehlner-Gardiner, C., Nadin-Davis, S., Robardet, E., Cliquet, F., Vuta, V., Hostnik, P., Mettenleiter, T.C., Beer, M., Höper, D. In-depth genome analyses of viruses from vaccine-derived

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