WORK PROGRAMME of EURL for RABIES

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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) 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)

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

Please, provided 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 *(Evaluation of antigen detection using DRIT)*

Objectives:
The EURL wishes to organise an inter-laboratory trial (ILT) to evaluate laboratories in the handling of the Direct Rapid Immunohistochemical Test (DRIT) which 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 immunohistochemical diagnostic assay. It is already in routine use in North America for support of oral wildlife rabies vaccination programs. This technique is as specific and sensitive as the FAT. Because of its recent inclusion in the 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 antibodies preparations have been 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:
Obtain 2 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.

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. Inter-laboratory tests will include a technical questionnaire to be filled by any participant to help to understand, in case of discordant result, the factors affecting the result. The different steps of the trials 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. 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's 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 will be organised every two years)

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 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

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 Immunohistochemical Test (DRIT) which 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 immunohistochemical diagnostic assay. It is already in routine use in North America for support of oral wildlife rabies vaccination programs. This technique is as specific and sensitive as the FAT. Because of its inclusion in the 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:

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 national reference laboratories 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

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 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. The primary tests prescribed in 2018 by the World Organisation for Animal Health (OIE) for rabies diagnosis are: the direct fluorescent antibody test, the direct rapid immunohistochemistry 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 (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.

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 method, 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 or not the real-time PCR provides similar performances when used by different laboratories.

Duration: 2020

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 in maintaining a high-quality level of harmonised techniques within the network.

Duration: 2019-2020 (annual activity)

Sub-activity 1.7 *(Annual review of the NRLs data)*

Choose a building block.

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 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 and to share and compare results of rabies programs 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)

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.***
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- *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 analysis 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 4 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 test).

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)

Sub-activity 2.2 *(Website 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, 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 cases 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)

Sub-activity 2.3 *(Organising 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)

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

Please, provided 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: 2019-2020 (annual activity)

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)

Sub-activity 3.3 *(Participation in meetings 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 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)

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. In the frame of rabies, despite the fact that the EU managed to nearly 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 archives, from different periods and different representative areas. Virus samples will be

firstly tested in the NRL by reference 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 a part of this context as it involves the Romanian strain analysis with the latest molecular tools then using the latest techniques in bioinformatics. This will make possible to trace the route of the different rabies strains in a EU country, a 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

4 REAGENTS AND REFERENCE COLLECTIONS

Please, provided 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 collection.

Description: The choice of new rabies virus strains to be included in the virus collection will depend on outbreaks and collaboration opportunities. Approximately 4 new strains will be included in the collection each year. 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)

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation: Nil

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

Objectives: Description: Expected Output: Duration:

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

Objectives: Description: Expected Output: Duration:

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

Objectives: Description: Expected Output: Duration:

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

Objectives: Description: Expected Output: Duration:

REMARKS

No remarks