

THE EXPECTATION OF THE DEVELOPMENT AND OPERATION OF RABIES NETWORK IN ASIA FROM THE PERSPECTIVE OF OIE/WHO/EU REFERENCE LABORATORY

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Nancy Laboratory
for Rabies and Wildlife



WHO Collaborating Centre
for Research and Management
in Zoonoses Control



OIE
Reference Laboratory
for Rabies



European Union
Reference Laboratory
for Rabies



European Union
Reference Institute for
Rabies Serology

Launching meeting of OIE twining project for rabies

17 - 18 October 2018, Taipei, TAIWAN

EURL IN THE FIELD OF ANIMAL HEALTH AND LIVE ANIMALS

Institut für Virologie des Tierärztlichen
Hochschule Hanover
• Classical swine fever



DTU National Veterinary
Institute
• Fish diseases



Interbull Center
• Zootechnics



Animal Health and Veterinary Laboratories
Agency (AHVLA)

- Avian influenza, Newcastle disease,
Crustacean disease



Pirbright Institute for animal health

- Bluetongue, Foot and mouse disease, Swine vesicular
disease

Anses-Nancy

- Rabies serology, rabies

Laboratoire Ifremer

- Bivalve molluscs diseases



Anses-Alfort

- Brucellosis, Equine diseases other than AHS

Anses-Sofia

- Bee health

Laboratorio de sanidad y producción animal

- African horse disease

Centro de investigación en Sanidad Animal

- African swine fever



Visavet

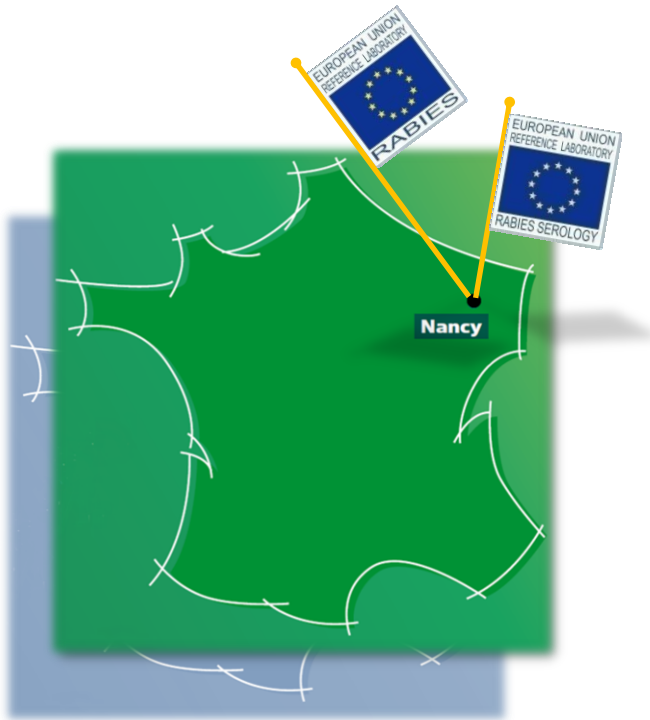
- Bovine

Istituto Superiore di Sanità
• Parasitology



EURL FOR RABIES SEROLOGY AND EURL FOR RABIES

- ✓ Council Decision of 20 March 2000 designating a **specific institute** responsible for establishing the criteria necessary for standardising the **serological tests** to monitor the effectiveness of rabies vaccines (2000/258/EC).
- ✓ Commission Regulation (EC) No 737/2008 of 28 July 2008 designating the Community Reference Laboratories for crustacean diseases, **rabies** and bovine tuberculosis, laying down additional responsibilities and tasks for the Community reference laboratories for rabies and bovine tuberculosis and amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council.



**Nancy Laboratory
for Rabies and Wildlife**

RESPONSIBILITIES OF EURLS

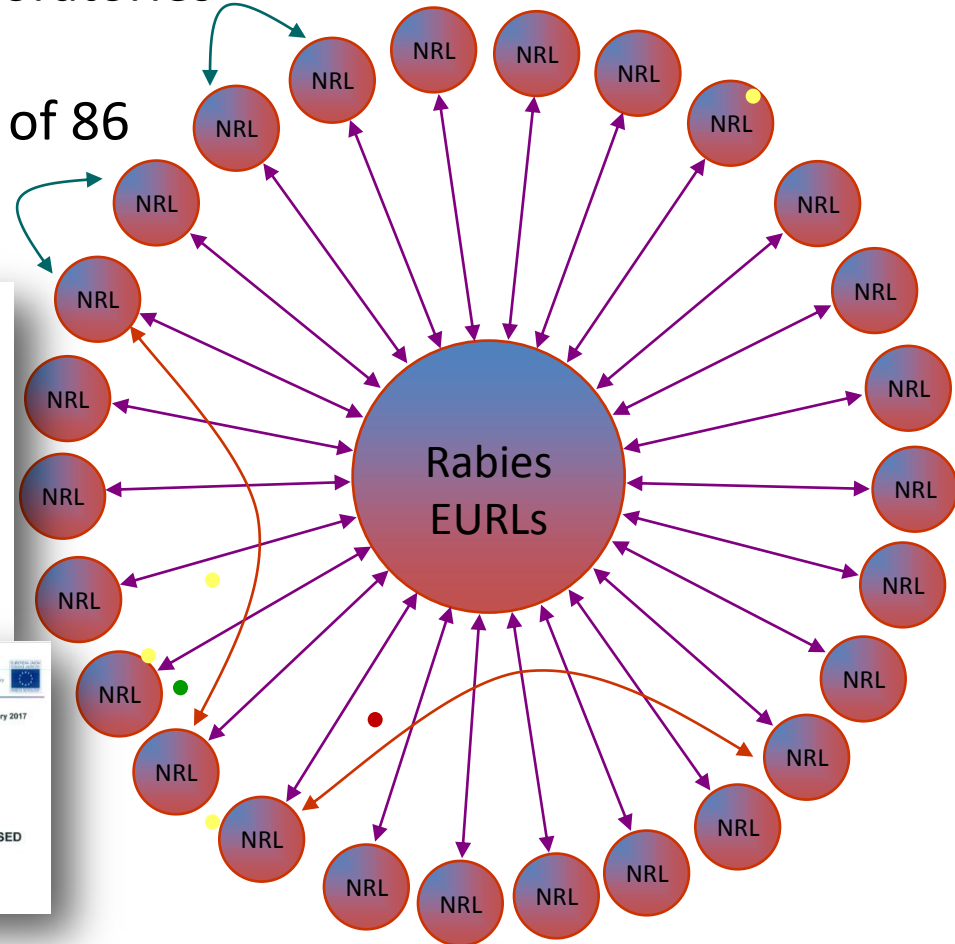
Concept of EURLs and National Reference laboratories (NRLs) are laid down in the regulation (EC) No 625/2017 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (Article 94).

- ✓ **Coordinating** the methods employed in the Member States for **diagnosing** diseases and informing NRLs of advances in this field – coordinating or performing tests to check quality of reagents used for diagnosis;
- ✓ **Organising** regular proficiency tests and informing EC of the results and follow-up to proficiency tests;
- ✓ **Maintaining** reference strains, reference collections, up to date lists of reference substances and reagents - maintaining a QA system, biosafety and biosecurity;
- ✓ **Providing** NRLs with details and guidance on the methods of laboratory analysis, testing or **diagnosis** – providing reference materials and information on international research activities;
- ✓ **Assisting** actively in the **diagnosis** of disease outbreaks in Member States by carrying out confirmatory **diagnosis**, characterisation and epizootic studies;
- ✓ **Conducting** training courses for the benefit of staff from national reference laboratories and of experts from third countries and from other official laboratories.
- ✓ **Collaborating** with laboratories in third countries and with EFSA, EMA and ECDC – collaborate to develop new methods;

RABIES EURLs NETWORKS

✓ EURL for rabies: Network of 64 laboratories

✓ EURL for rabies serology: Network of 86 laboratories*



NRLs from Member States and third countries

* In 2018



THE EUROPEAN UNION: FROM 6 TO 28 COUNTRIES!

60 years of European evolution

1950



France and Germany announce plans for an economic union pooling coal and steel production

1957

EEC
6



The Treaty of Rome is signed. **The European Economic Community (Common Market) is born**

1973

EEC
9



The UK, Denmark and Ireland join

1981

EEC
10



Greece joins

1986

EEC
12



Portugal and Spain join. The Single European Act sets the goal of creating a single market

1995

EU
15



Austria, Finland, Sweden join. The Maastricht Treaty (1992) lays the basis for a single currency. The Schengen Area enters force

2004

EU
25



Enlargement to the east. The euro replaces national currencies (2002)

2007

EU
27



The Treaty of Lisbon streamlines how the EU operates. Rise in Euroscepticism. 2009 financial crisis

2013

EU
28



Croatia joins. UK leader David Cameron promises a referendum on EU membership

2016

EU
27



Brexit: In the thick of Europe's most serious migration crisis since WWII, the UK votes to leave the EU

© AFP

NRLs from EU constitute a network!

RABIES SEROLOGY NETWORK



Location of approved laboratories for rabies serology in September 2018

- Europe
 - ↪ 33 labs from 28 EU countries
 - ↪ 8 labs from other countries (Russia, Serbia, Switzerland, Ukraine)
- America
 - ↪ 3 labs from USA
 - ↪ 1 lab from Mexico
 - ↪ 3 lab from Brazil
 - ↪ 1 lab from Chile
- Africa
 - ↪ 1 lab in Morocco
 - ↪ 1 lab in South Africa
- Oceania
 - ↪ 1 lab from Australia
- Middle East
 - ↪ 1 lab from Turkey
 - ↪ 1 lab from Israel
 - ↪ 1 lab from United Arab Emirates
- Asia
 - ↪ 1 lab from China
 - ↪ 2 labs from Japan
 - ↪ 4 labs from South Korea
 - ↪ 2 labs from Taiwan

RABIES DIAGNOSIS NETWORK



Location of evaluated laboratories for rabies diagnosis in May 2017

- Europe
 - ↪ 25 labs from 28 EU countries
 - ↪ 6 labs from other countries (Balkans, Moldavia, Norway)
- America
 - ↪ 1 lab from USA
 - ↪ 1 lab from Mexico
 - ↪ 1 lab from Colombia
 - ↪ 1 lab from Chile
 - ↪ 1 lab from Peru
- Oceania
 - ↪ 1 lab from Australia
- Middle East
 - ↪ 1 lab from Turkey
 - ↪ 1 lab from Israel
- Asia
 - ↪ 1 lab from Sri-Lanka
 - ↪ 1 lab from Philippines
 - ↪ 1 lab from Indonesia
- Africa
 - ↪ 2 labs in South Africa
 - ↪ 2 labs in Morocco
 - ↪ 1 lab in Nigeria
 - ↪ 1 lab from Egypt

OIE LABORATORIES FOR RABIES



Animal and Plant
Health Agency
Weybridge
ADDLESTONE, UNITED
KINGDOM



Anses – Laboratory for
rabies and wildlife
MALZEVILLE, FRANCE

Friedrich Loeffler Institute
GREIFSWALD-INSEL
RIEMS, GERMANY



Animal and Plant
Quarantine Agency
GYEONGSANGBUK-
DO, REP .OF KOREA



Centre of Expertise
for Rabies
CFIA/ACIA-
NEPEAN, CANADA



Diagnostic
Laboratory for
Rabies and Wildlife
Associated Zoonoses
CHANGCHUN, CHINA



Centers for disease
Control and Prevention
ATLANTA, UNITED
STATES OF AMERICA



Centro Nacional de
Servicios de
Diagnostico en Salud
Animal
TECAMAC, MEXICO



Onderstepoort
Veterinary Institute
ONDERSTEPSOORT, SOUTH AFRICA

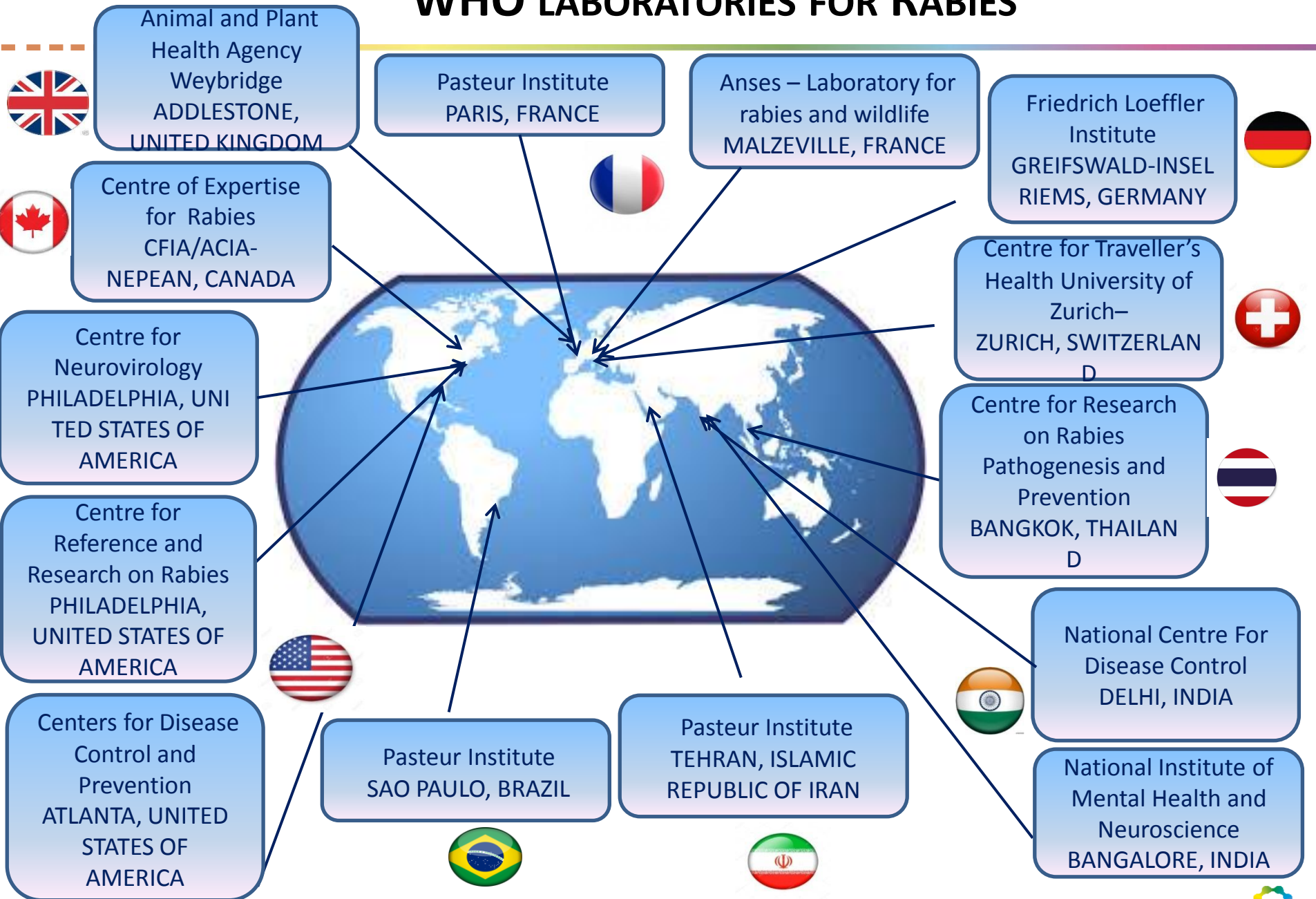


Kimrin Veterinary
Institute
BEIT DAGAN, ISRAEL



- ✓ **Coordinating** or/and carrying out scientific and technical studies in collaboration with other laboratories, centres or organisation;
- ✓ **Recommending** and promoting diagnostic methods validated according to OIE standards;
- ✓ **Providing** diagnostic testing facilities and scientific and technical advice on **disease control** measures – providing trainings;
- ✓ **Developing**, standardising and validating new procedures for **diagnosis** and **disease control** – developing reference material;
- ✓ **Storing** and distributing biological reference products and any other reagents used in **diagnosis** and **disease control**;
- ✓ **Organising** proficiency tests with laboratories other than OIE ref labs to ensure equivalence of results – organising and participating in scientific meetings on behalf of OIE;
- ✓ **Maintaining** a network with other OIE ref labs – maintaining a QA system, biosafety and biosecurity.

WHO LABORATORIES FOR RABIES





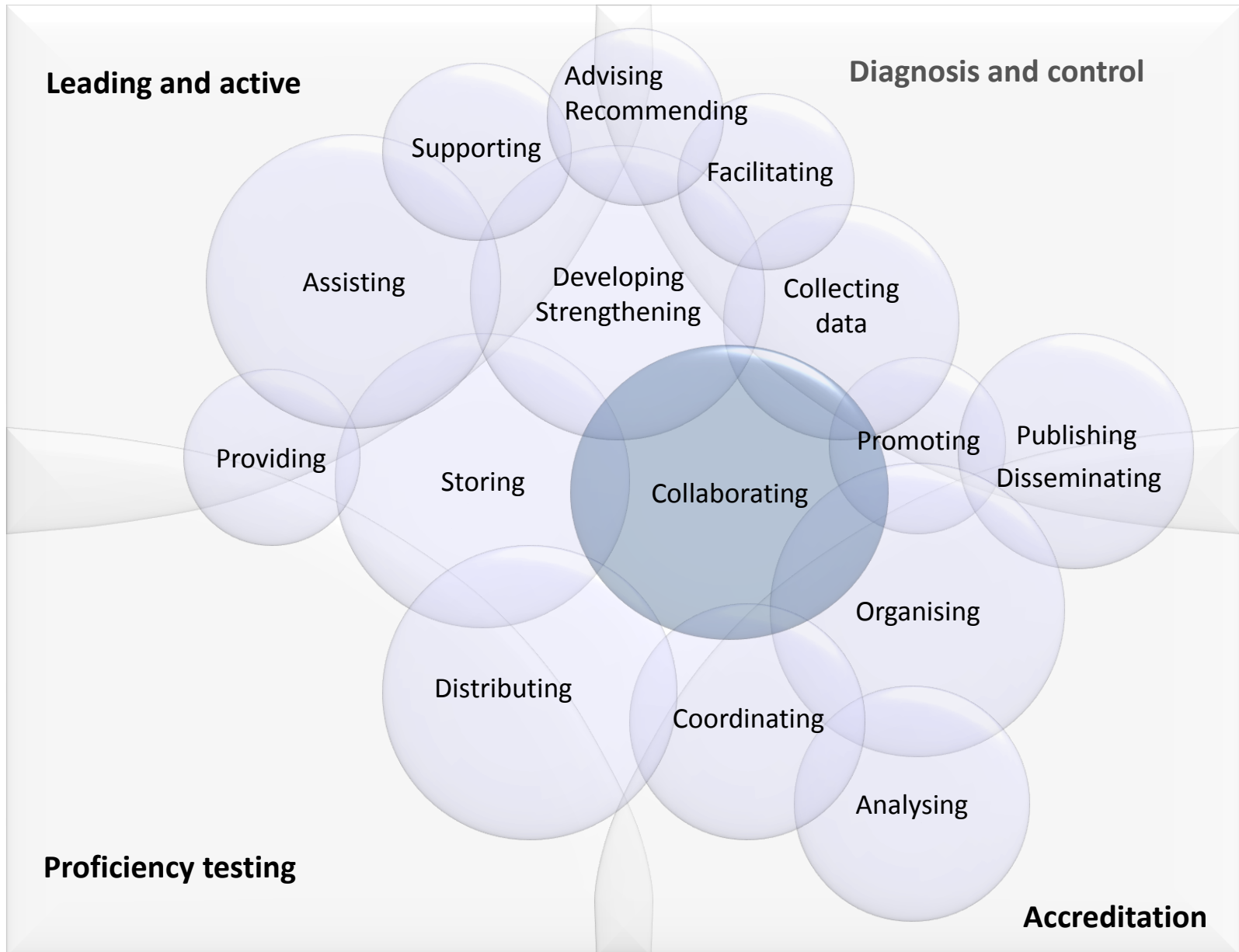
RESPONSIBILITIES OF ANSES - NANCY WHO COLLABORATING CENTRE

- ✓ **Designing**, conducting and evaluating basic, operational and applied research projects for the control of zoonoses, more specifically on rabies and Echinococcosis;
- ✓ **Strengthening** networking among other WHO Collaborating Centres;
- ✓ **Providing** training – providing biological reference materials and laboratory services;
- ✓ **Assisting** countries to develop their surveillance against rabies and Echinococcosis - assisting with the preparation and dissemination of educational materials.

On going programme :

- ✓ Surveillance programs of *Echinococcus granulosus sensu lato* in North Africa.
- ✓ Surveillance programs of *Echinococcus multilocularis* in Eastern Europe.
- ✓ Collaboration on regional and international surveillance and control programs of vulpine and canine rabies.
- ✓ Collaboration with WHO collaborating Centres.
- ✓ Training activities.

KEY ACTION TERMS COMMON TO OIE/EU/WHO REFERENCE MANDATES



OTHER RABIES INTERNATIONAL NETWORKS

- **GARC (PRP):**

- To set up, coordinate and support networks at the global and regional levels, bringing stakeholders together to collaborate, acquire new tools, and share experiences.
- Members of the Partners for Rabies Prevention (PRP), a group consisting of the major international rabies prevention stakeholders, including WHO, FAO, OIE, WHO rabies CCs, research scientists, the UBS Optimus Foundation, animal welfare NGOs and representatives from industry.

- **European Rabies Task Force:**

- To monitor disease in EU countries to improve the cost-benefit ratio of eradication programmes co-funded by the EU.
- Members are EU countries and Commission representatives.
- To give tailored technical assistance to EU countries.

- **OMCL network** (Official medicines Control Laboratories – EDQM):

- To support regulatory authorities in controlling the quality of medicinal products for human veterinary use available on the market.
- Members are EU countries and non-European countries can participate as associate members of the work programme.

Reference material Reference methods



PRODUCTION OF A OIE REFERENCE STANDARD SERUM OF DOG ORIGIN

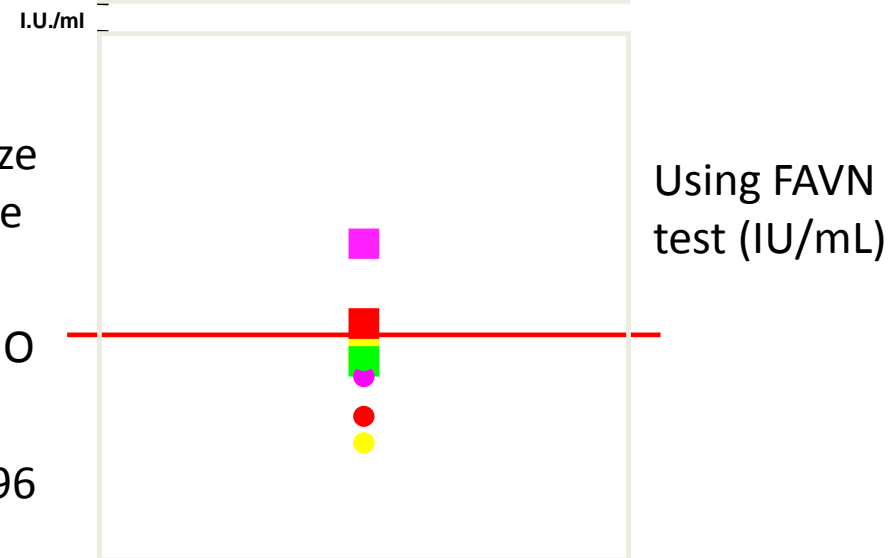
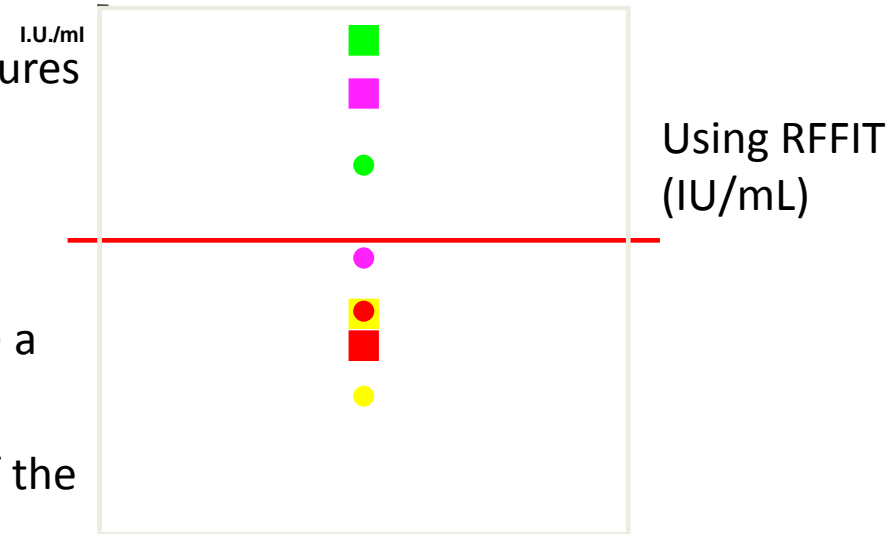
✓ At the beginning of 1990s :

- Expert groups to study on alternative measures of animal quarantine.
- Serology proven as a reliable indicator of effective vaccination (Aubert, 1992).
- Request of OIE to produce and characterize a reference serum of dog origin for rabies.
- Collaborative study to establish the titre of the OIE serum using RFFIT.

✓ In 1994 – 1995 :

- Development of the FAVN test to standardize serological tests to be used for assessing the ability of pets to travel with their owners.
- 4 workshops organised among the OIE, WHO and European networks.
- FAVN test has become referenced since 1996 (OIE) and 2005 (WHO).

OIE serum titration (8 OIE/WHO laboratories)

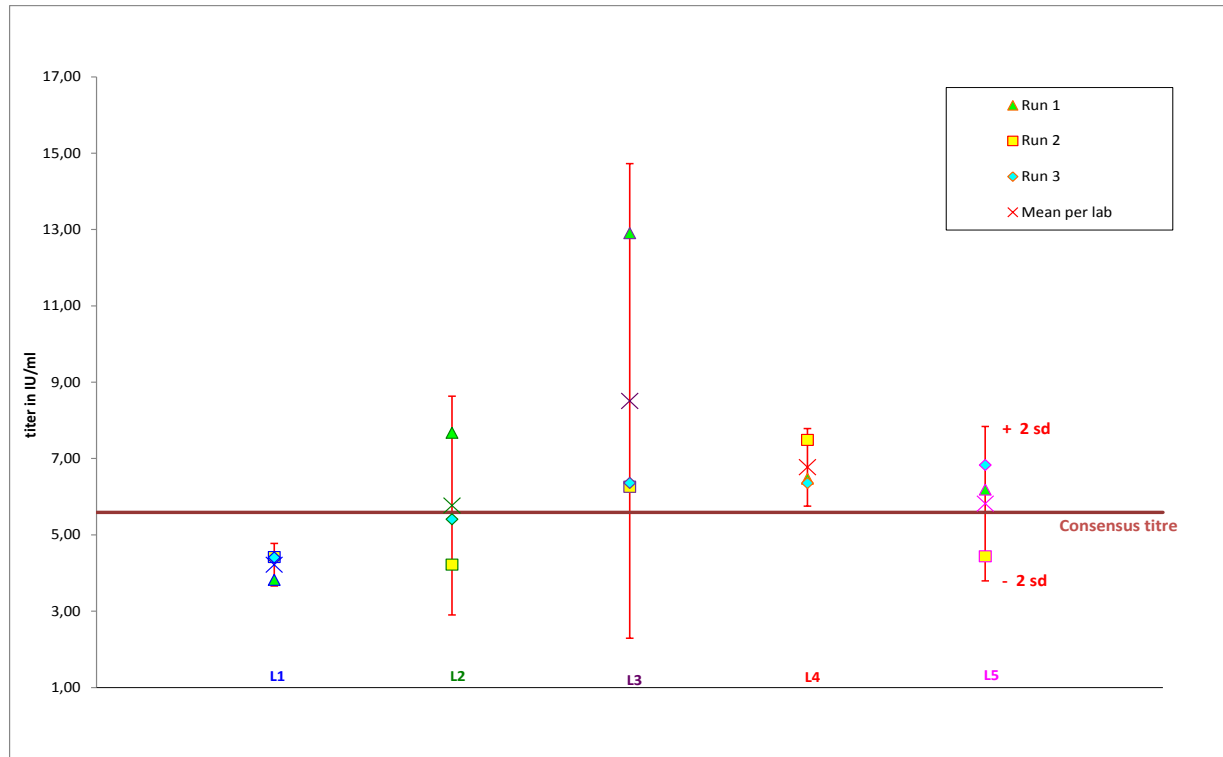


Ref : Cliquet et al., 1998

1 point = mean of 3 tests within the same laboratory

NEW BATCH OF OIE STANDARD SERUM REFERENCE OF DOG ORIGIN

Results of the inter-laboratory titration (5 OIE participating laboratories)



Consensus titre established at 5.59 IU/mL

- Technical and statistical data submitted to the OIE for assessment.
- In February 2014, the OIE Biological Standards Commission adopted this serum as an OIE-approved standard reagent for rabies serology.

Rev. Sci. Tech. Off. Int. Epiz., 2017, 36 (3), 779-788

Production and calibration of the second batch of OIE anti-rabies positive reference serum

M. Wasniewski ^{(1)*}, J. Barrat ⁽¹⁾, A.R. Fooks ⁽²⁾, R. Franka ⁽³⁾, T. Müller ⁽⁴⁾, C. Sabeta ⁽⁵⁾ & F. Cliquet ⁽¹⁾

Ref :Wasniewski et al., Rev Sci Tech Off Int Epiz, 2017

(1) Agency for Food, Environmental and Occupational Health and Safety (ANSES) – Nancy Laboratory for Rabies and Wildlife, Technologie Agricole et Vétérinaire CS 40009 54220 Malzéville, France (World

COORDINATION OF THE METHODS EMPLOYED FOR RABIES DIAGNOSIS



Laboratory diagnosis is the key for surveillance of the disease and also to possibly initiate rational post-exposure rabies treatment decision: an appropriate and accurate diagnosis is consequently fundamental.

- ✓ Rabies methods (in compliance with OIE standards)
 - FAT is based on the ability of a rabies FITC antibody conjugate to bind to rabies antigen and allow its visualisation using fluorescent microscopy technique.
 - RTCIT is based on the propagation and isolation of the rabies virus in relevant cell cultures.
- ✓ Harmonisation of methods and elaboration of standardised procedures following identification of the critical points of the methods

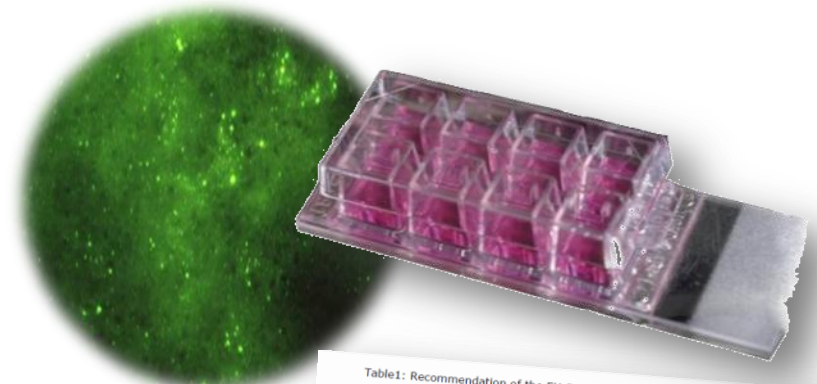


Table 1: Recommendation of the EU-RL for rabies diagnosis using FAT

Procedure step	Strong recommendation	Recommendation	At laboratory decision
1. Part of the brain	Brainstem and cerebellum or Ammon's horn /... indicates per		
	/ smear	15-30 min at Room Temperature	
		Acetone: -20°C; 30min	
		15-30 min at RT	
			Addition of Evan's blue to conjugate
			Soaking 2 x 5min in PBS
8. Mounting	Buffer mounting > or equal to pH 8.5 A strong concentration of glycerol may provide diminished staining		
9. Reading	Read slides within 2 hours 2 independent trained readers	Final magnification from 200 to 400	
10. Controls	Include positive and negative controls in each session		

Journal of Virological Methods 177 (2013) 15–25
Contents lists available at ScienceDirect
Journal of Virological Methods
journal homepage: www.elsevier.com/locate/jviromet

International interlaboratory trials on rabies diagnosis: An overview of results and variation in reference diagnosis techniques (fluorescent antibody test, rabies tissue culture infection test, mouse inoculation test) and molecular biology techniques
E. Robardet*, E. Picard-Meyer, S. Andrieu, A. Servat, F. Cliquet

Headline Publishing Corporation
Bioscience Resource International
Volume 2013, Article ID 639313, 11 pages
http://dx.doi.org/10.1155/2013/639313

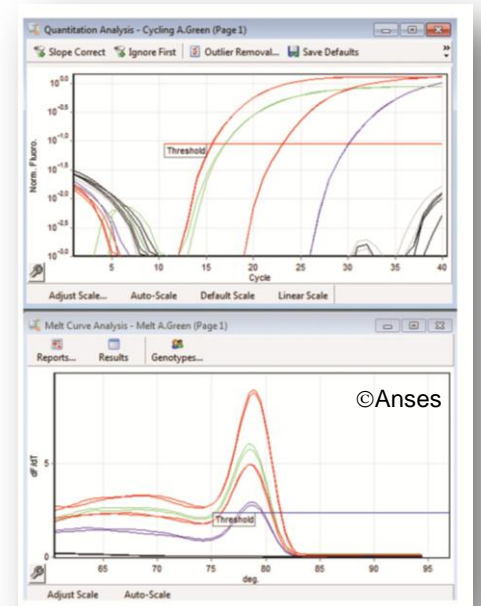
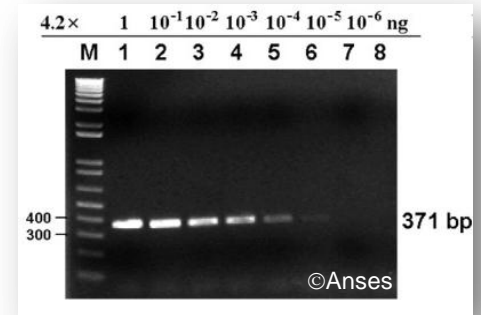
Research Article
Cross-Platform Evaluation of Commercial Real-Time SYBR Green RT-PCR Kits for Sensitive and Rapid Detection of European Bat *Lyssavirus* Type 1
Evelyne Picard-Meyer, Carine Peytavin de Garam, Jean Luc Schereffer, Clotilde Marchal, Emmanuelle Robardet, and Florence Cliquet
Anses, Laboratory for Rabies and Wildlife, WHO Collaborating Centre for Research and Management in Zoonoses Control.

MOLECULAR BIOLOGY TECHNIQUES: RT-PCR & qPCR

- Secondly commonly used confirmatory tests.
- Detects RNA even in degraded samples. RT-PCR useful for *intra vitam* diagnosis in humans (saliva samples or skin biopsies).
- Identifies the virus species (specific primers or typing).
- RT-qPCR quantifies DNA (less cross-contaminations).
- High sensitivity and specificity.
- High amount of analysis in a short time.
- Multiplicity of existing protocols, machines, primers...

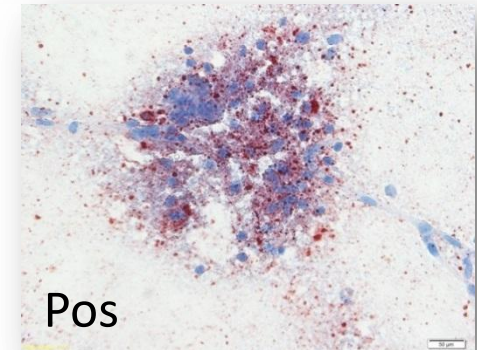
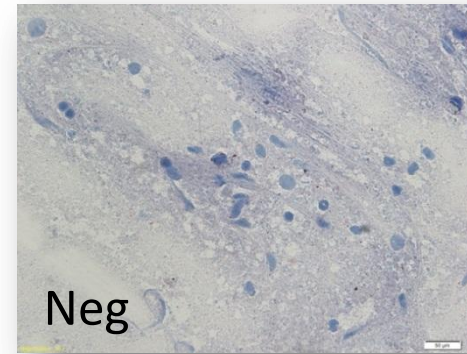


- High technological laboratory requirement.
- Cross-contamination and false positive risks.
- Stringent quality assurance.



DIRECT RAPID IMMUNOHISTOCHEMICAL TEST

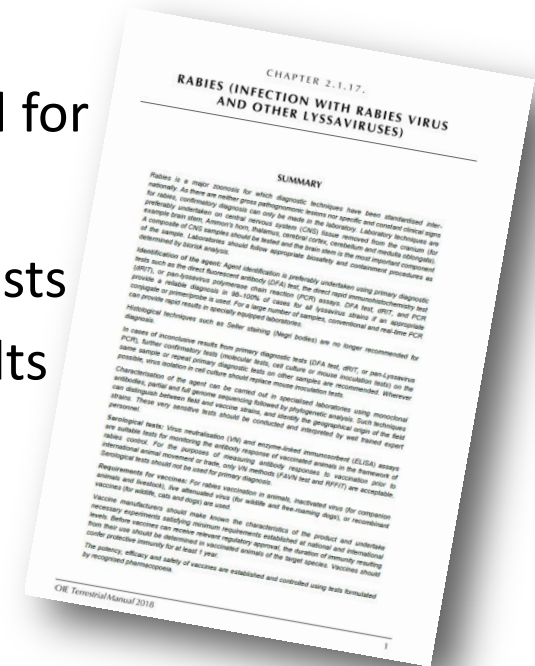
- dRIT developed at the CDC in the 2000's.
- Detects viral antigens present in the CNS (RABV and all other lyssaviruses) with similar sensitivity and specificity to FAT.
- Similar to FAT except the use of streptavidin-biotin peroxidase staining with monoclonal or polyclonal antibodies either from OIE/WHO ref. labs. or self-produced; so fluorescence microscope not required.
- Already in routine use in North America for support of oral wildlife rabies vaccination programs.



- Requires basic laboratory equipment, reagents and training for application.
- Should improve decentralized lab-based surveillance in developing countries where the burden of rabies is important.

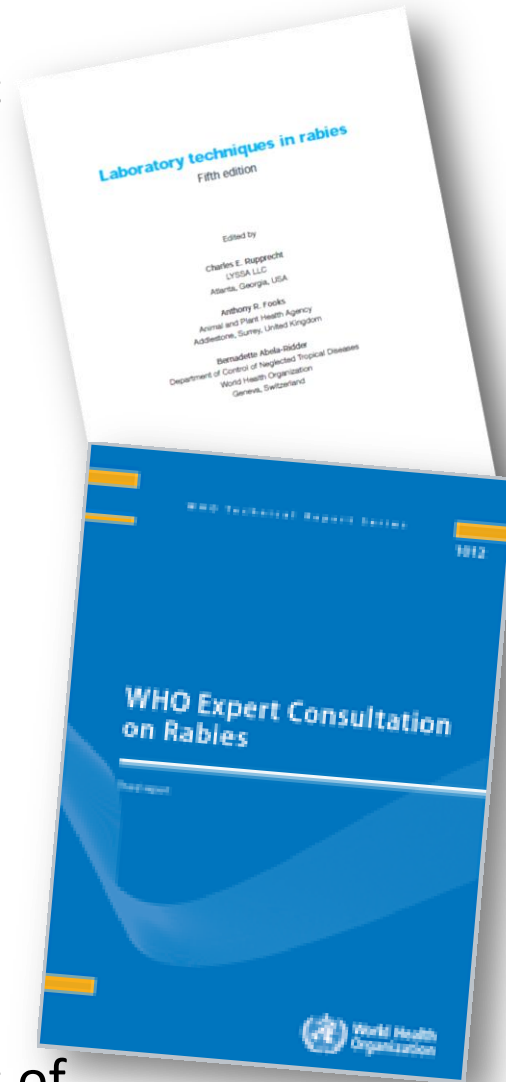
CHAPTER 2.1.17 RABIES*

- Full revision, new chapter adopted in May 2018
- Update existing diagnosis tests:
 - FAT, dRIT, pan-lyssavirus PCR recommended as primary diagnostic tests
 - Conventional and real time PCR recommended for large number of samples
 - RTCIT, MIT (discouraged) or other molecular tests are recommended in case of inconclusive results
- Update vaccine's section
 - Injectable
 - Oral use (wildlife and dog)



* Updated and published in 2018

- Update on laboratory techniques (standard diagnostic tests and tests introduced since the WHO Laboratory techniques in rabies in 1996 and in 2018).
- Role of oral vaccination of dogs during mass vaccination campaigns.
- Template dossier for validation and verification that dog-mediated rabies has been eliminated.
- Promoting the reporting of surveillance data through national, regional or international databases or platforms (i.e. DHIS).
- Guidelines for palliative care of infected patients, safe techniques for human and animal immunization, strategies to reduce over-use of biologicals, potentials of new biologics.

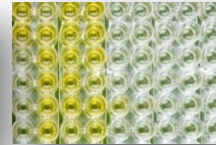


* Updated and published in 2018

COLLABORATIVE STUDIES

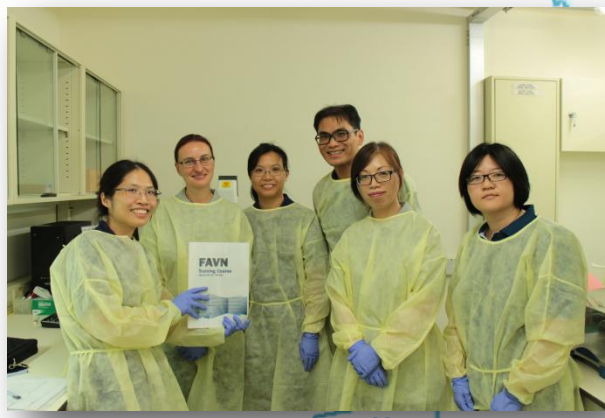
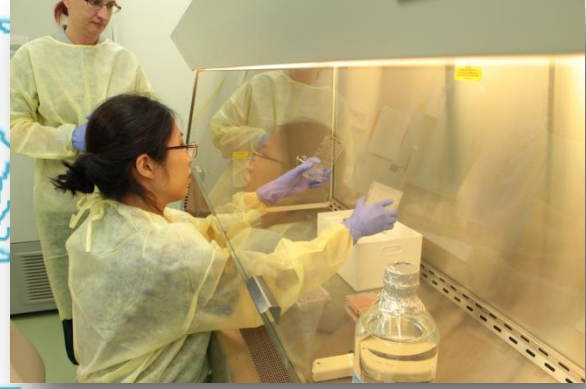
- To evaluate a new method (in development or published) in comparison with a reference method
- To evaluate or to compare reagents or kits for rabies diagnosis, serology or molecular biology
- To evaluate or to compare equipment/machines

- **Examples:**



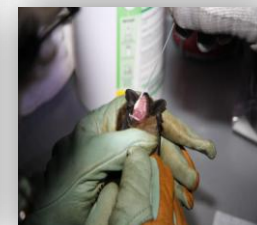
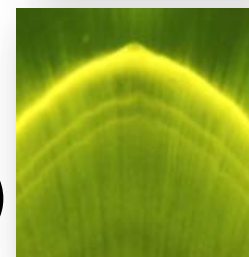
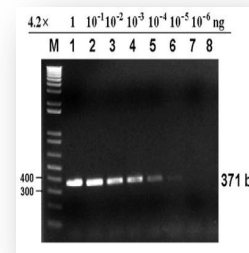
- Evaluation of ELISAs for use in fox and raccoon dog samples to assess the efficacy of oral vaccination campaigns
- Comparison of FAT results among 12 NRLs from EU using various conjugates
- Comparison of conventional RT-PCR performances in 16 NRLs from EU
- Determination of the titre of a new reference vaccine

Trainings



TRAININGS : VARIOUS FORMATS! (1/2)

- In France or in the laboratories
- Individual (on request or request from our lab) or several people (workshop)
- Laboratory methods:
 - Rabies serology (FAVN test and ELISA BioPro kit)
 - Rabies diagnosis (FAT and RTCIT)
 - Molecular biology (PCR, RT-PCR, real time RT-PCR)
 - Titration of oral vaccine baits
 - Potency of inactivated vaccines (NIH test)
 - Determination of biomarker (tetracycline) in oral vaccine baits
 - Others
- Field work (capture of bats, vaccination of dogs, dog counting)
- Rabies epidemiology (analysis of data)



TRAININGS : VARIOUS FORMATS! (2/2)

Organisation of workshops:

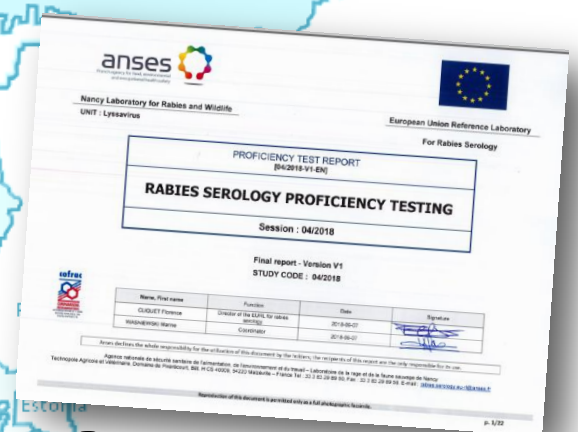
- Serology (4 workshops for the FAVN test in Nancy (1995 – 1999), one for ELISA (2004) and one in Taiwan (AHRI) in 2017)
- Molecular biology (in Morocco in 2008 and 2015)
- Detection of biomarker in vaccine baits (in 2012)



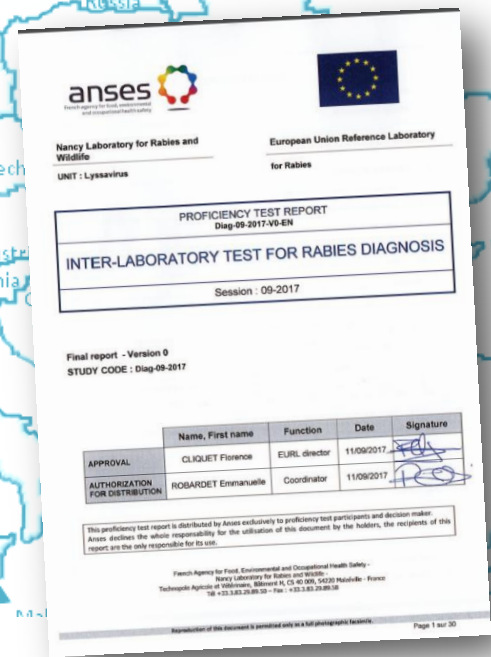
A good way for creating networks and make them working!

Follow up of trainings:

- Satisfaction questionnaires
- Procurement of reagents, panels, calculation sheet models, SOPs, etc...
- Support for data and results analysis and interpretation



Proficiency tests



GENERAL PRINCIPLES

- Blind samples analysed in participating laboratories as if they are samples received for routine analysis
- All involved laboratories test the same samples with the same method (tests referenced by the OIE)
- In the context of rabies serological testing for pet movements, proficiency tests are regulated by the EC¹ with the delivery of an annual approval (https://ec.europa.eu/food/animals/pet-movement/approved-labs_en)

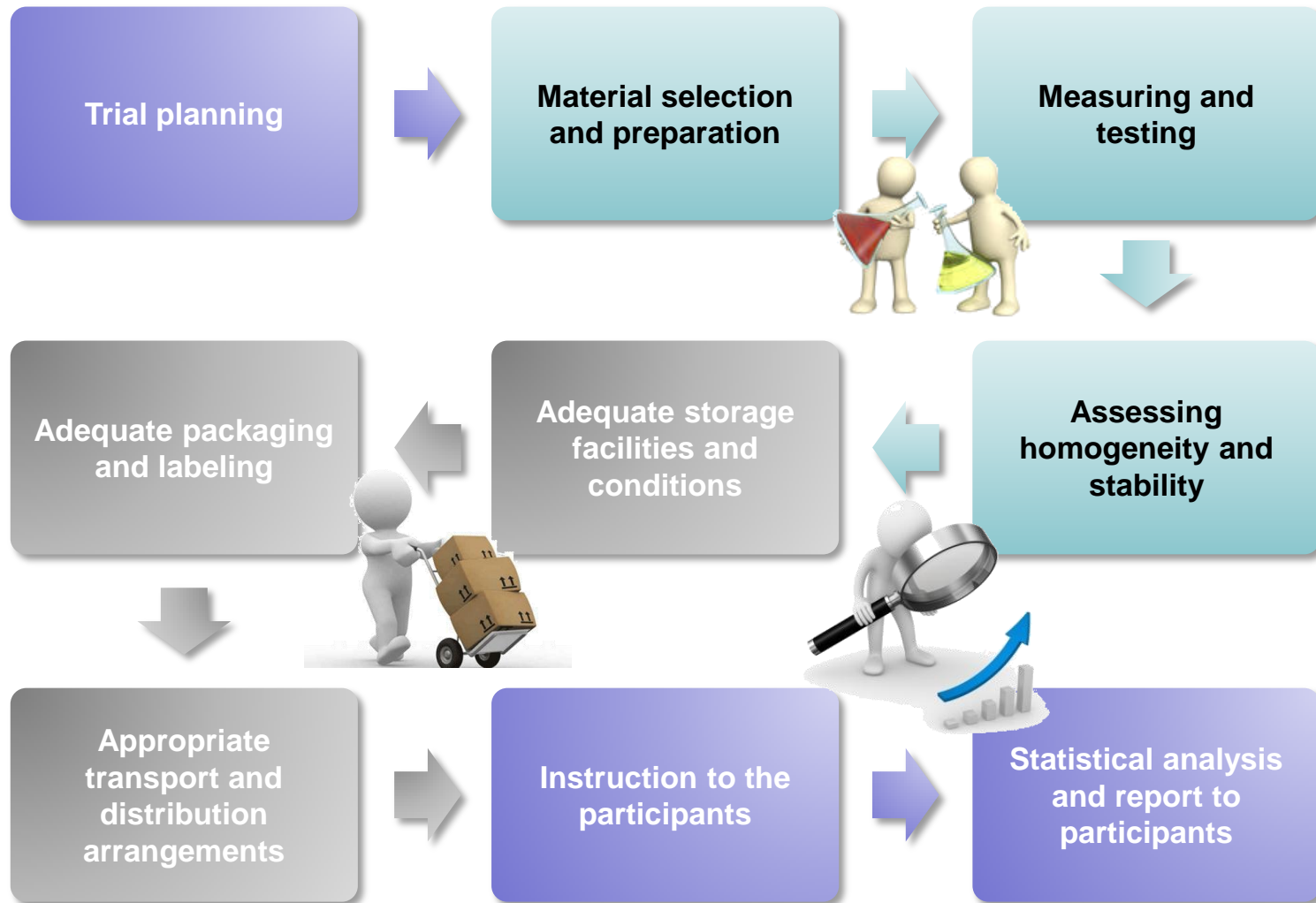


The overall objective is to assess the degree of confidence of results (positive and negative) produced by the laboratories



1 : Regulation of European Parliament and of council 2013/576/EC; Council Decision 2000/258/EC; Commission Decision 2010/436/EU

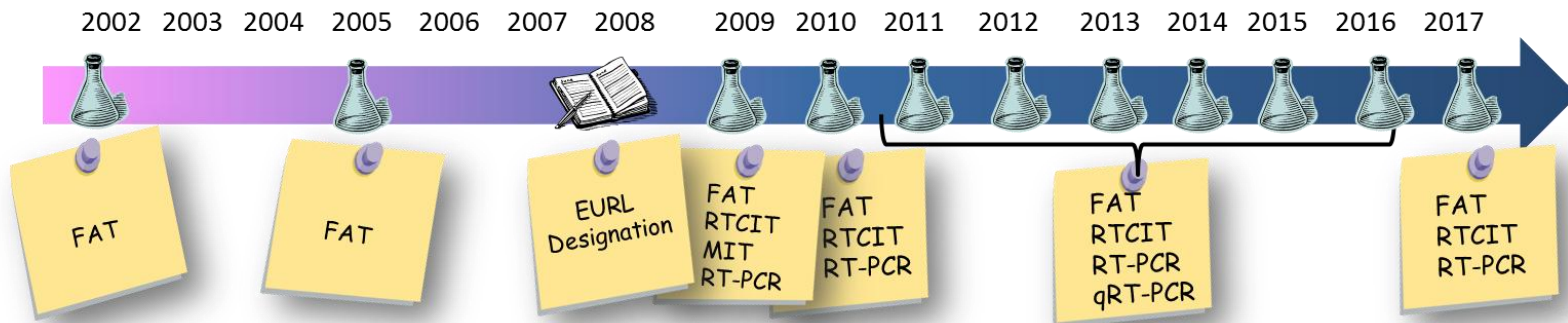
PROCESS OF AN INTER-LABORATORY COMPARISON



- Confidentiality (labs and samples are randomly coded)
- Collusion and falsification of results

PROFICIENCY TESTS ORGANIZED IN ANSES-NANCY

- Rabies diagnostic tests: FAT, RTCIT, PCR and real time PCR (OIE referenced tests) and dRIT from 2019



- Rabies serological test: FAVN test and original RFFIT (OIE referenced tests) : every year since 1999

- Tetracycline and age detections of orally vaccinated animals (foxes):

- Biomarker incorporated into the oral vaccine-bait matrix used in Europe for wildlife
- To analyse occurrence of tetracycline in teeth of wildlife to evaluate bait uptake



RABIES DIAGNOSTIC TESTS

TEST ITEMS COMPOSITION

- 8 to 10 different batches of homogenate of brain tissues

*To mimic as much as possible the standard rabies diagnosis conditions:
virus is produced in vivo and a single panel is sent for all the tested techniques*

The panel composition changes from year to year:

- Negative sample (homogenate of red fox brain tissue from French territory)
- CVS 27 (fixed strain, *in vivo* production)
- RABV (field strain, *in vivo* production)
- EBLV-1; EBLV-2; DUVV; BBLV; etc... (*in vivo* production)
- + various samples to avoid collusion between laboratories (since 2016)

- ✓ Rule of the FAT, RTCIT, RT-PCR test:

At least one discordant result ⇨ the participant fails the test

- ✓ Since 2017: Additional “Rabies Diagnosis Conclusion” evaluation

- For each applied test, a technical questionnaire is filled by the participants.

(Evaluation of method variabilities and support in the interpretation of discordant results)

ANSES INTERLABORATORY TEST FOR RABIES DIAGNOSIS
RTCIT
Technical Questionnaire
(Rabies Tissue Culture Infection Test)

2.3. Tissue suspension

Please specify:
Grinding medium used: _____
Final suspension after adding the grinding medium to the tissue (in %): _____

Is a freezing/defrosting step included in the procedure? Yes No
Is the tissue suspension centrifuged? Yes No

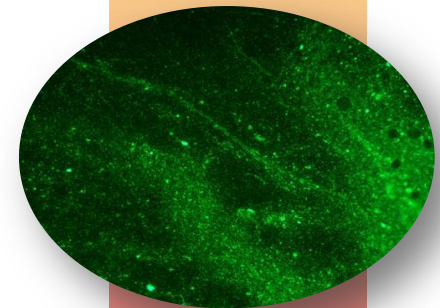
If the tissue suspension is centrifuged, please specify:
Duration (in min): _____
Force (in g): _____
Temperature (in °C): _____

Is serum added to the grinding medium? Yes No

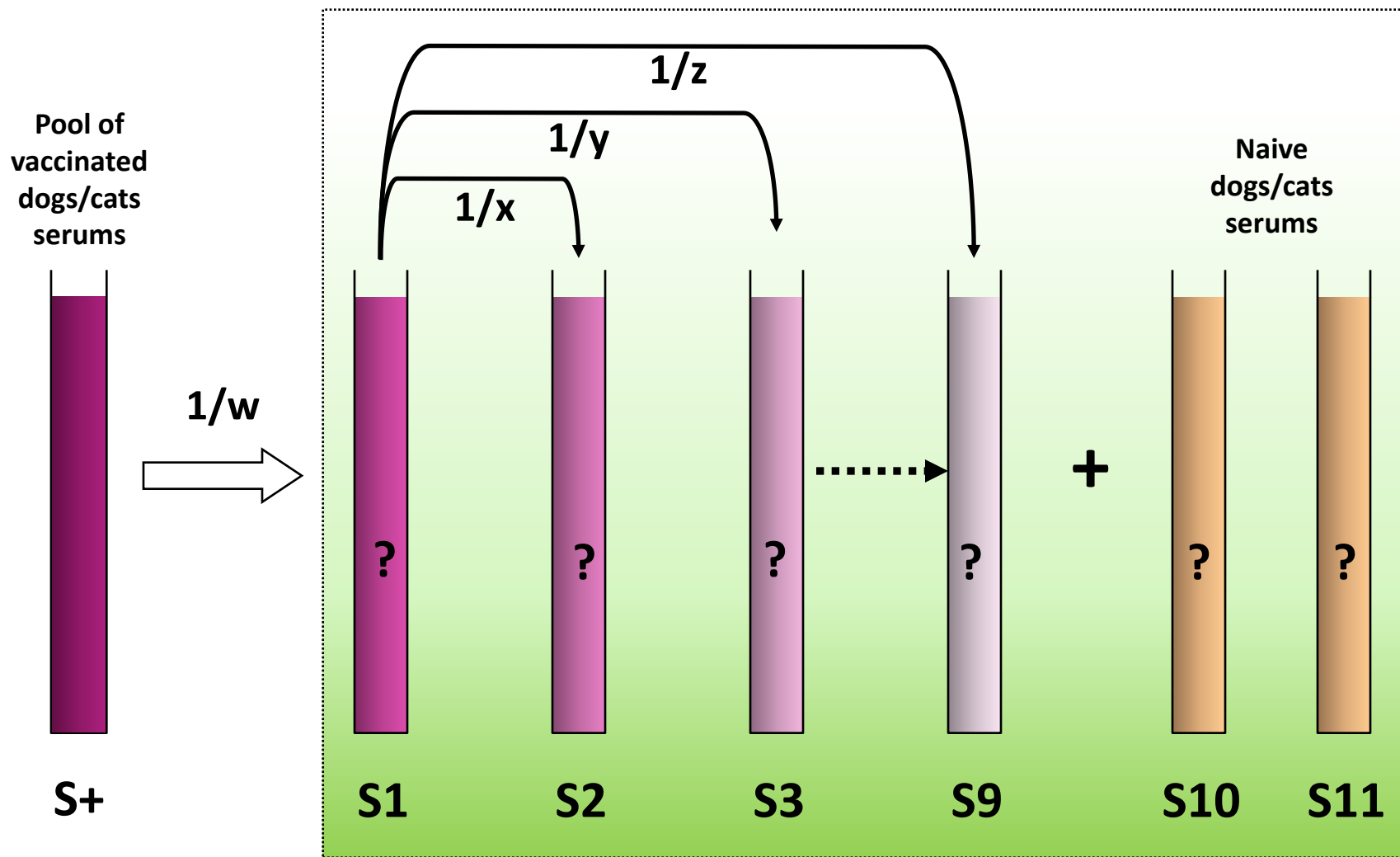
EUROL for rabies - Spring 2013



Results as positive
or negative



RABIES SEROLOGICAL TEST : COMPOSITION OF THE PANEL



One sample in 3 replicates



One sample for avoiding the collusion

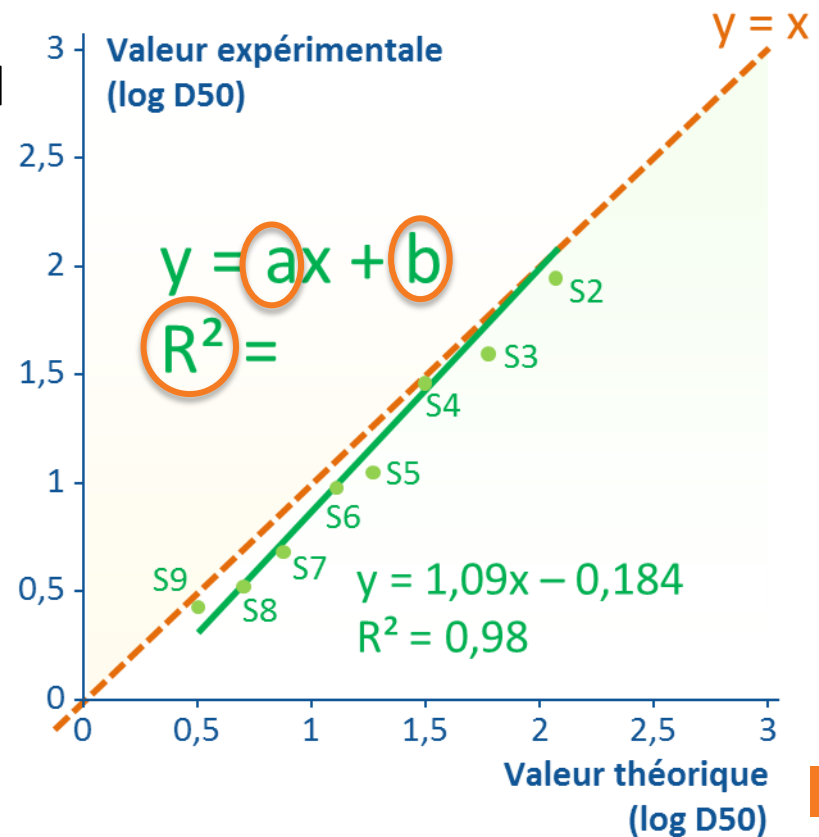
PROFICIENCY TEST PANEL

RABIES SEROLOGICAL TEST: PASS/FAIL CRITERIA (1/2)

- **Specificity:** Ability to identify serum from naïve dog/cat. Each laboratory should determine such serum as being strictly $< 0,5$ IU/mL
- **Intra-laboratory consistency:** Comparison of the values given by a laboratory with the values that this laboratory should have found (using the scale of dilutions)

The degree of consistency is measured using the linear regression:

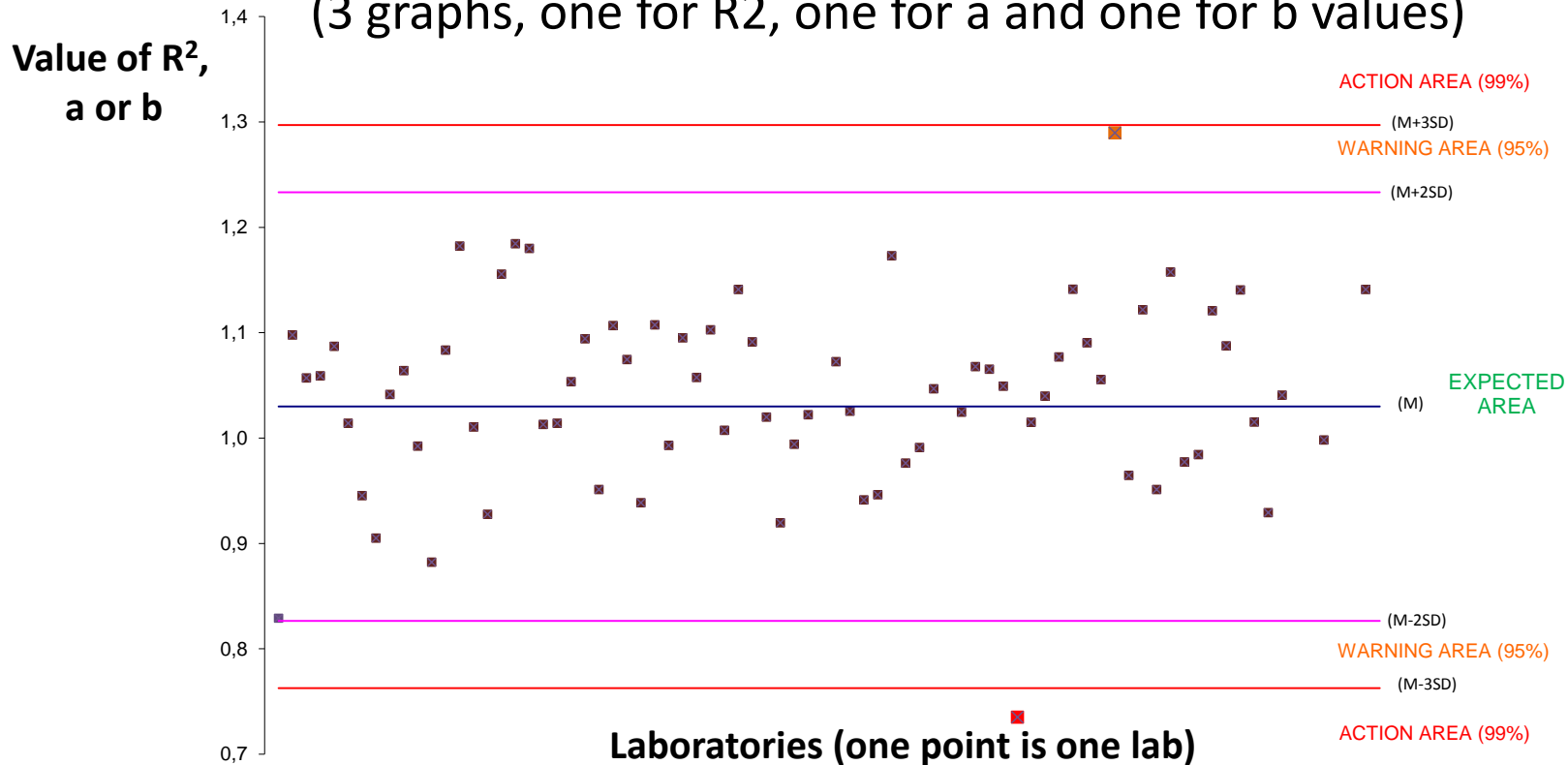
- Value of the coefficient of determination R^2 ,
 - Value of the y-intercept a ,
 - Value of the slope b .
- These values are defined by the historical data and the current campaign



RABIES SEROLOGICAL TEST: PASS/FAIL CRITERIA (2/2)

Example of graph

(3 graphs, one for R2, one for a and one for b values)



Three areas are defined:

- Success: Values of R^2 , a and b are in the **expected area**
- Close examination: Values of R^2 , a and b are in the **warning area**
- Failure: Values of R^2 , a and b are in the **action area**



TETRACYCLINE AND AGE DETERMINATIONS

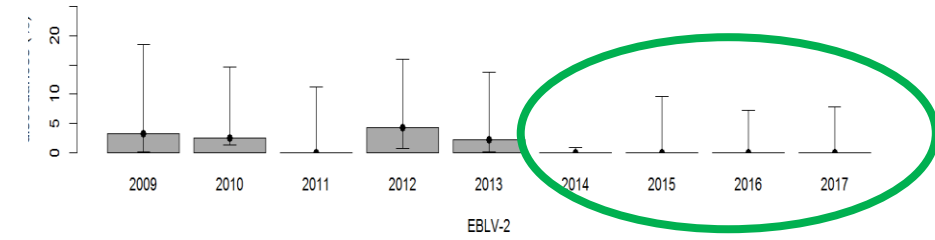
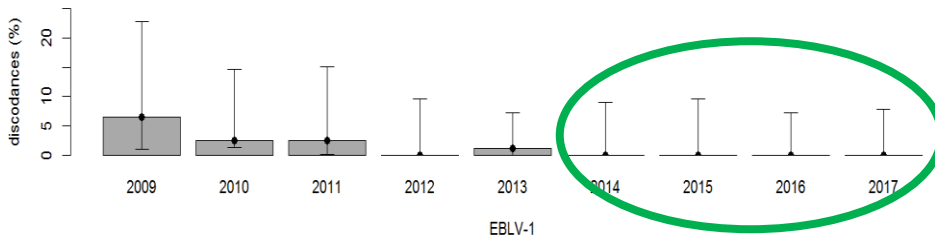
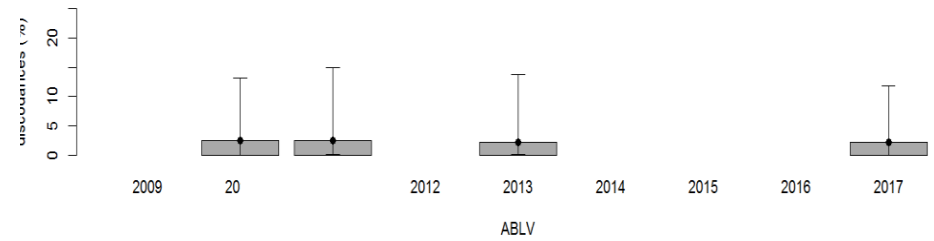
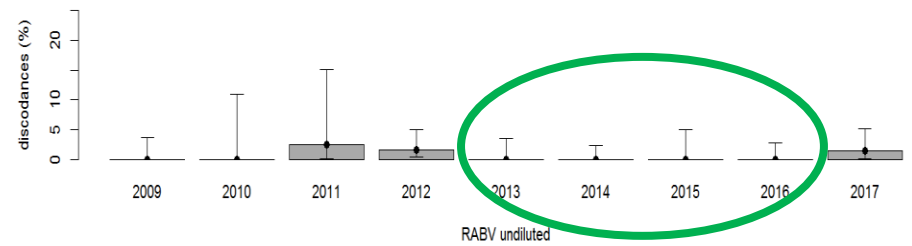
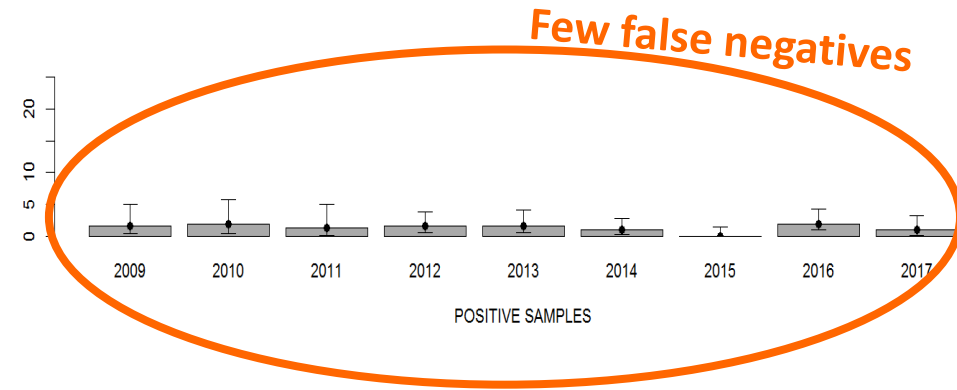
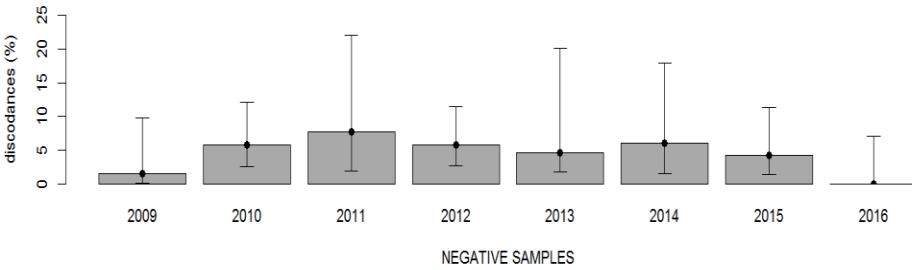
- Jaws collected from red foxes sampled in the field in an infected country following oral vaccination and in France for negative samples.
- Jaw divided in two parts:
 - One to determine the status of the sample in Anses (2 readers).
 - The other one to be sent to participating laboratory.

PANEL TEST COMPOSITION: 6 half red fox jaws

- 1 adult weak positive jaw,
 - 3 adult positive jaws,
 - 1 juvenile negative jaw,
 - 1 adult negative jaw.
- Panel sent in dry ice to avoid Tetracycline signal deterioration under UN3373 conditions.
 - Testing:
 - Determination of tetracycline (results as positive or negative).
 - Determination of animal age (juvenile/ adult) on the same samples

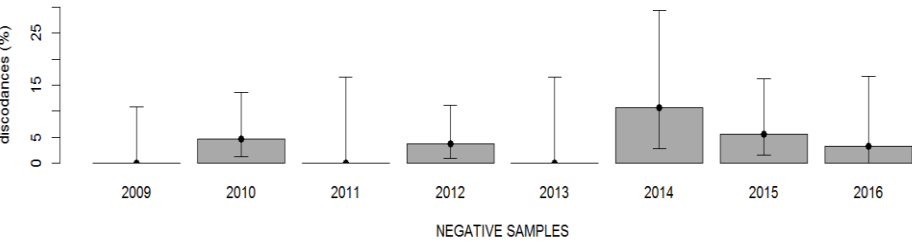


FAT RESULTS TREND



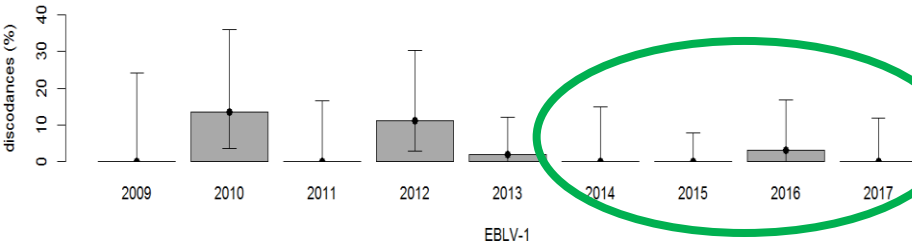
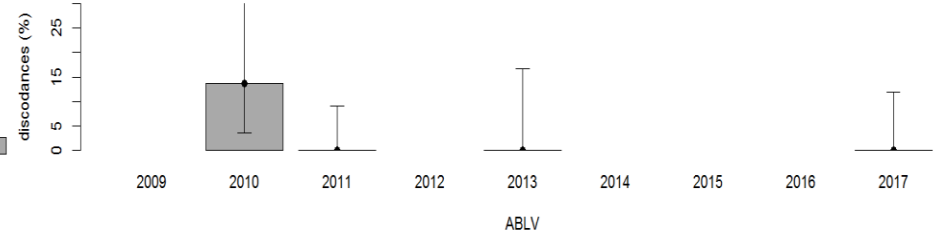
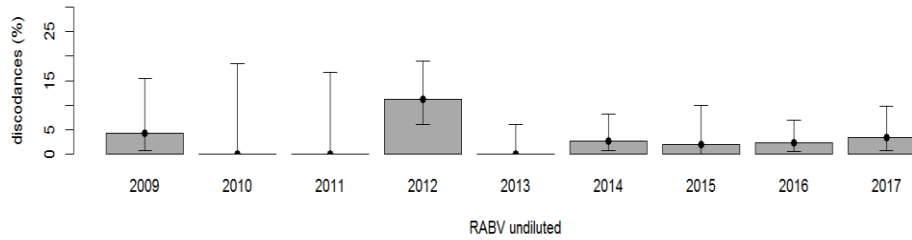
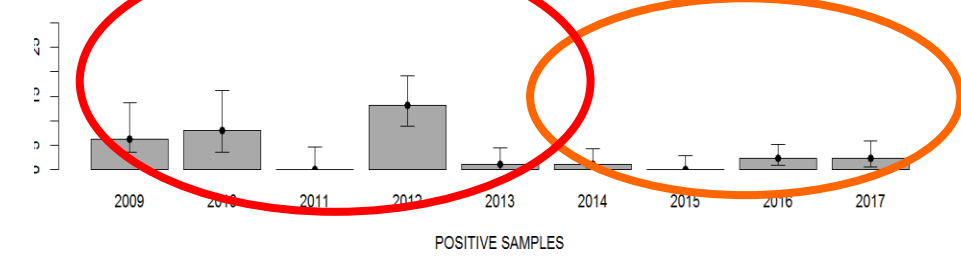
Very few false negatives on undiluted RABV and bat samples!

RTCIT RESULTS TREND



False negatives

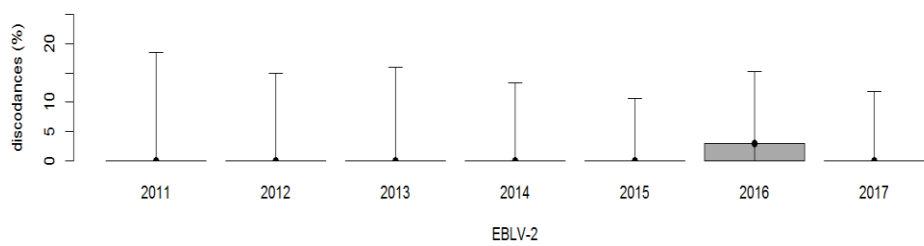
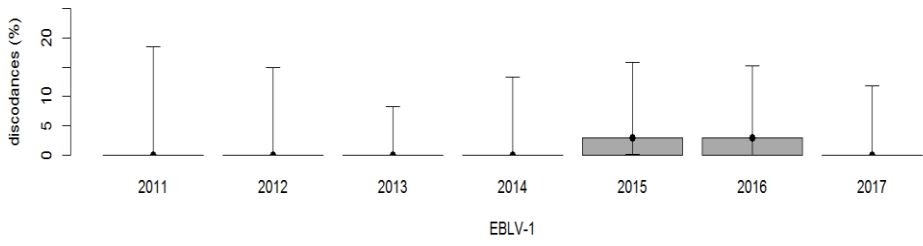
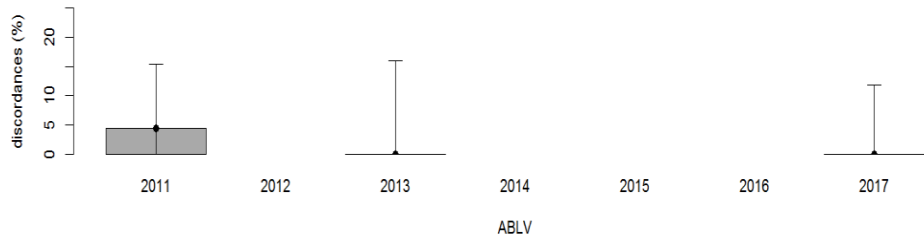
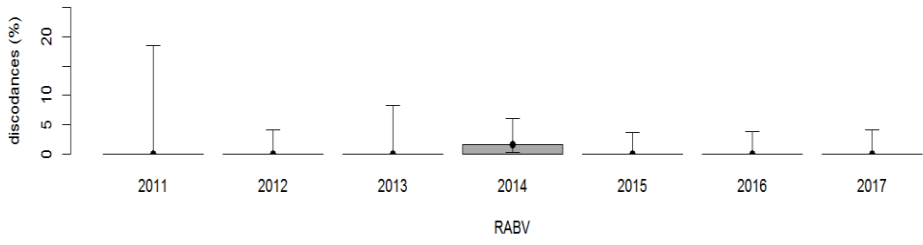
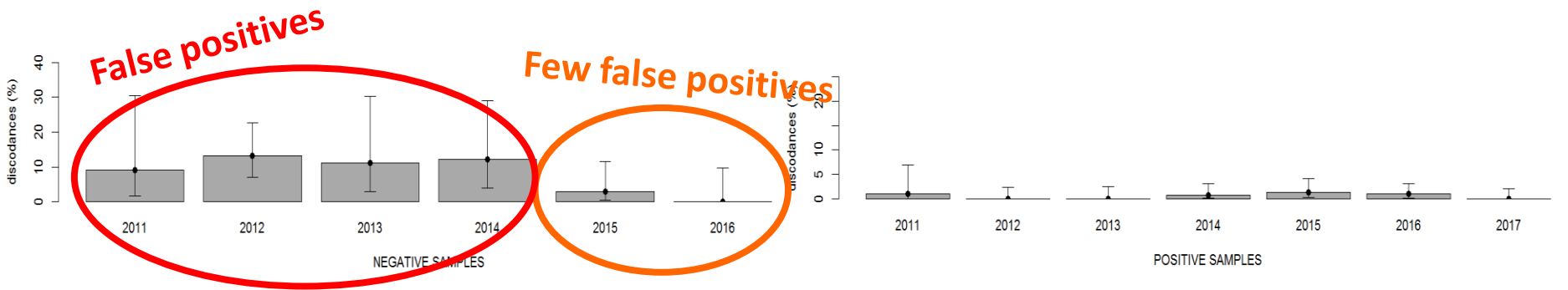
Few false negatives



Very few false negatives on bat samples!



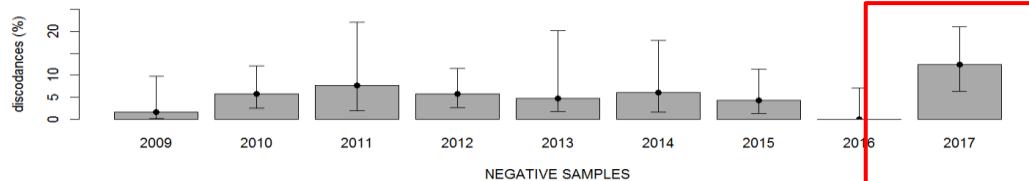
RT-PCR RESULTS TREND



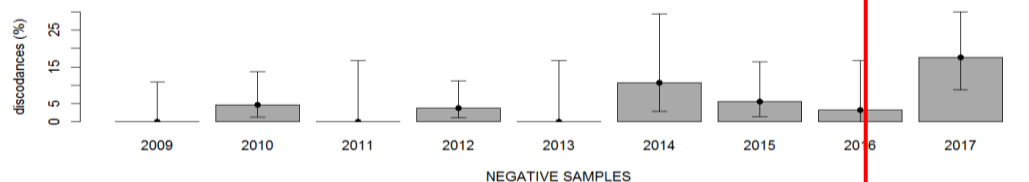
Very few false negative results!

CONCLUSIONS ON THE TREND FOR RABIES DIAGNOSIS

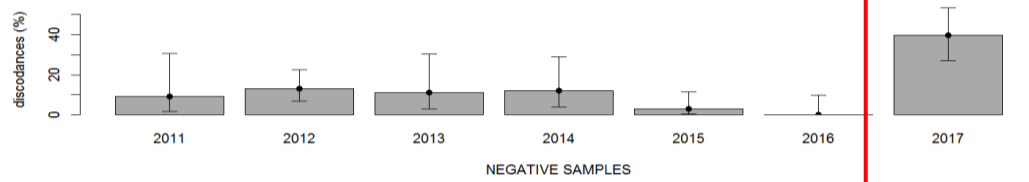
FAT



RTCIT



RT-PCR

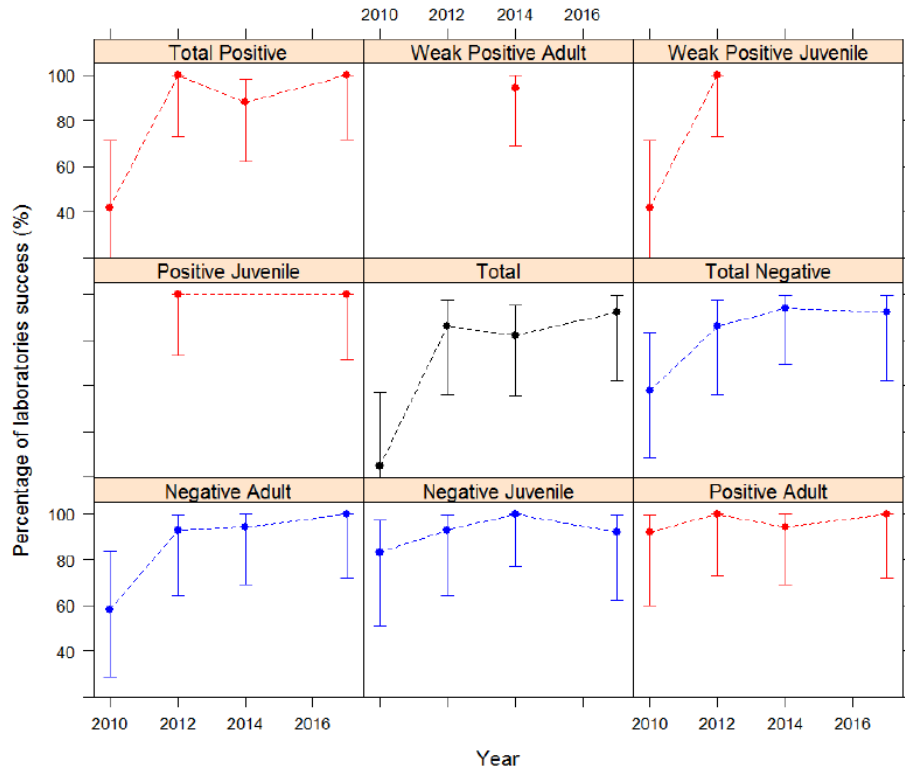


Batch contamination of the negative sample in 2017

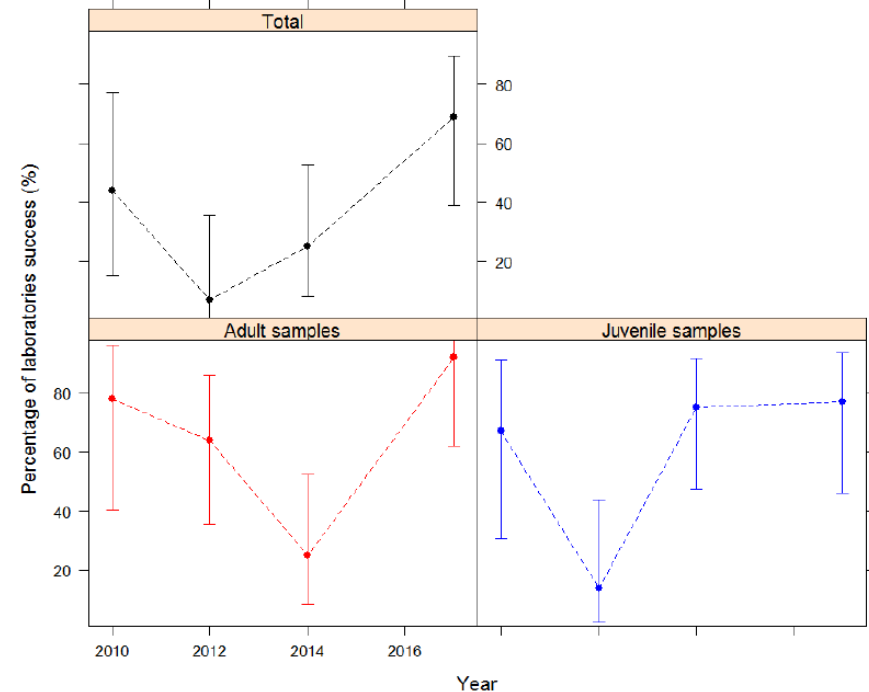
- The 2017 session presented the best performances ever observed on positive samples
- Questions raised within the network:
 - With the use of more and more sensitive tests (real time PCR), how to effectively discriminate true positive samples from contaminated material?
 - In view of new OIE reference tests, which test to consider as a confirmatory test?
 - Need to harmonize rabies diagnosis decision tree.

RESULTS AND CONCLUSIONS ON THE TREND FOR BIOMARKER AND AGE DETERMINATIONS

Biomarker determination

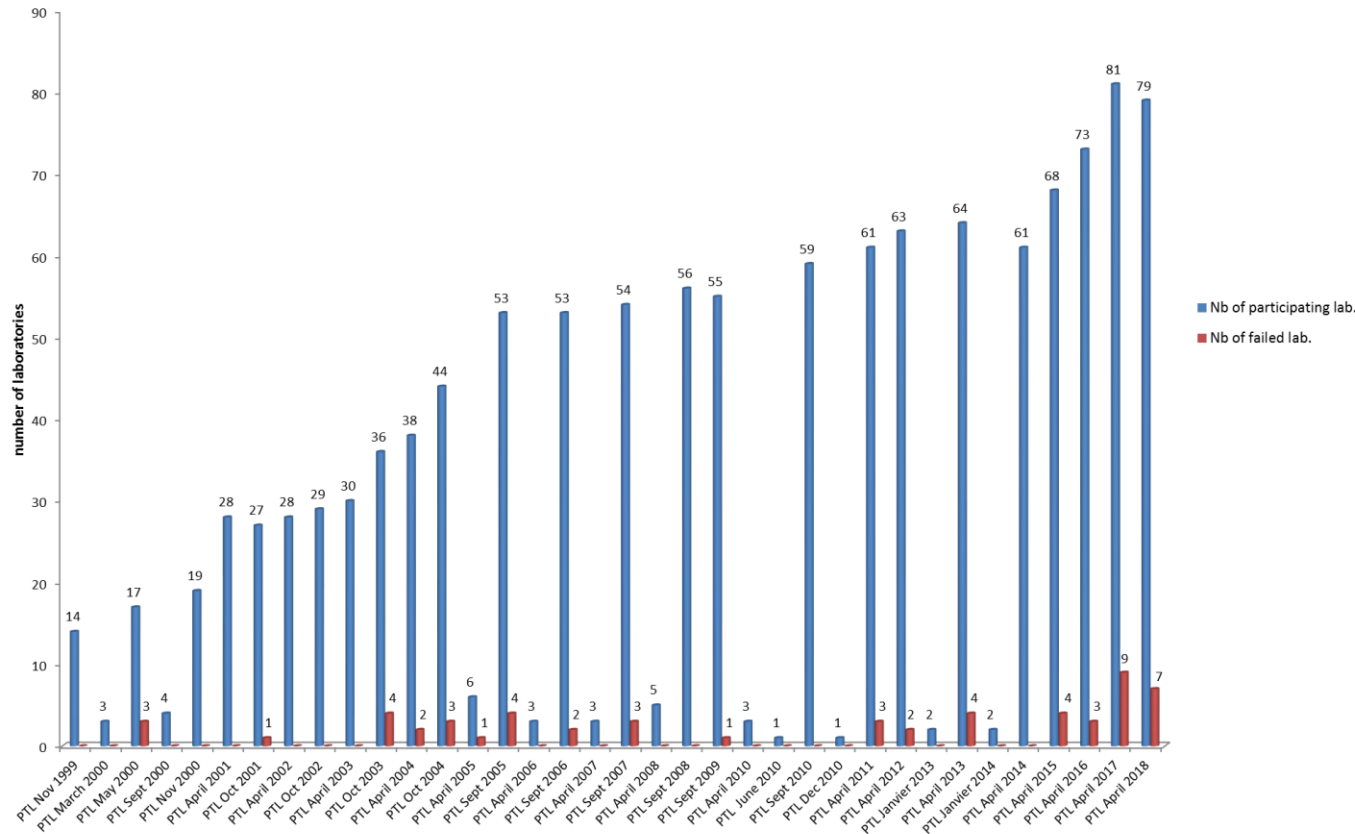


Age determination



- Higher success rate of laboratories for tetracycline detection in 2012, 2014 and 2017 compared to 2010 (92% in 2017 vs 25% in 2010).
- Significant lower rates of discordant results in 2017 for age and tetracycline determinations compared to 2012 and 2010, respectively.
- High level of performances of the laboratories and improvement, particularly for age determination.

OVERVIEW OF SEROLOGICAL RESULTS (SUCCESS/FAILURE) 1999 - 2018



Since 2017, proficiency tests conducted according to ISO/CEI 17043:2010 and ISO 13528:205

- From 1999 to 2017, the average failure is 4,10%, no trend.
- Proficiency testing is part of the quality assurance of laboratories (ISO/CEI 17025).
- This system of laboratory approval for those which are involved in titrations of serum samples from pets for international movements is globally satisfactory.

Meetings - Consultations



OIE AND WHO EXPERT GROUPS WORK

WHO:

- Expert consultations and regular phone meetings
- Laboratory techniques in rabies book: update (fifth edition, September 2018)

OIE:

- Working groups
- Manual of standards for diagnostic tests and vaccines: regular update of rabies chapter (chapter 2.1.17, May 2018)
- Terrestrial Code: regular update (chapter 8.14) (in progress)



EU RABIES TASK FORCE AND GARC

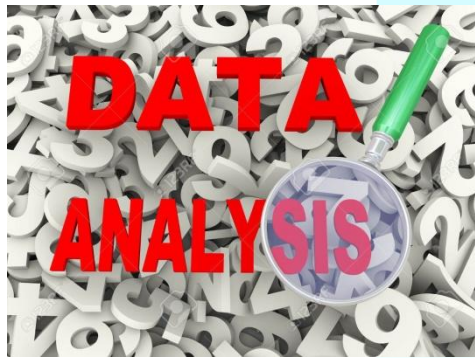
- EU rabies task force
 - Regular meetings in different infected countries (Romania in 2017, Hungary in 2015)
 - Facts and findings report with practical recommendations
- GARC
 - Annual meetings of the PRP (Banna, Italy 2008 – 2012; Wolfsberg, Switzerland 2013 – 2018)
 - Support for MEEREB (MERACON), ARACON and PARACON meetings



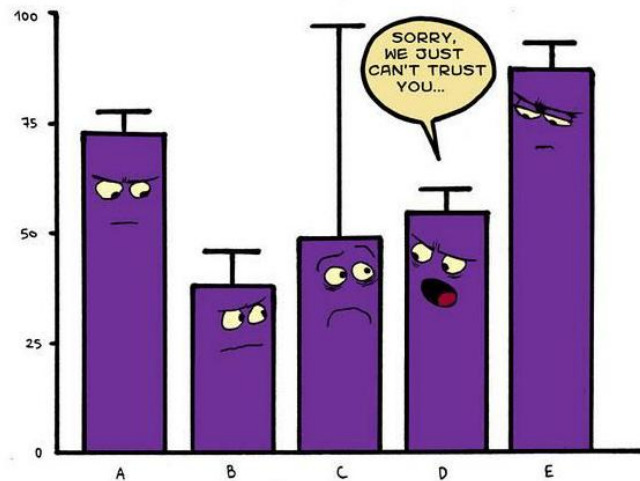
EURL WORKSHOPS

- Organised every year for EURL for **rabies** with Member States and certain European and non European from third countries
- Organised every 3 years for EURL for **rabies serology** with all international laboratories approved for rabies serology





Sharing and disseminating epidemiological data



EPIDEMIOLOGICAL DATA

- Rabies diagnosis based on laboratory investigations of dead animals (suspect).
- Diagnostic data = Surveillance data (rabies incidence) = Epidemiological data if analysed.
- Essential component for short and long term analysis of rabies situation, for deciding the strategy of control, evaluation of control measures and for understanding spatiotemporal dynamics of virus spread.
- These epidemiological data are used by the decision makers.

- Sharing the data
 - From the local, regional to national level,
 - To the network of neighbouring countries, if existing,
 - To national and international databases (WHO, OIE, DHIS2, RBE, etc...)



Rabies is a transboundary disease, exchanging data is transparency for the same objective: elimination of rabies!

NETWORKS UNIFYING CONTINENTS AGAINST THE FIGHT AGAINST RABIES¹

PARACON, ARACON and MERACON aims to ...



- **Unify** countries and rabies control networks of the worlds dog rabies endemic regions and promote co-operation in a **One Health approach** towards rabies control and elimination.



- Create sustainable **platforms** to showcase countries' **successes** towards achieving rabies control and elimination as well as any **lessons learnt** and **challenges** faced.



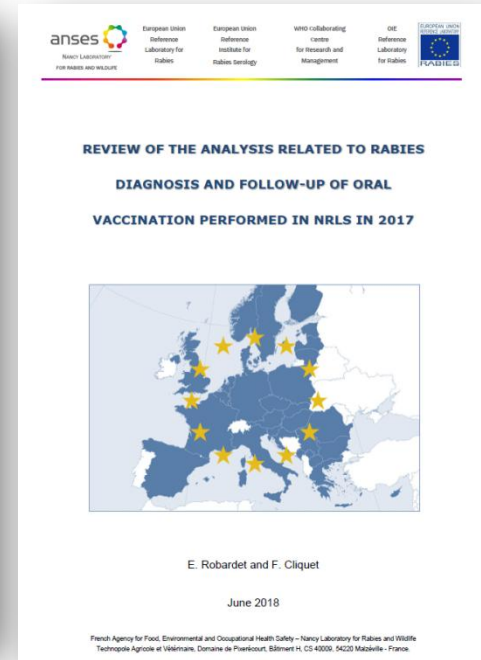
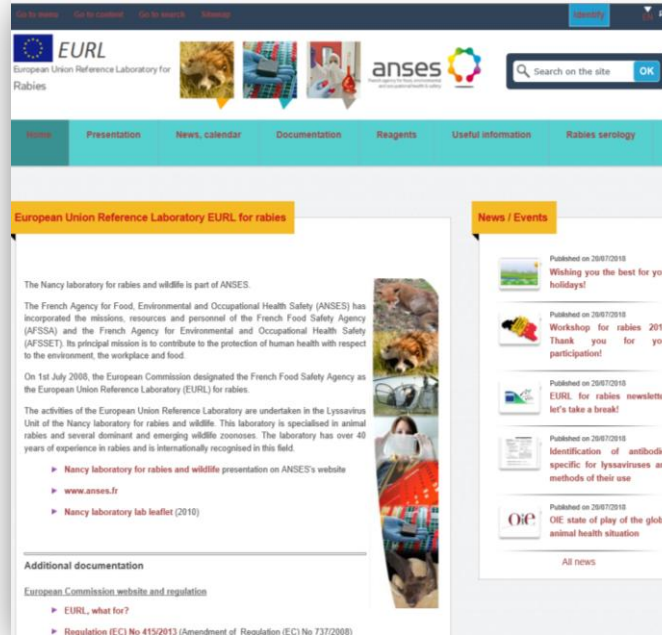
- Promote the **implementation** of **tools** for rabies control such as the **Rabies Blueprint/SARE, National Workplans, Educational initiatives** such as **GEP** and the **Rabies Epidemiological Bulletins**

In Europe : Rabies Bulletin Europe

1 : Slide partly from Louis Nel, GARC

RABIES EURL NETWORKS

- Website (network presentation, news, calendar of activities, reports, events, reagents catalogue, etc...)
- Bimестrial Newsletter (news, calendar, rabies alerts, list of new publications, etc...)



- Annual report of laboratory analysis for rabies surveillance and control in EU countries

ORAL RABIES VACCINATION EVALUATION RESULTS IN EUROPE



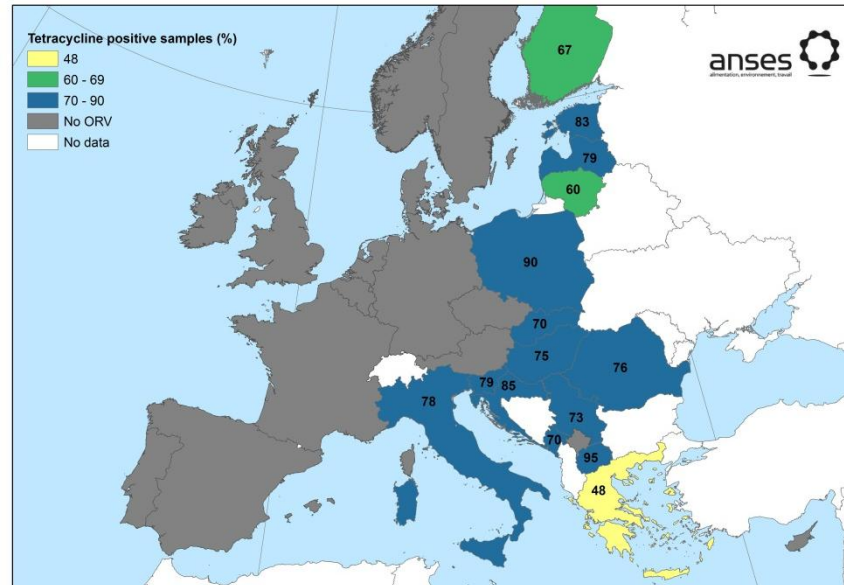
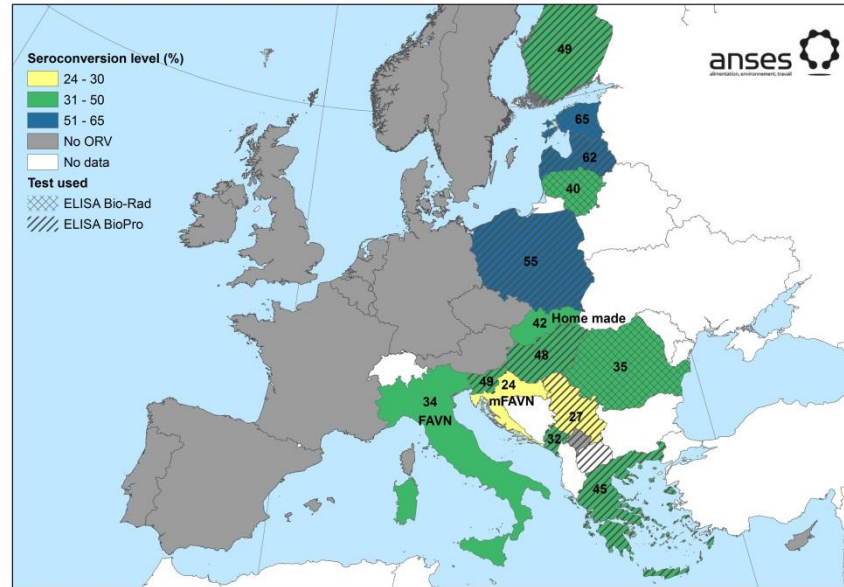
REVIEW OF THE ANALYSIS RELATED TO RABIES DIAGNOSIS AND FOLLOW-UP OF ORAL VACCINATION PERFORMED IN NRLS IN 2016



E. Robardet and F. Cliquet

July 2017

French Agency for Food, Environmental and Occupational Health Safety – Nancy Laboratory for Rabies and Wildlife
Technique Agricole et Vétérinaire, Domaine de Plœucourt, Bâtiment H, CS 43008, 54220 Malzeville - France.



Rabies serology:
Multiple tests,
Comparability of results?

Biomarker determination:
Unique standardized procedure



Discussion



STRATEGIC PLAN « ZERO BY 2030 » IN A ONE HEALTH APPROACH

Objective 1: To efficiently prevent and



World Health



Food and Agriculture

OIE



respo
vaccin
techn

Objec
meas
guida
data
makin

Objec
and r
provi
rabies
feasib
engag
instit

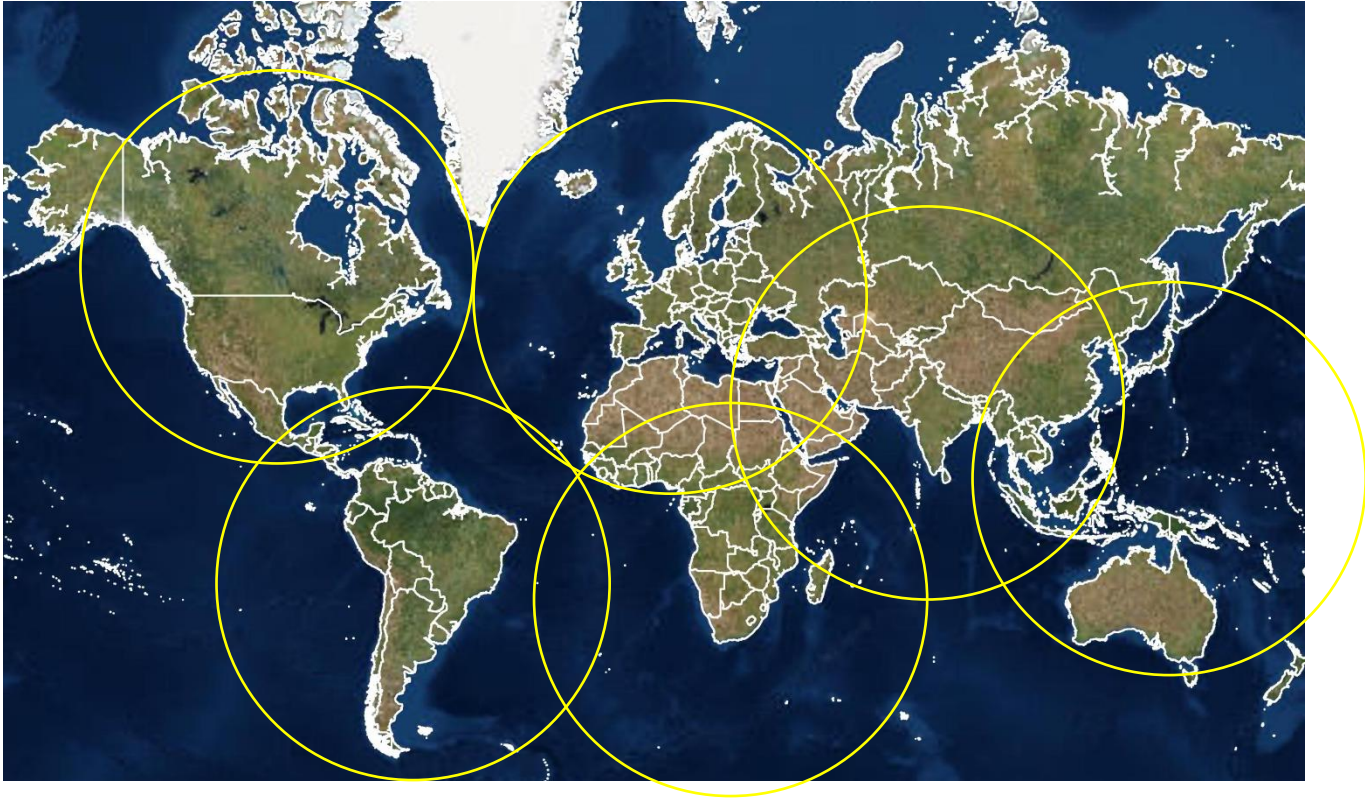
in the fight to end rabies.

**Our common objective :
to end human deaths
from
dog-mediated rabies by
2030**

COUNTRIES
leading elimination efforts

OWN

EVOLUTION OF RABIES DIAGNOSIS PROFICIENCY TESTS ORGANISATION



NETWORKS OF ORGANIZERS *SUPPORTED BY BOTH OIE AND WHO*

- Increase of participation availabilities
- Reduction transport costs
- Need to built a network of PT organisers with comparable PT process

PROFICIENCY TESTS ORGANISATION IN EAST-ASIA



- Experienced in Asian Networking (Workshop, APEC, etc..)
- Successful participation in rabies proficiency tests
- Central geographical position
- Building and staff with high capacities

SOME AREAS OF COLLABORATIONS

- Rabies control and surveillance (irrespective of the rabies status of the country):
 - Exchanging data
 - Creating shared databases
 - Organising regular meetings
 - Possibly confirming certain positive cases by another laboratory of the network
- Proficiency tests:
 - Production of reference materials - Exchanging of reagents, materials
- Trainings/workshops
 - Exchanging or receiving staff of laboratories
- Communication, result valorisation:
 - Creating tools (website, newsletter)
- Research activities in collaboration

The OIE Twinning project AHRI – Anses could be an opportunity to improve and strengthen Asian laboratories collaboration

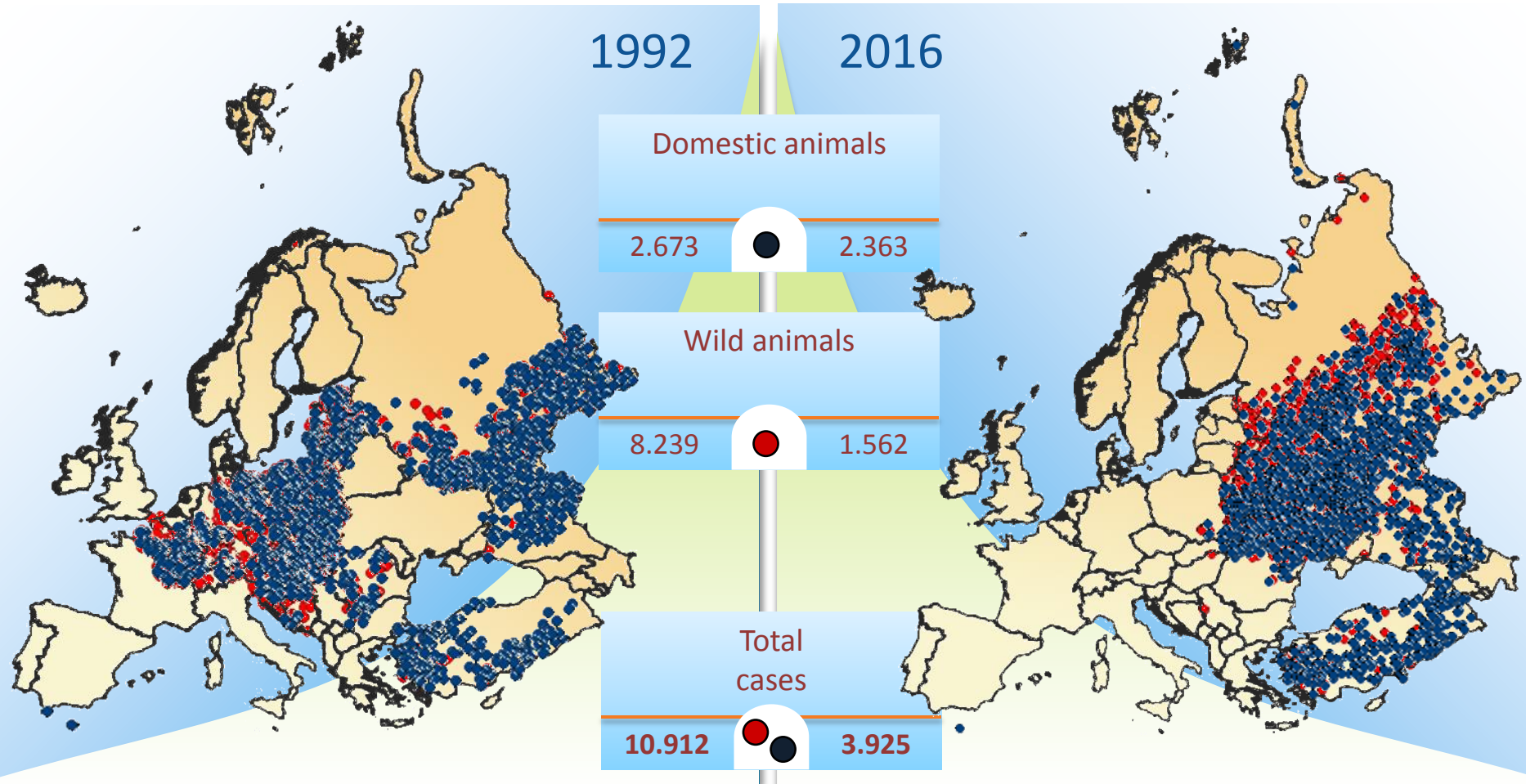
AS A PRE-CONCLUSION

- We do not know:
 - How countries from Asia are organized on the local, regional and national levels
 - How the relations between different countries/regions from Asia are established
 - Existing networks, even not recognized, involved in rabies surveillance and control activities
- We only know that..... being part of network(s), more or less large, more or less recognized is a win-win deal (mutually beneficial)
- We humbly hope this talk will provide you with some items you could develop, adapt or use



RABIES SITUATION IN NON FLYING MAMMALS IN EUROPE 1992 - 2017

In 2017 : six cases (2 cases in Hungary, 2 cases in Poland and 2 cases in Romania)



CONCLUSION

- Reasonable hope to achieve rabies elimination in Europe in the next years
 - Political engagement from EU countries, with associated co-funding from EC
 - Efficient EU network
- With the One Health approach and 2030 as a common objective of OIE, WHO, GARC and FAO for “No human case worldwide by 2030”
 - All efforts to be gathered for this objective from all *ad hoc* sectors on the local, regional and national levels – OIE/WHO/GARC networks
 - Building of networks at the “local” level
- The OIE Twinning is a first stage of the steps to build an Asian network aiming at rabies elimination

***If you want to go fast, go alone, if you want to go far, go together
(African proverb)***

感謝您的關注



Thank you for your attention



LYSSAVIRUS TEAM - ANSES-NANCY