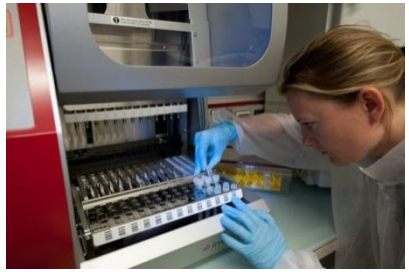
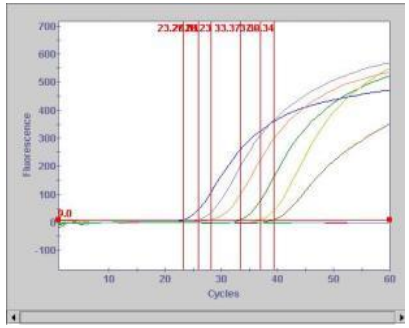
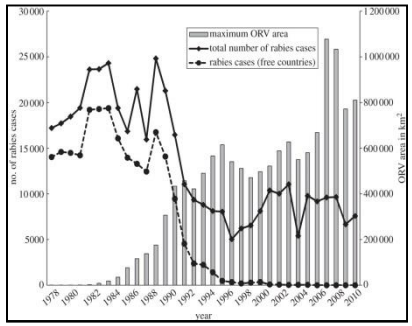
anses
agence nationale de sécurité sanitaire
alimentation, environnement, travail

Connaître, évaluer, protéger





Rabies diagnostic and epidemiological data



Dr Florence Cliquet

Nancy laboratory for rabies and wildlife

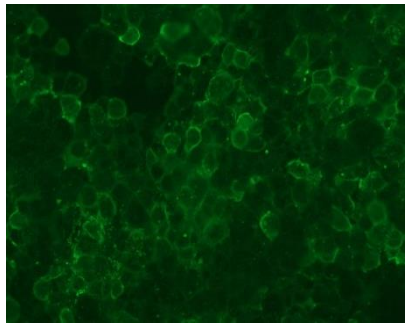
WHO Collaborating Centre for Research and Management in Zoonoses Control

OIE Reference Laboratory for Rabies

European Union Reference Laboratory for Rabies

European Union Reference Institute for Rabies Serology

National reference laboratory for rabies



Middle East, Eastern Europe, Central Asia

and North Africa Rabies Expert Bureau (MEEREB) Meeting

4th Edition, Fondation Mérieux, Les Pensières, Center For Global Health, Veyrier-du-Lac (France) – 23 - 25 April 2018

Reference Books for Laboratory Techniques

- Meslin, F.X., Kaplan, M.M., Koprowski, H., 1996, Laboratory techniques in rabies, Vol Fourth edition, World Health Organization, Geneva, 476 p.

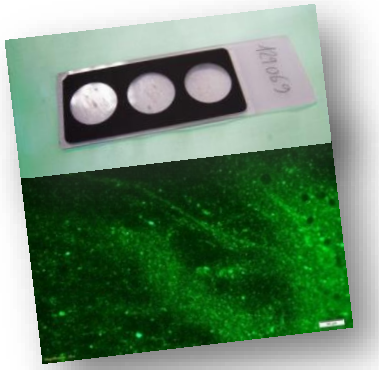
- OIE, 2013, Manual of standards for diagnostic tests and vaccines, Rabies, Chapter 2.1.13. 26 p.
  (review in process).

- Rupprecht C, Nagarajan T., 2015, Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention, 1-366 p.

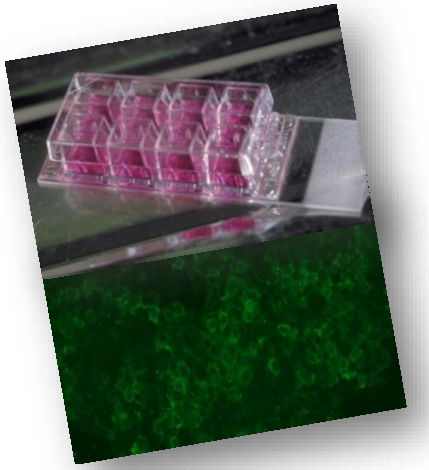
CURRENT OIE/WHO REFERENCE METHODS FOR RABIES DIAGNOSTIC

The Fluorescent Antibody Test (FAT): gold standard (99% SE; 95% SP)

- Detects rabies antigen on fresh, frozen or fixed material.
- Based on the staining of a tissue smear with a FITC labelled anti-rabies immunoglobulin.
- The stained smear is washed in buffer and read under blue light fluorescence to detect the characteristic green fluorescence associated with rabies antigen corpuscles.



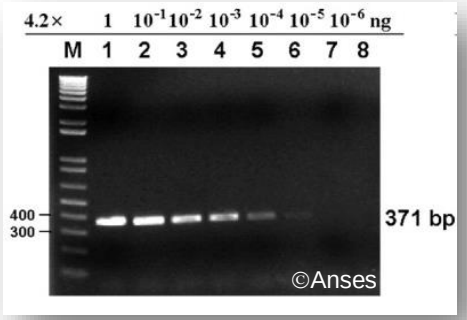
The Rapid Tissue Culture Infection Test (RTCIT)

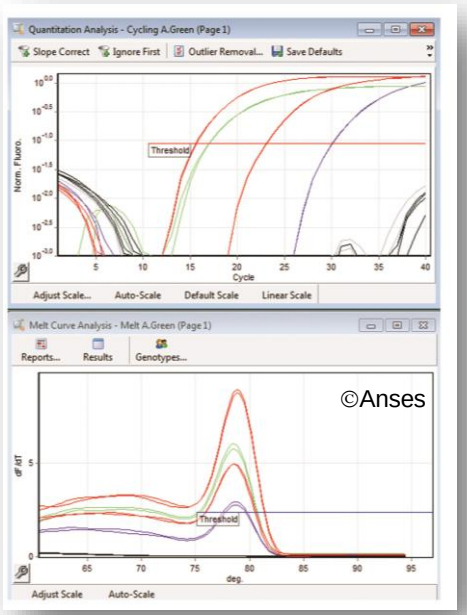


- Detects rabies infectious particles.
- Most commonly used confirmatory test (96% SE; 97% SP) especially in case of negative or uncertain results.
- *In vitro* test using Neuro2a cells (more ethical than the Mouse Inoculation Test).
- Used for lyssavirus isolation.

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…



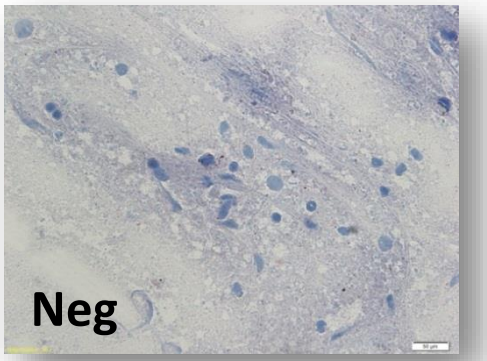


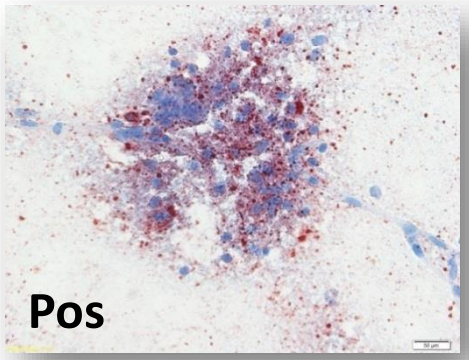
- High technological laboratory requirement.
- Cross-contamination and false positive risks.
- Stringent quality assurance.

Ref : Crepin *et al*., 1998; Dacheux *et al.* , 2008, 2010, 2016; Faye *et al*., 2017; Hayman *et al*., 2011; Hoffmann *et al*., 2010; Mani *et al*., 2014, 2016; Nadin-Davis *et al*., 2009; Picard-Meyer *et al*., 2004; Wacharapluesadee *et al*., 2008; Wadhwa *et al*., 2017; Wakeley *et al*., 2006

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.





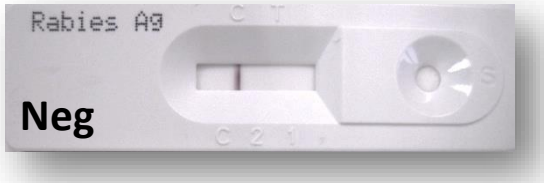
- Requires basic laboratory equipment, reagents and training for application.
- Should improve decentralized lab-based surveillance in developing countries where the burden of rabies is important.

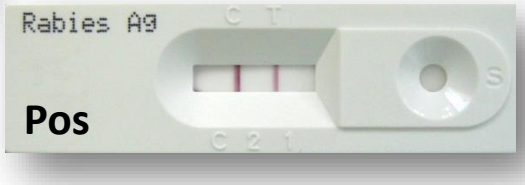
Ref: Coetzer *et al*., 2014; Dürr *et al*., 2008; Lembo *et al*., 2006;Madhusudana *et al*., 2012; Rupprecht *et al*., 2014; Saturday *et al*., 2009; Tao *et al*., 2008

LATERAL FLOW ASSAYS/RAPID IMMUNODIAGNOSTIC TESTS

- LFA/RIDT, or immunochromatographic tests, were first described in the 1960s. Most well-known application is home pregnancy test.
- Adaptation of LFA for rabies was first described in the mid-2000s.
- Detects rabies virus antigen.
- Enables a direct observation of results by the naked-eyes and provides a one-step, rapid (generally from 5 to 20 minutes) and low-cost tool.
- Low technological and low containment requirements; can be used at point of sampling.
- Promising for surveillance under field conditions.

- Need for better standardization and quality controls of the kits (currently, performance highly heterogeneous between the commercialized kits, and between batches).
- Cannot substitute the current reference techniques, but might be helpful in developing countries where rabies surveillance is lacking.
- Prior to routine use, the kit(s) should be locally validated by comparing results with those of FAT on a collection of positive and negative samples.





Ref : Eggerbauer *et al.*, 2016; Kang *et al.*, 2007; Lechenne *et al.*, 2016; Nishizono *et al.*, 2008; Servat *et al.*, 2012; Voehl *et al.*, 2014; Zhang *et al.*, 2009.

DATABASE MANAGEMENT

- A national database containing quantitative and qualitative information relating to the animals tested and to the result of the diagnostic:

Animal tested:

- Species,
- Age and sex,
- Geographical location (GPS data if possible),
- Date of sampling,
- History (found dead, clinical signs, human contamination),
- Biological quality of the sample,
- Sample procedure (passive *versus* active).

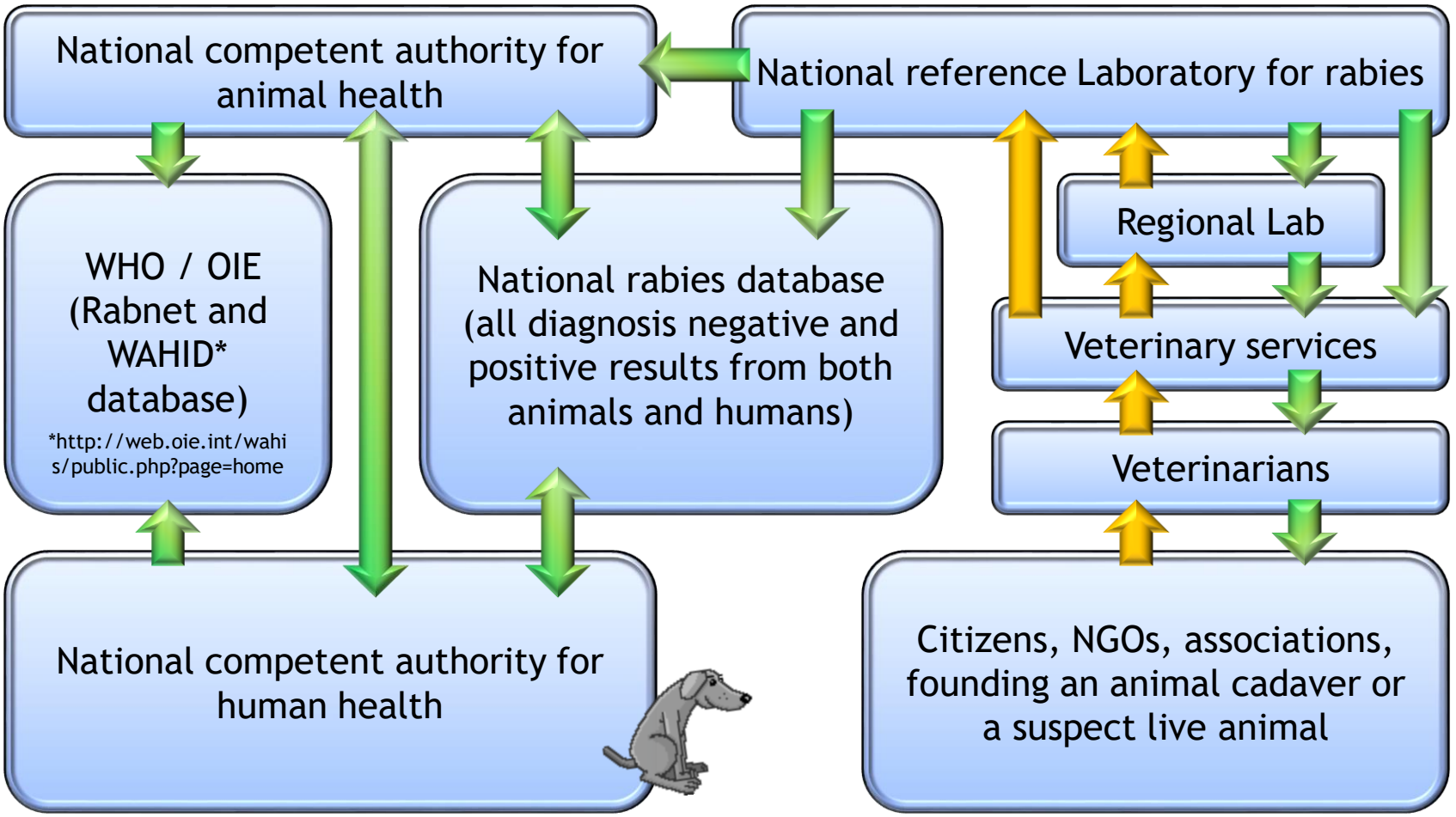
Result of the diagnostic testing:

- Detection of viral antigen:
  - Test(s) used (FAT/RTCIT/dRIT) - Result pos/neg.
- Detection of viral RNA:
  - Conventional PCR/RT-PCR/RT-qPCR - Result pos/neg.
  - Conventional PCR - partial or full gene (N) sequencing.
  - New generation sequencing (NGS) – Full genome sequencing.

Ref: EFSA report, 2010

EXAMPLE OF LABORATORY-BASED SURVEILLANCE NETWORK

SUCH NETWORK MUST HAVE A LEGAL BASIS (LEGISLATION ARTICLE)



Ref: Cliquet *et al.*, 2012

ONLINE NATIONAL, REGIONAL AND INTERNATIONAL RABIES DATABASES

National (examples):

- Canada:
  http://www.inspection.gc.ca/
- USA:
  http://www.cdc.gov/rabies/location/usa/surveillance/

Regional:

- Animal Disease Notification System (ADNS)
- https://ec.europa.eu/food/sites/food/files/animals/docs/ad_adns_outbreaks-per-disease.pdf
- European rabies database of the Rabies Bulletin Europe (RBE)
  www.who-rabies-bulletin.org
- SIRVERA/SIEPI rabies database from PANAFTOSA (PAHO,
  sirvera.panaftosa.org.br/index.php
- Pan African Rabies Bulletin from the Pan-African Rabies Control Network
  paracon.rabiesalliance.org/bulletin/

International:

- OIE World Animal Health Information Database (WAHID)
  http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasehome
- WHO Global Health Observatory / Rabies
  apps.who.int/gho/data/node.main.NTDRABIES?lang=en

KEY POINTS

Irrespective of the status of the countries (free or infected):

- Rabies should be a notifiable disease.
- Adequate and effective surveillance network in place for both human and animal.
- National Reference Laboratory for rabies and possibly regional laboratories:
  - Reference tests for rabies diagnosis (equipment, reagents, trained staff).
  - Collaboration between national and regional labs.
  - Quality assurance scheme or a system guaranteeing the traceability of the analysis from sample receipt to result sending.
  - Rabies testing algorithm (methods to be used and decisions).
  - Timely reporting of both positive and negative results to the competent national authorities.
  - To guarantee the reliability of results, if possible, involvement in annual international proficiency testing.

- The primary challenge in assessing the overall impact of rabies is the need of laboratory diagnosis reliable data.
- Personnel in charge of gathering and analyzing timely the national data.

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

Rabies surveillance – Estimation of the disease burden

- **Overall evaluation of surveillance:** Tables with total number of positive cases (incidence) and total number tested per administrative unit

Animals tested negative: 436 records

Region	Domestic animals									Wildlife														bat	total
	dog	cat	cattle	equine	goat sheep	pig	stray dog	other	sub total	fox	racoon dog	racoon	wolf	badger	marten	other mustelides	other carnivores	wild boar	roe deer	red deer	fallow deer	other	sub total		
Aitoloakarnania									0	1													1		1
Argolida									0														0		0
Arta									0														0		0
Attiki									0	1												14	15		15
Chalkidiki									0	2													2		2
Drama									0	4			1				1						6		6
Evoia									0														0		0
Evros									0	2													2		2
Evrytania									0														0		0
Florina	4	1							5	14				1								2	17		22
Fokida									0														0		0
Grevena									0	155													155		155

Example: RBE - Bulletin Trimester 4th 2017 RABIES / Greece, data kindly given by Dr Marilina Korou (Greece)

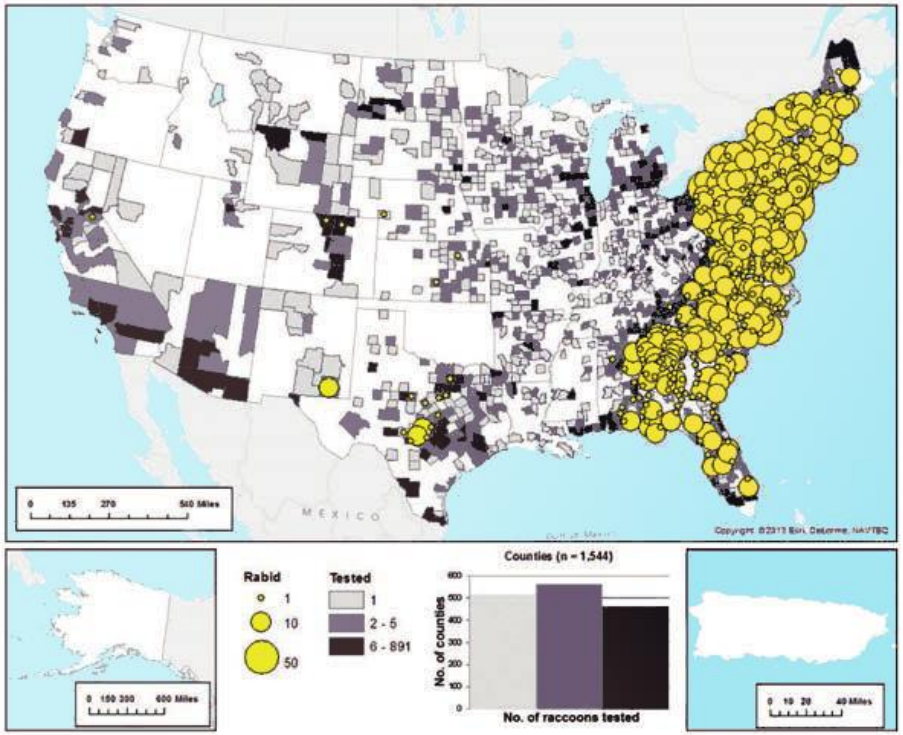
- **Spatio-temporal patterns: Epidemiological situation at a certain time and in a certain area:** Maps recording individual positive and negative cases. Distinctions can be done for:
  - Cases in wild animals,
  - Cases in domestics,
  - Cases per species,
  - Cases in bats,

- **Epidemic curve and disease trend monitoring:** Distribution of cases by times unit to show the dynamic of the disease.

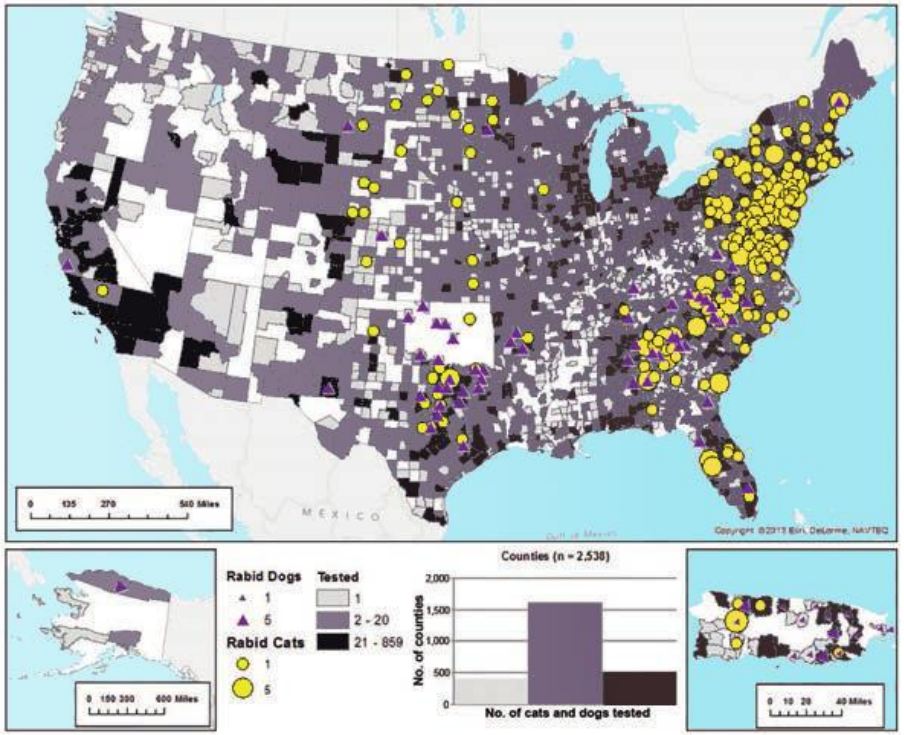
RABIES CASES IN RACCOONS AND DOMESTIC CARNIVORES IN 2012

Rabies cases in USA and Puerto Rico in 2012

Raccoon

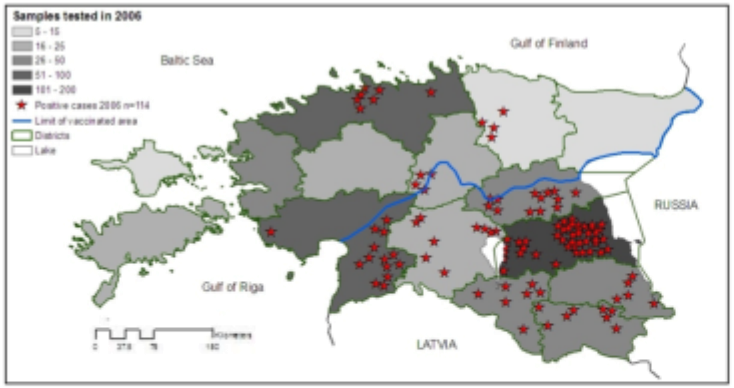


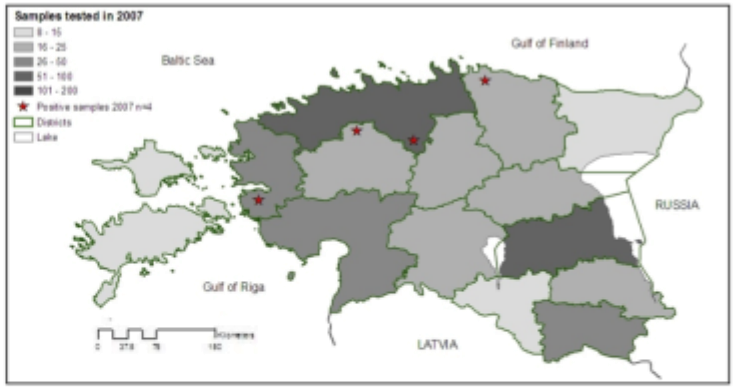
Dog and cat

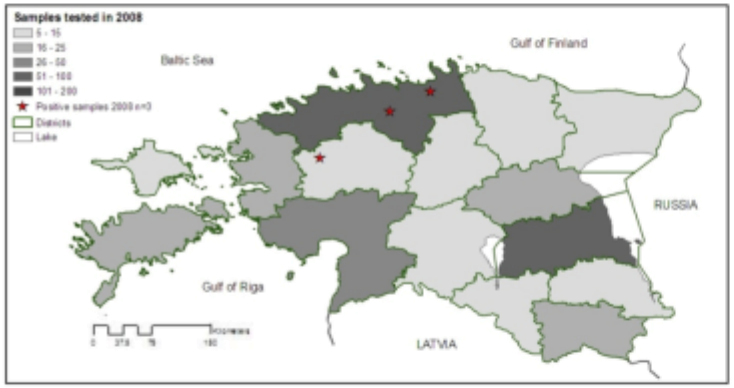


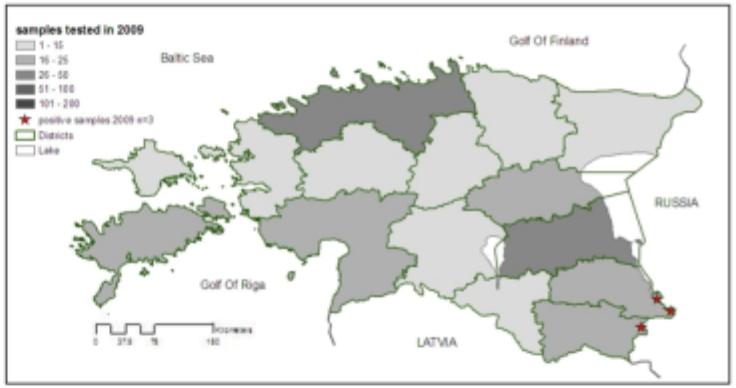
Source : Dyer *et al.*, 2013

PASSIVE SURVEILLANCE AND LOCATION OF RABIES CASES IN ESTONIA

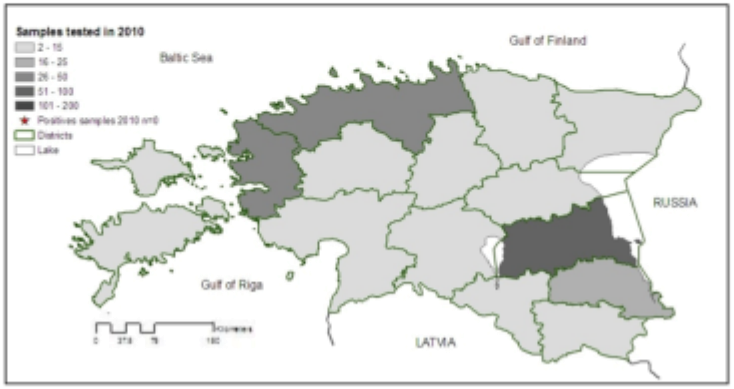








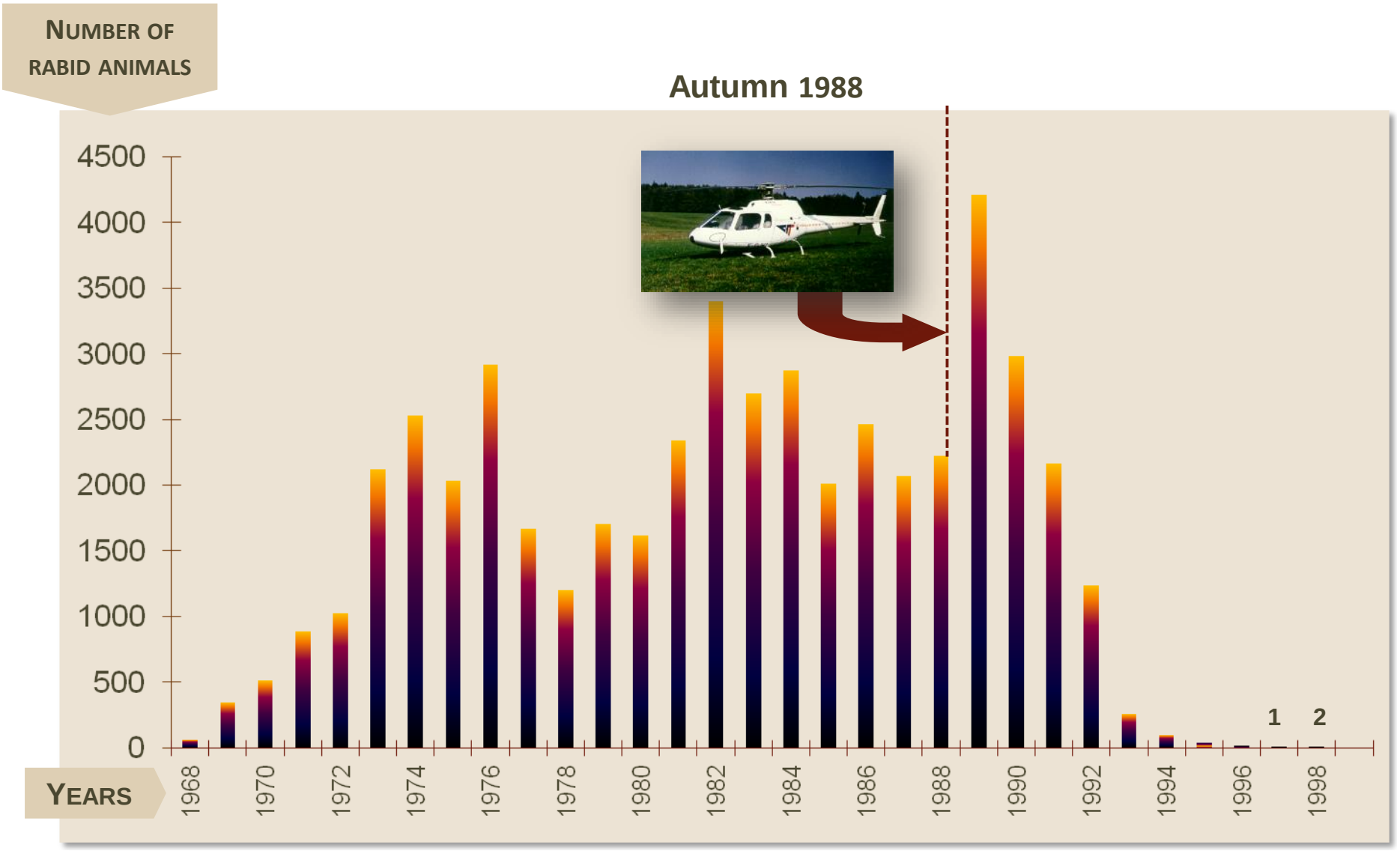
from 2006



to 2010

Ref : Cliquet *et al.*, 2012

RABIES EVOLUTION IN FRANCE FROM 1968 TO 1999



OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

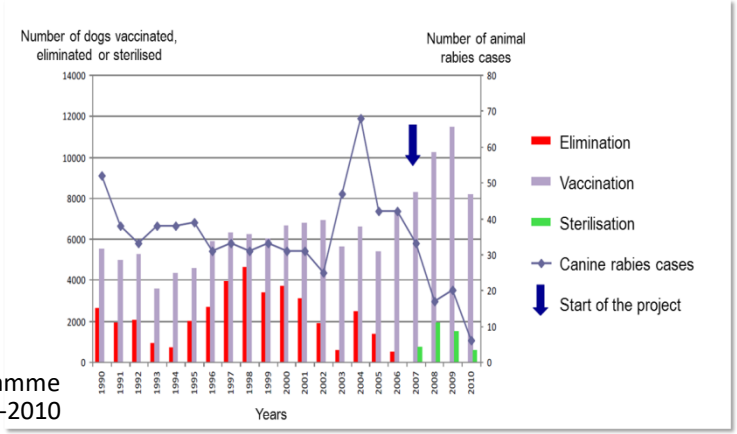
Evaluation of effectiveness control measures

- Efficacy of rabies vaccination both in humans and in animals

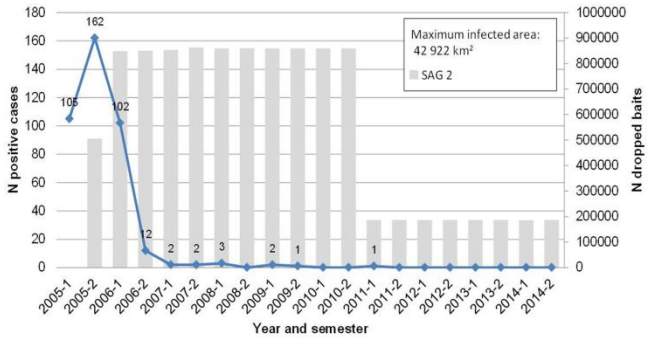
Evaluation of vaccination campaign in Mexico

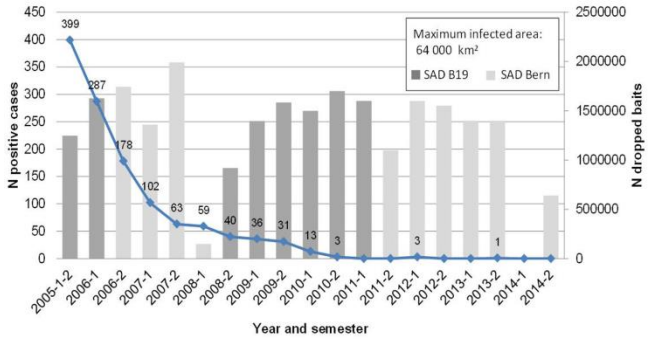
- Establishment or adaptation of the strategy based on surveillance data analysis

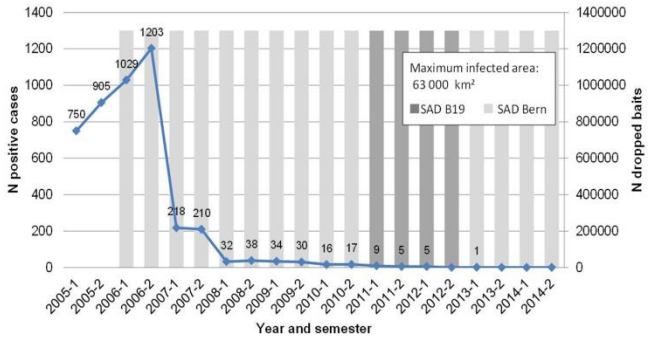
- Evaluation of other measures such as sterilisation of dogs, elimination of stray dogs



Evaluation of the rabies control programme in Sri-Lanka, Colombo, 1990-2010







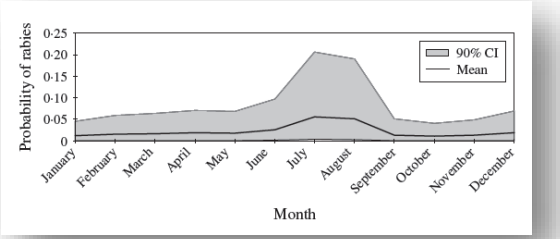
Ref : E. Robardet *et al.*, 2016

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

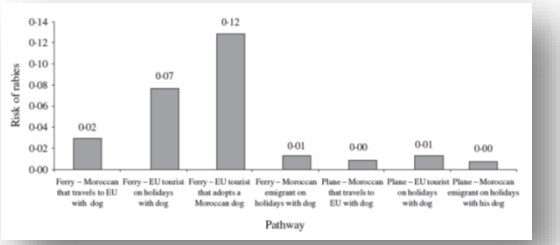
Ensure appropriate management of cases and outbreaks - Risk assessment

- Rabies-free countries: surveillance should be maintained.
  - Those that are still engaged in oral wildlife vaccination at their borders; surveillance data with negative results.
  - Effectiveness of rabies control and management (e.g. vaccination of pets, import policy, PEP).

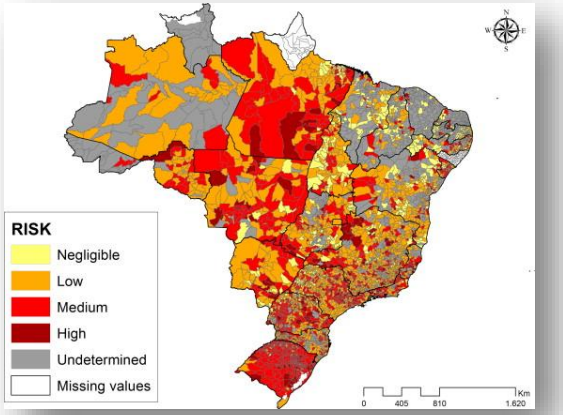
- Risk assessment: identifying the risks and predicting patterns of disease introduction and spread to define national strategies and aid policy decisions.
  - Mathematical models of disease transmission based on surveillance data
  - Identify the high-risk entry routes of canine rabies in a country/area to develop a surveillance strategy and incursion response plans.
  - Map rabies risk areas.



Monthly probability of rabies introduction into the EU through dogs coming from Morocco.



Relative contribution of the different pathways to the annual risk of rabies introduction into the EU through dogs coming from Morocco.



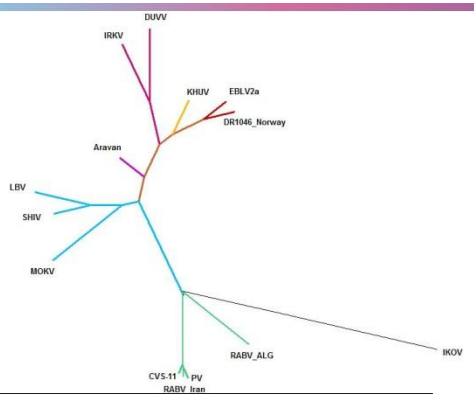
Qualitative risk levels of cattle rabies occurrence by Brazilian municipalities in 2010.

Ref : Napp *et al.*, 2010; Jones *et al.*, 2005; Hudson *et al.*, 2017; Braga *et al.*, 2014

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

Molecular biology tools

- Determination of the lyssavirus species virus and types of variants



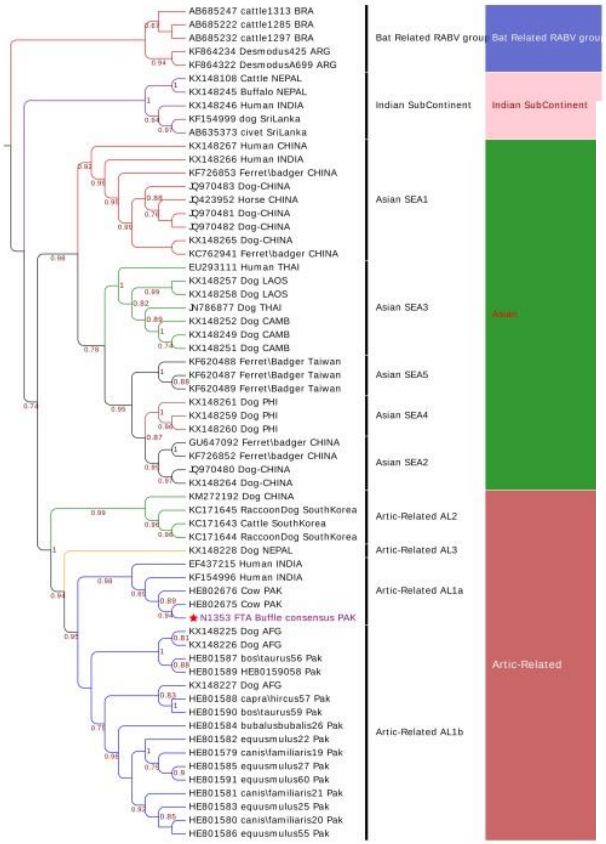


Table 1 | **The *Lyssavirus* genus**

Species	Virus	Countries of virus isolation*	Most common reservoir based on virus detection
Phylogroup I			
Rabies lyssavirus	Rabies virus (RABV)‡	Global	All mammals
Aravan lyssavirus	Aravan virus (ARAV)	Kyrgyzstan	Bats
Australian bat lyssavirus	Australian bat lyssavirus (ABLV)‡	Australia	Bats
Bokeloh bat lyssavirus	Bokeloh bat lyssavirus (BBLV)	France and Germany	Bats
Duvenhage lyssavirus	Duvenhage virus (DUVV)‡	Kenya and South Africa	Bats
European bat 1 lyssavirus	European bat lyssavirus 1 (EBLV-1)‡	Belgium, Denmark, France, Germany, the Netherlands, Poland, Russia, Slovakia, Spain and Ukraine	Bats
European bat 2 lyssavirus	European bat lyssavirus 2 (EBLV-2)‡	Denmark, Finland, France, Germany, the Netherlands, Norway, Switzerland and the United Kingdom	Bats
Gannoruwa bat lyssavirus	Gannoruwa bat lyssavirus (GBLV)	Sri Lanka	Bats
Irkut lyssavirus	Irkut virus (IRKV)‡	China and Russia	Bats
Khujand lyssavirus	Khujand virus (KHUV)	Tajikistan	Bats
Phylogroup II			
Lagos bat lyssavirus	Lagos bat virus (LBV)	Central African Republic, Ethiopia, France[§], Ghana, Nigeria, Senegal, South Africa and Zimbabwe	Bats
Mokola lyssavirus	Mokola virus (MOKV)‡	Cameroon, Central African Republic, Ethiopia, Nigeria, South Africa and Zimbabwe	Rodents and domestic animals
Shimoni bat lyssavirus	Shimoni bat virus (SHIBV)	Kenya	Bats
Phylogroup III			
Ikoma lyssavirus	Ikoma lyssavirus (IKOV)‖	Tanzania	African civet
Lleida bat lyssavirus	Lleida bat lyssavirus (LLEBV)‖	Spain	Bats
West Caucasian bat lyssavirus	West Caucasian bat virus (WCBV)	Russia	Bats

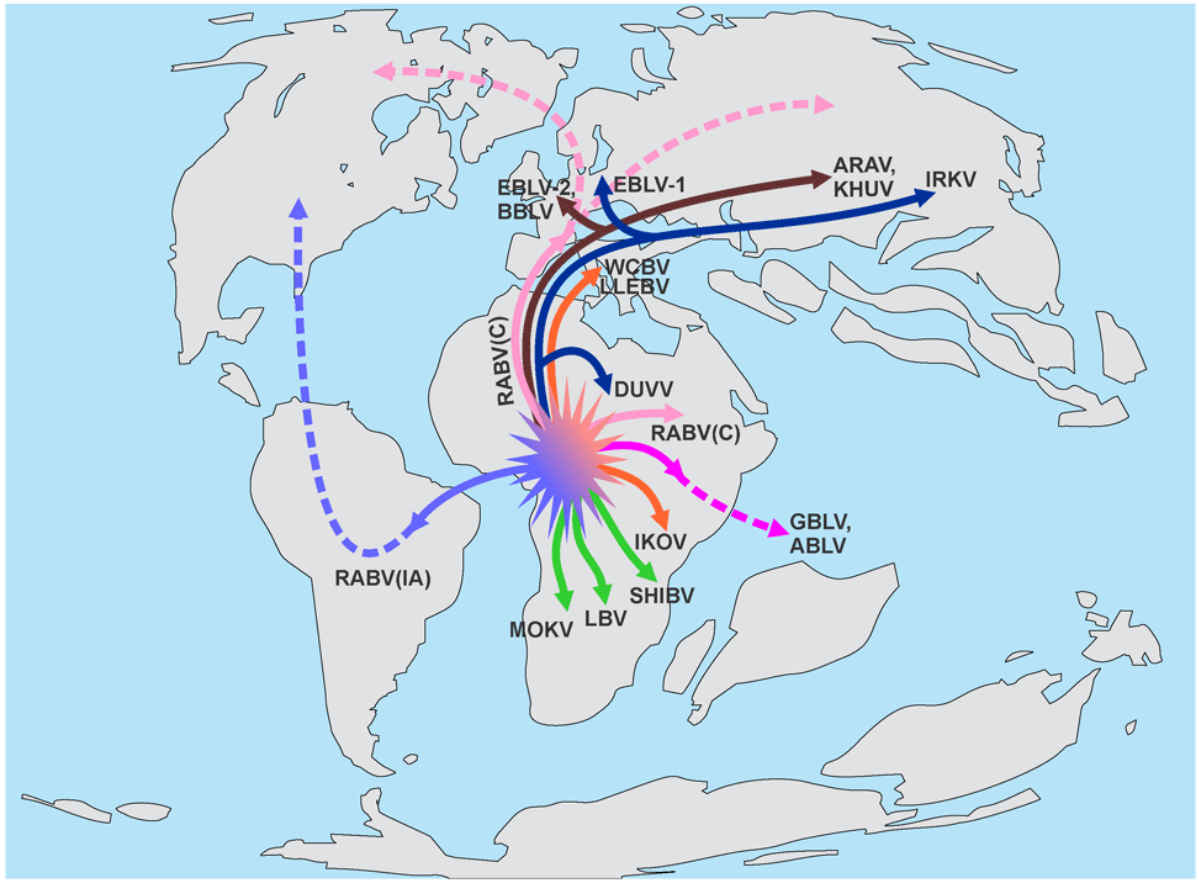
Based on the International Committee on Taxonomy of Viruses classification[194]. *Countries where the virus was isolated. ‡Associated with human fatality. §It is unknown whether the infected animals arrived in France from Egypt or Togo. ‖Could be single member of new phylogroup.

Ref : Anses Nancy; Fooks *et al.*, 2017

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

Molecular biology tools

- Phylogeographic evolution of rabies virus



Proposed lyssavirus radiations

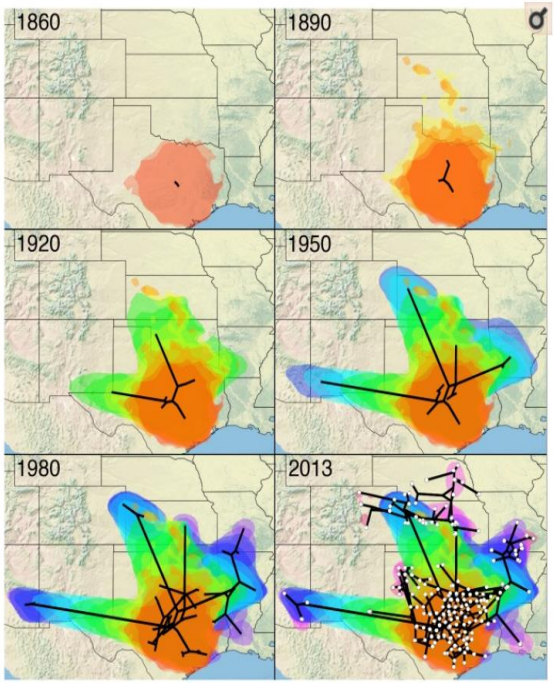
Ref : Bourhy *et al.*, 2008; Rupprecht *et al.*, 2017; Talbi *et al.*, 2010; Kuzmina *et al.*, 2013; Fooks *et al.*, 2014; Troupin *et al.*, 2017

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

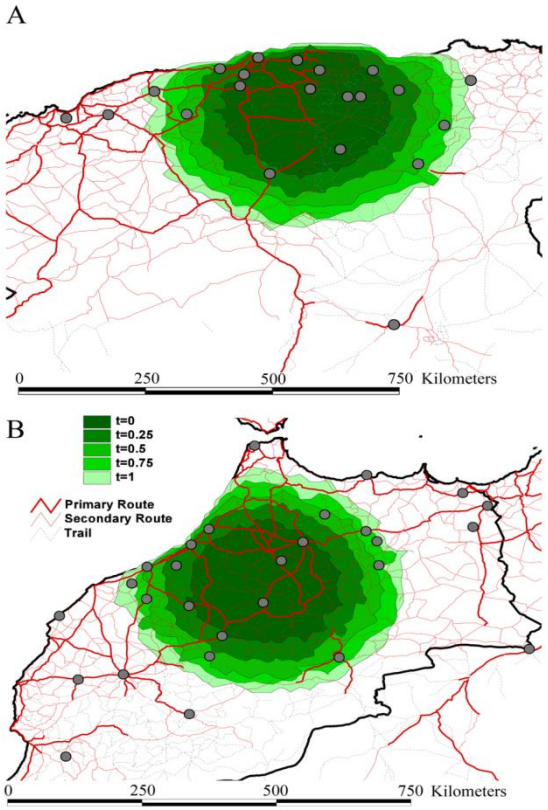
Molecular biology tools

- Spatiotemporal spread and dynamics of rabies virus

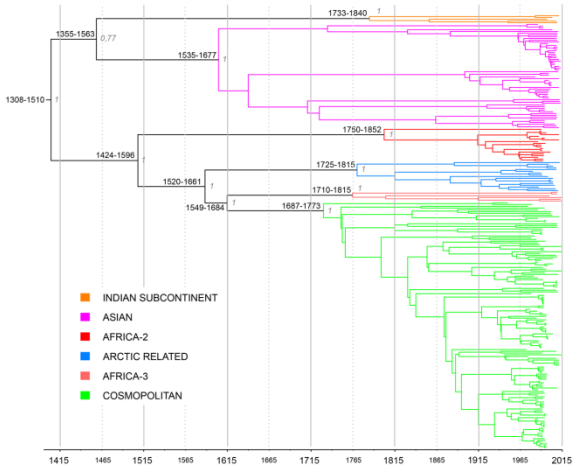
Spatiotemporal diffusion of skunk rabies virus in North America



Spatial simulation based on the inferred evolutionary histories and epidemiological parameters of rabies spread in Algeria (A) and Morocco (B).



Temporal dynamics and spread of the dog-related RABV

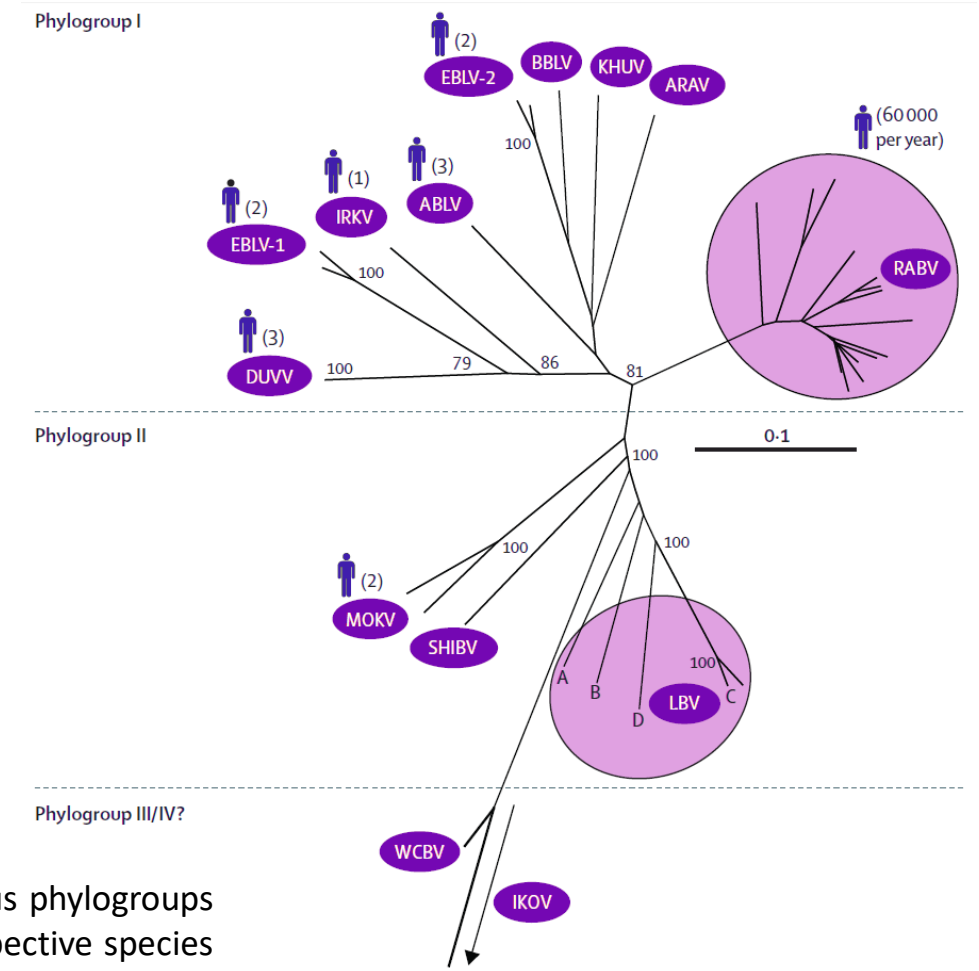


Source: Bourhy *et al.*, 2008; Rupprecht *et al.*, 2017; Talbi *et al.*, 2010; Kuzmina *et al.*, 2013; Fooks *et al.*, 2014; Troupin *et al.*, 2017

OBJECTIVES OF EPIDEMIOLOGICAL DATA ANALYSIS

Molecular biology tools

- Segregation of lyssaviruses into phylogroups for anticipating their pathogenicity, hence efficacy of current vaccines



Phylogenetic tree of the lyssavirus phylogroups and their respective species

Ref : Bourhy *et al.*, 2008; Rupprecht *et al.*, 2017; Talbi *et al.*, 2010; Kuzmina *et al.*, 2013; Fooks *et al.*, 2014; Troupin *et al.*, 2017

CONCLUSION

- 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.
- Different ways of presenting the data depending on analysis objectives.
- At least one person should be dedicated to regular analysis of surveillance data, with experts of the laboratory involved in the analysis.
- All data should be included in the analysis, including those from human cases, and including all positive and negative cases.
- These epidemiological data are used by the decision makers.

TANK YOU FOR YOUR ATTENTION!

Lyssavirus group