



LA RECHERCHE AGRONOMIQUE  
POUR LE DÉVELOPPEMENT

## Support from OIE PPR Reference Laboratory to countries

**Geneviève Libeau & Olivier Kwiatak**

*Regional training workshop on the OIE procedures  
for the official status recognition and endorsement  
of national official control programmes with regard  
to peste des petits ruminants (PPR) and foot and  
mouth disease (FMD), and maintenance of FMD  
official free status"*

*16 - 18 April, 2019, Almaty, Kazakhstan*

Introduction

Role of the laboratory

Current diagnostic tests

Laboratory networking

Conclusion



# Introduction

Control and prevention of peste des petits ruminants depends on:

- Capacity to sample, detect and confirm disease at the earliest possible time for the design of appropriate response measures
- Diagnostic measures in place at national level should ideally combine with networks to allow for a regional approach in PPR management.

# Role of the Laboratory

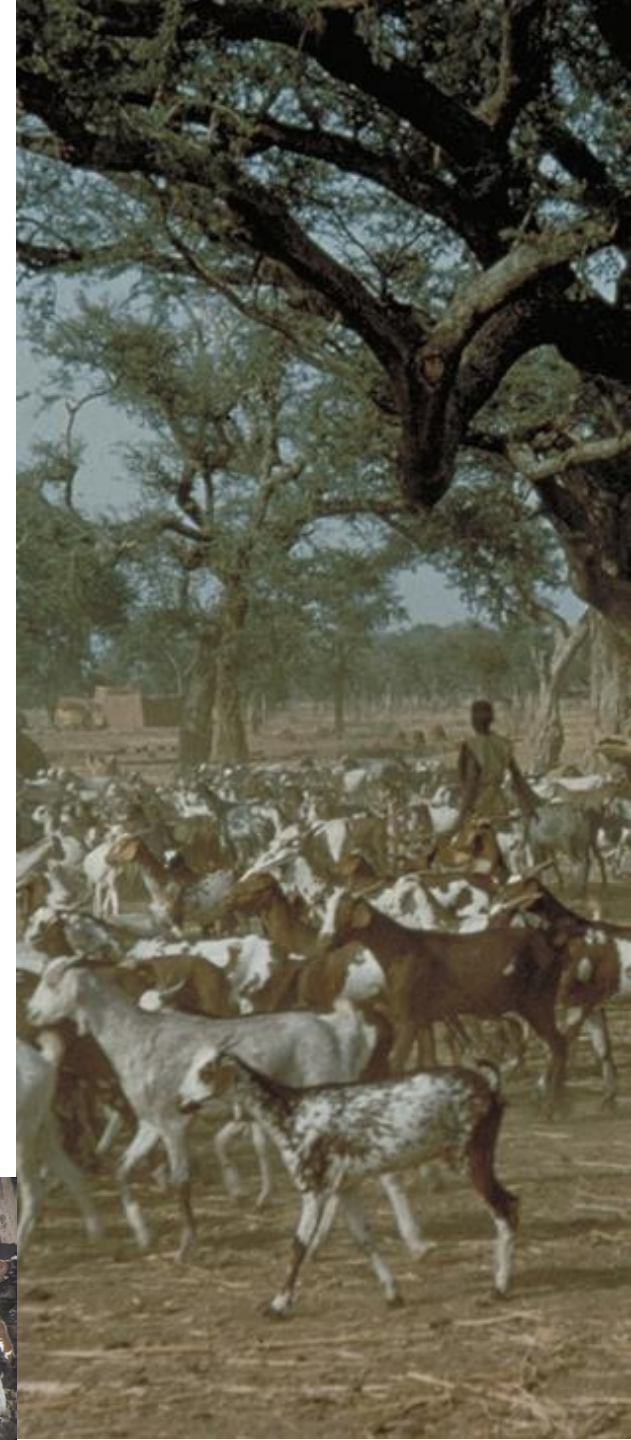
- Establish virological/serological diagnosis to complete observations of clinical symptoms;
- Implement quality diagnosis with standardised methods to deliver reliable PPR diagnosis results;
- Share information and expertise with veterinary services on:
  - Epidemiology of the disease,
  - Surveillance activities whether clinical, serological or virological
  - Sampling workplans, allowing to estimate disease circulation



# Laboratory confirmation of clinical cases of PPR is compulsory

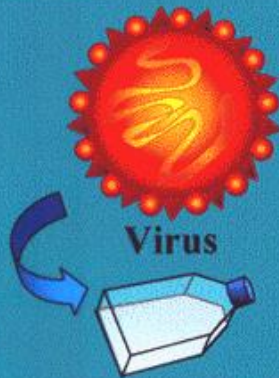
PPR can be easily confused with other diseases such as:

- Bluetongue, CCPP, Pasteurellosis,
- Definitive diagnosis of PPR is demonstrated when laboratory results are combined with clinical observations and epidemiological data.
- Essential that diagnosis whether for virology, serology, rely on validated, sensitive and specific tools

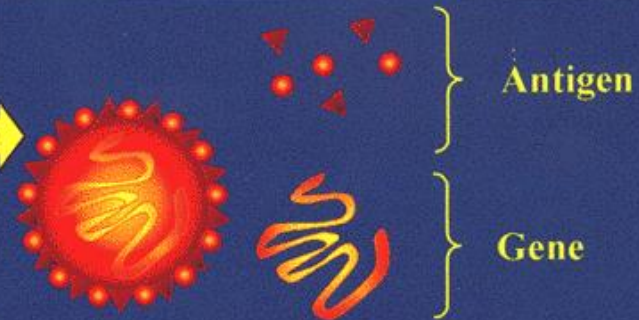


# LABORATORY DIAGNOSIS

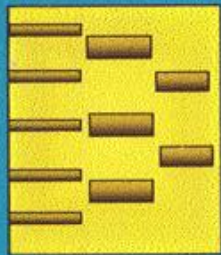
## ISOLATION



## DIRECT IDENTIFICATION



## CHARACTERIZATION



Field

## RETROSPECTIVE DIAGNOSIS



Antibody

CONVALESCENTS

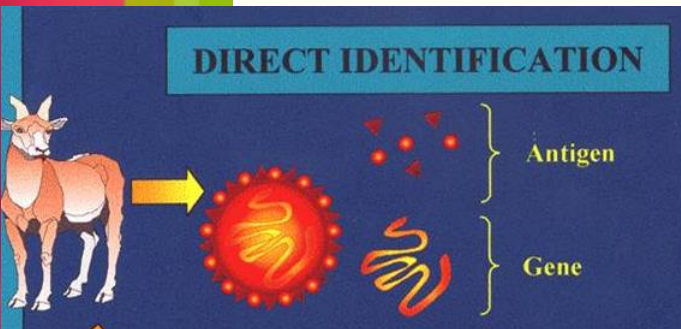
VACCINATED

SEROSURVEILLANCE

EFFICACY OF  
VACCINATION  
CAMPAIGN



# Current laboratory tests

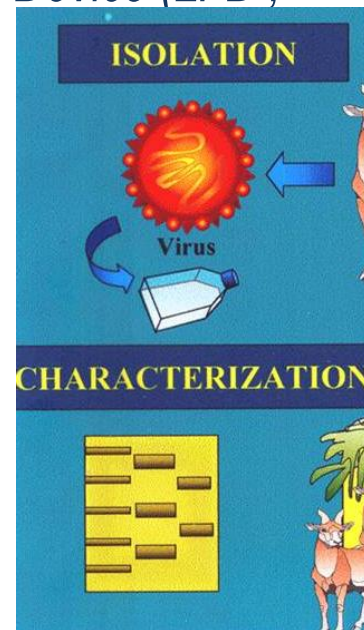


## ■ Virology tests : **ANTIGEN** and **GENE** detection

- ✓ Antigen Capture Elisa; Lateral Flow Device (LFD ; field test)
- ✓ Conventional RT-PCR
- ✓ Real-time RT-PCR
- ✓ LAMP PCR (field test)

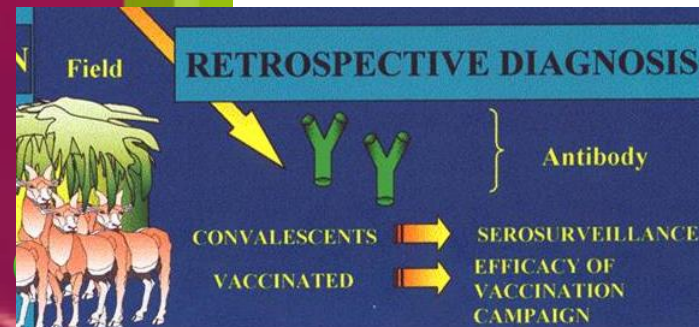
### **VIRUS**

- ✓ Isolation on Vero cells
- ✓ Isolation on Vero Slam cells
- ✓ Characterization by sequencing



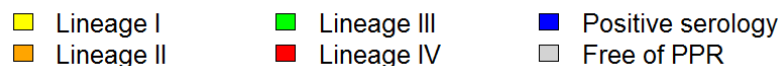
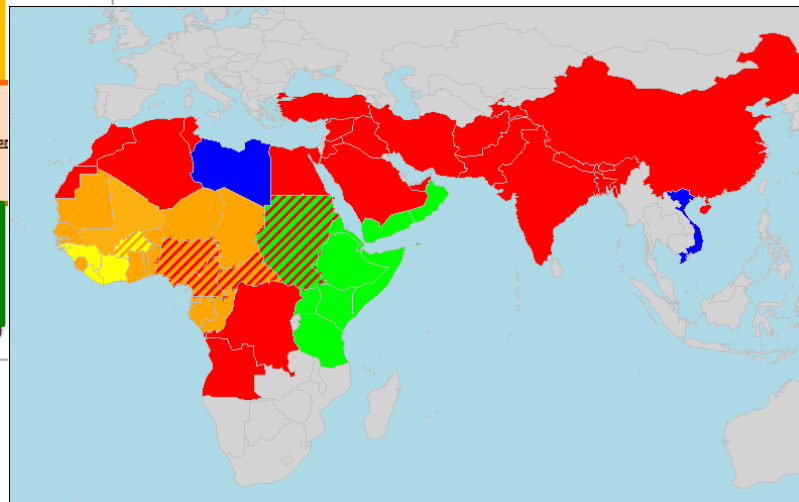
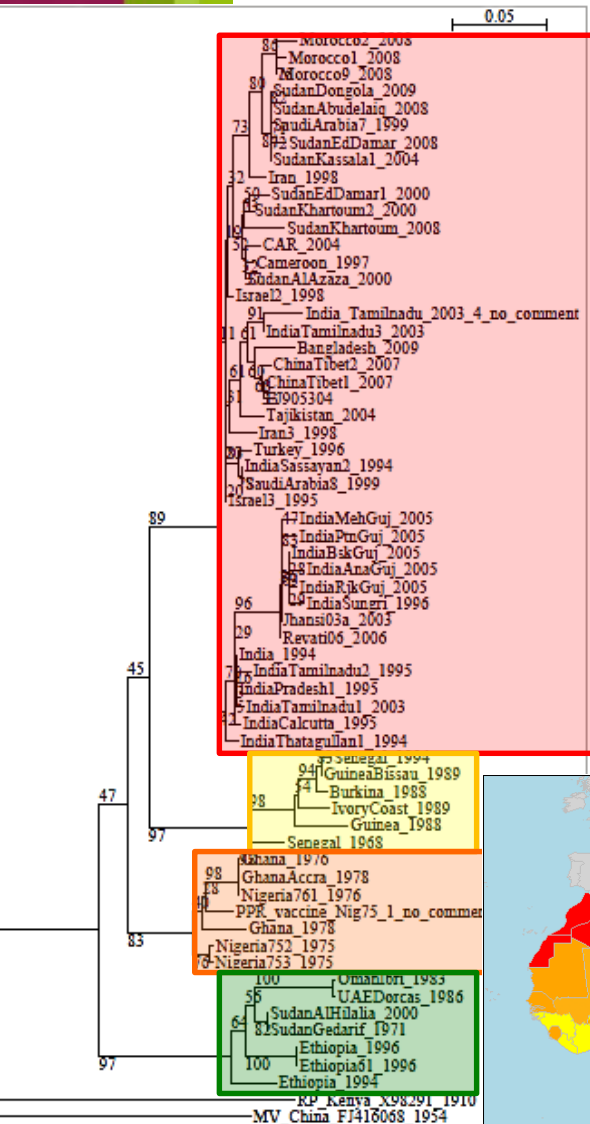
## ✓ Serology tests : **ANTIBODY** detection

- ✓ VNT (OIE prescribed test for international trade)
- ✓ c-Elisa,



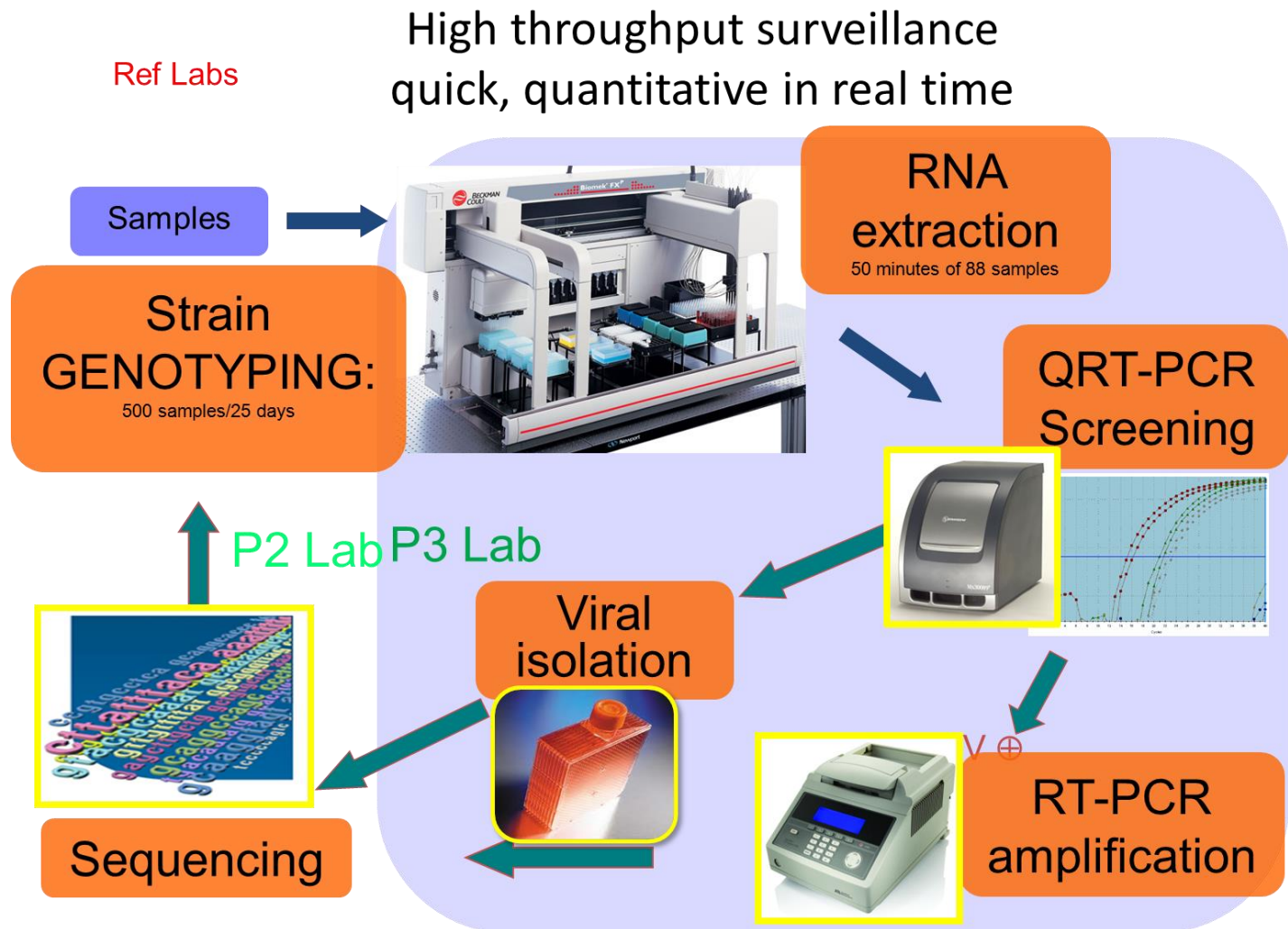
# Virology tests

- It is crucial to provide laboratories with efficient tools allowing the early detection of PPR emergence/re-emergence and to conclude on the origin of the virus.
- Conventional RT-PCR, now widely implemented in labs, allows direct sequencing and thus for the genotyping of strains.
- With recent technical breakthroughs on NGS, diversity of field strains is established, facilitating source tracking and understanding disease diffusion pathway.



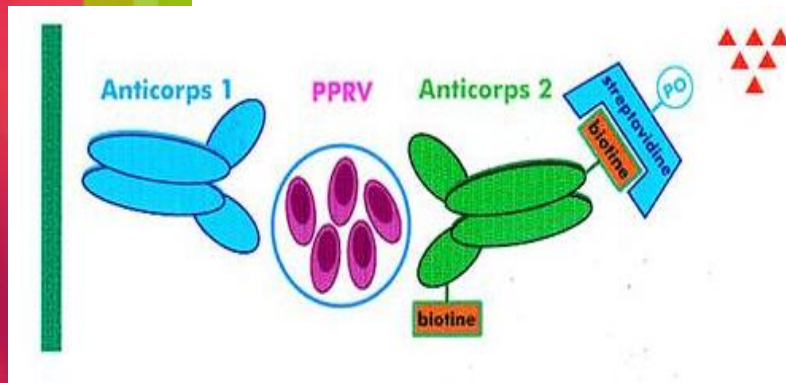


# Virology tests

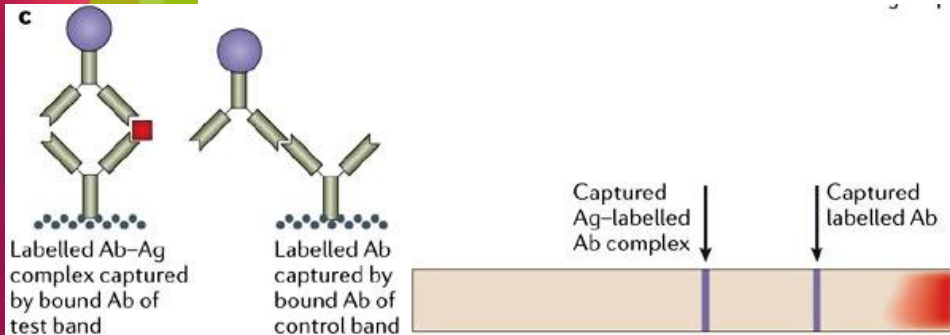


High capacity labs: different steps::Real time RT-PCR can be used as a screening tests and RT-PCR in association with viral isolation allows for strain genotyping.

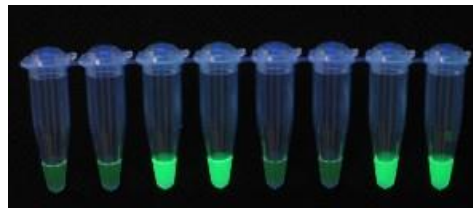
# Virology tests



## LFD pen-side tests



Bst: *Bacillus  
stearothermophilus*



- Oppositely, in many laboratories or resource limited, simple, rapid and robust assays can be adopted as routine techniques, able to detect viral:

- ✓ **Antigen:** such as Antigen Capture Elisa,

Sensitivity = RTPCR

- ✓ Pen-side tests: LFD

- ✓ **Gene:** LAMP-PCR: RT- loop-mediated isothermal amplification at 63° C: obtained 60min, observed by the naked eye

Sensitivity = Q-RTPCR  
= 10-fold higher than  
conventional RT-PCR

# Serology tests



ELISA (developed 30 years ago)

- These tests are able to promptly detect new outbreaks of PPRV and to produce data on the prevalence in infected areas.

- A set of ELISAs were developed.

- ✓ Competitive ELISA (C-ELISA) are H or N-Mab-based, high degree of correlation to the VNT, the gold standard assay.

Well adapted (96 wells format)

 cirad to large scale serology studies



# OIE manual: Purpose of the methods

Method	Purpose					
	Target	<u>Confirmation of clinical cases</u>	<u>Population freedom from infection</u>	<u>Individual freedom from infection</u>	Prevalence of infection – <u>Surveillance</u>	Immune status in individual animals – <u>Vaccination</u>
ICE- ELISA	Protein	+++				
RT-PCR	Gene	+++				
QRT-PCR	Gene	+++				
Virus isolation	Virus	++				
VNT	Antibody		+++	+++	+++	+++
C-ELISA	Antibody		++	++	+++	+++

Key:     +++ = recommended method;  
++ = suitable method;

**Source:** Last version Chapter 2.7.11. – Peste des petits ruminants

# Veterinary laboratory networking: Main activities of these networks. Examples of laboratory networks

Laboratories are encouraged to collaborate notably:

- Diagnostic techniques harmonization,
- Implementation of quality assurance,
- Link with epidemiological surveillance networks to increase the number of samples collected and analyzed by:
  - ✓ Sharing with the veterinary services sampling workplans
  - ✓ Improving sample logistics and cold chain
- Specific network on PPR is being built
- In the frame of the networks
  - ✓ Technical training in field pathology, diagnostic techniques
  - ✓ Support for pathogen sequencing, quality assurance and biosafety/biosecurity practices

**RESOLAB**  
SOUS RÉSEAU RAGE

**RESOLAB**  
SOUS RÉSEAU PPR

**RESOLAB**  
SOUS RÉSEAU FMD

**RESECOP**  
FILIERES ANIMALES

**RESEPI**  
EPIDEMIO-SURVEILLANCE

**RESOLAB**

**IDENTIFY**



# Veterinary laboratory networking: Reference Laboratories & Collaborating Centres

- The OIE Reference laboratories (3 at present in the world for PPR) plays an important role in assisting and supporting the training and diagnostic activities within regional networks, giving them opportunities to:
  - ✓ Participate to ring trials,
  - ✓ Involved in OIE twinning projects,
- Providing training relating to PPR, supplying reagents, scientific and technical knowledge.

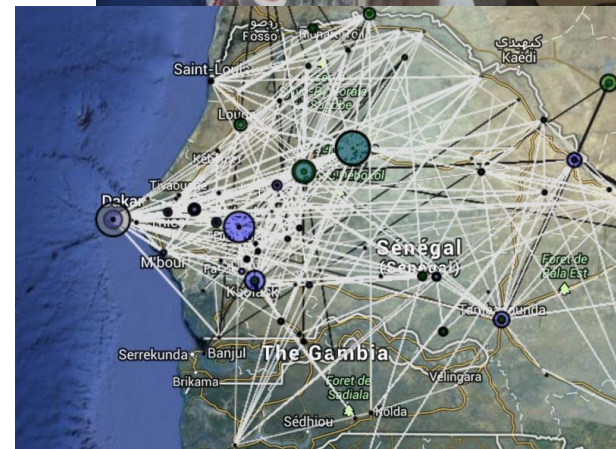


A major issue for the laboratory networks is the close involvement of international reference laboratories, Parent - candidate twinning consists in strengthened diagnostic / quality assurance (including metrology), in strengthened disease surveillance capabilities adapted to the specific epidemiological situation in the country.



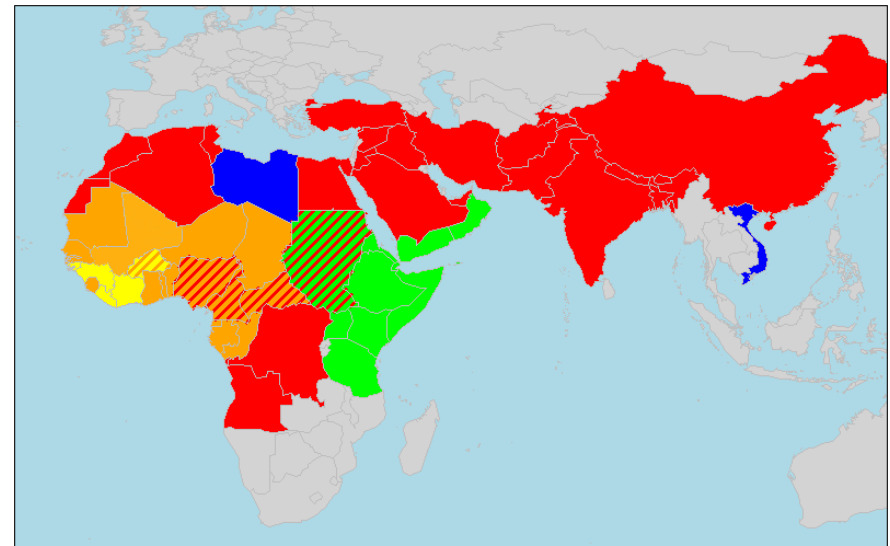
# Conclusion 1/2

- It is crucial that laboratories implement efficient diagnostics allowing the early detection of PPR.
- All these tests will allow to appreciate at national level:
  - ✓ the presence/spreading of the disease into new areas or to certify freedom from the disease.



## Conclusion 2/2

- It is important to integrate national/regional laboratories activities and epidemiological surveillance networks.
- Allow to :
  - ✓ Clarify the regional situation of peste des petits ruminants and understand PPRV diffusion pathway,
  - ✓ Map the health risk areas to improve the coordination of prevention and control measures.



# Team involved



**Arnaud Bataille**

PPR expertise, evolutionist,  
reverse genetics methodology



**Samia Guendouz**

PPR expertise, Lab techniques



**Renata Servan de Almeida**  
siRNA methodology, animal  
challenges

**Geneviève Libeau**  
PPR expertise, ELISA and  
vaccine development



**Olivier Kwiatek**  
PPR expertise, Lab techniques



**Tetiana Kwan Tat**  
Intellectual Property



**CIRAD (ASTRE, UMR117)**

Thank you

