Laboratory diagnosis and molecular epidemiology of PPR

Geneviève Libeau

Preparing SADC countries for the PPR progress towards PPR-Free areas (Disease identification, control and management)

10 to 12 June, 2013 Dar es Salaam, Republic of Tanzania

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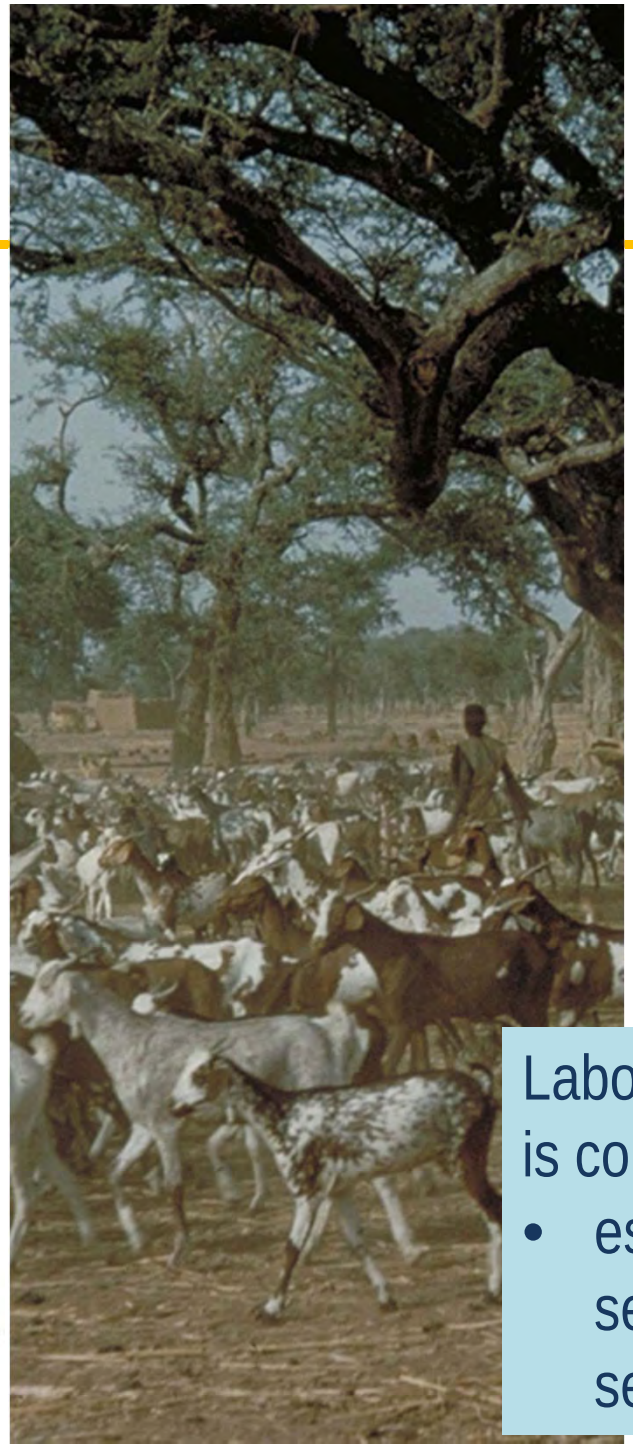

New diagnostic approach

Laboratory confirmation of clinical cases of PPR is compulsory:

- essential that diagnosis rely on validated, sensitive and specific tools either for virology, serology, molecular biology.

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Clinical differential diagnosis

PPR can be easily confused with other diseases such as:

- Bluetongue,
- Contagious caprine pleuropneumonia (CCPP) with similarity in respiratory signs,
- Pasteurellosis, also be a secondary complication of peste des petits ruminants.
- Rinderpest

Definitive diagnosis of PPR is demonstrated when laboratory diagnosis is made and combined with clinical observations and epidemiological data.

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Role of the Laboratory

- In establishing diagnosis to complete observations of clinical symptoms
- Implementing quality diagnosis with standardised methods to deliver reliable PPR diagnosis results
- Accompanying the implemetation of surveys for estimates of viral circulation:
  - ✓ Serosurvey
  - ✓ Virus sampling (update viral data).

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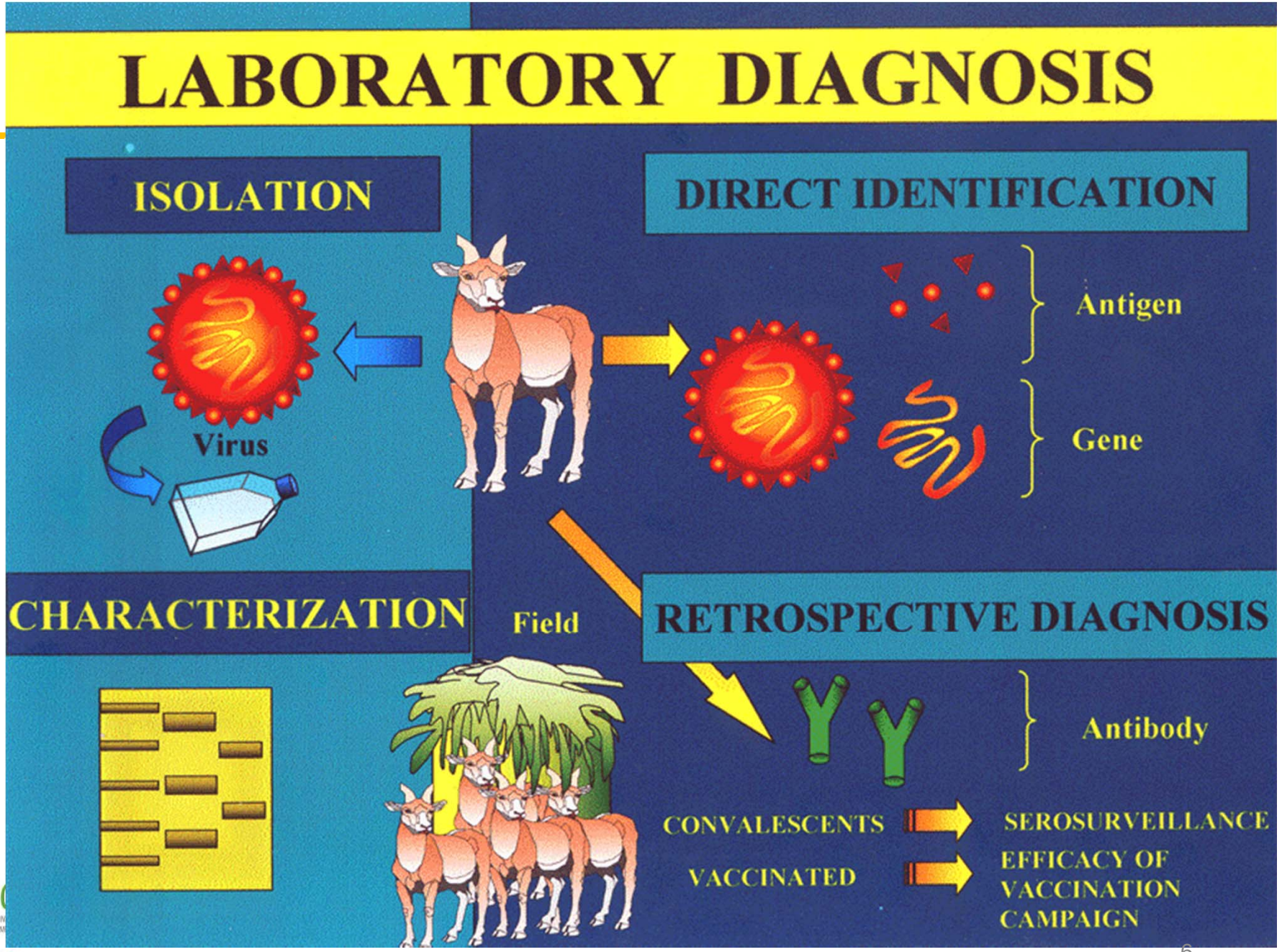
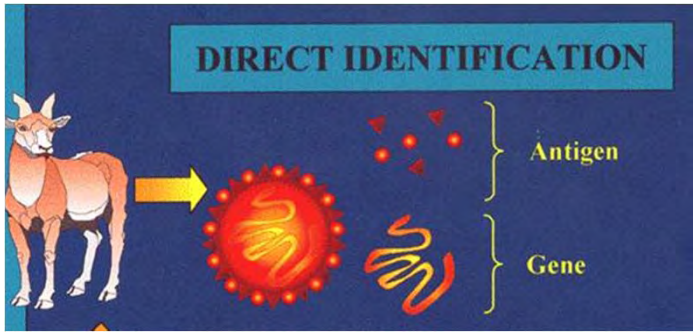
Other aims of the Laboratory:

- Plays also a role in the development of diagnostic tools
  - Improve sensitivity/specificity
  - Speed up the lab process
- Develop tests easily transposable to laboratories with low resources
- Innovative developments to ease field samples testing and sampling (non invasive)
- Organizing or participating to international proficiency testings. Make lab networks.


LABORATORY DIAGNOSIS
ISOLATION
DIRECT IDENTIFICATION
Virus
Antigen
Gene
CHARACTERIZATION
Field
RETROSPECTIVE DIAGNOSIS
Antibody
CONVALESCENTS
SEROSURVEILLANCE
VACCINATED
EFFICACY OF VACCINATION CAMPAIGN

Current laboratory tests

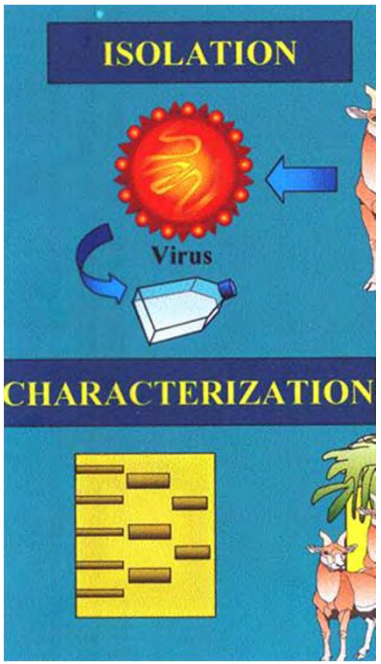
*Virology tests : **ANTIGEN and GENE detection***



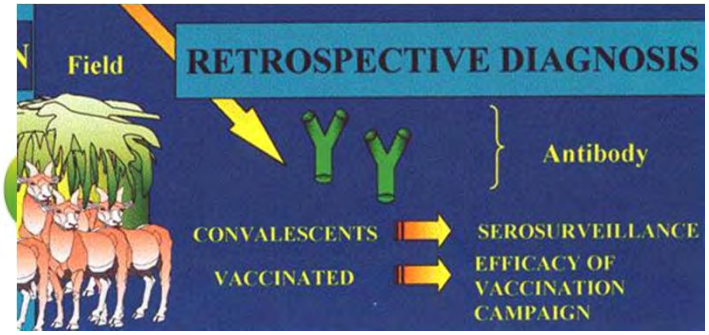
- Antigen Capture Elisa; *Lateral Flow Device (LFD ; field test)*
- *Conventional RT-PCR*
- *Real-time RT-PCR*
- *LAMP PCR (field test)*

VIRUS

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



*Serology tests : **ANTIBODY detection***

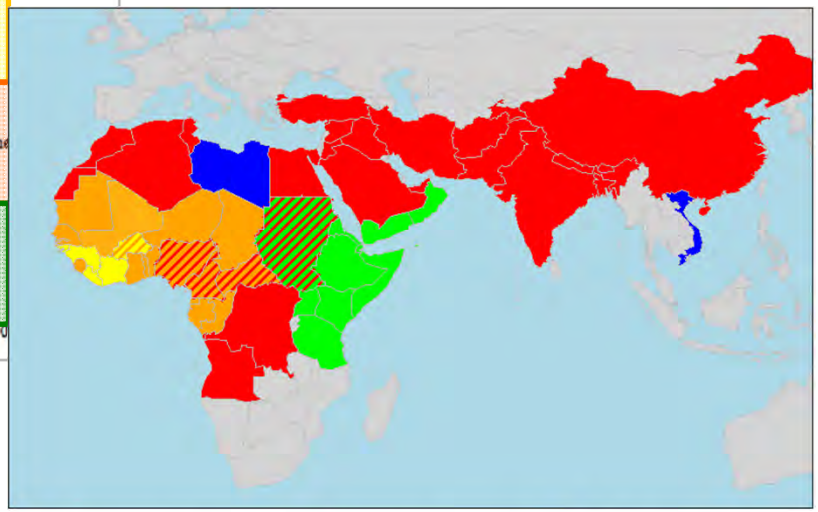


- *VNT (OIE prescribed test for international trade)*
- *c-Elisa,*
- *IFI*

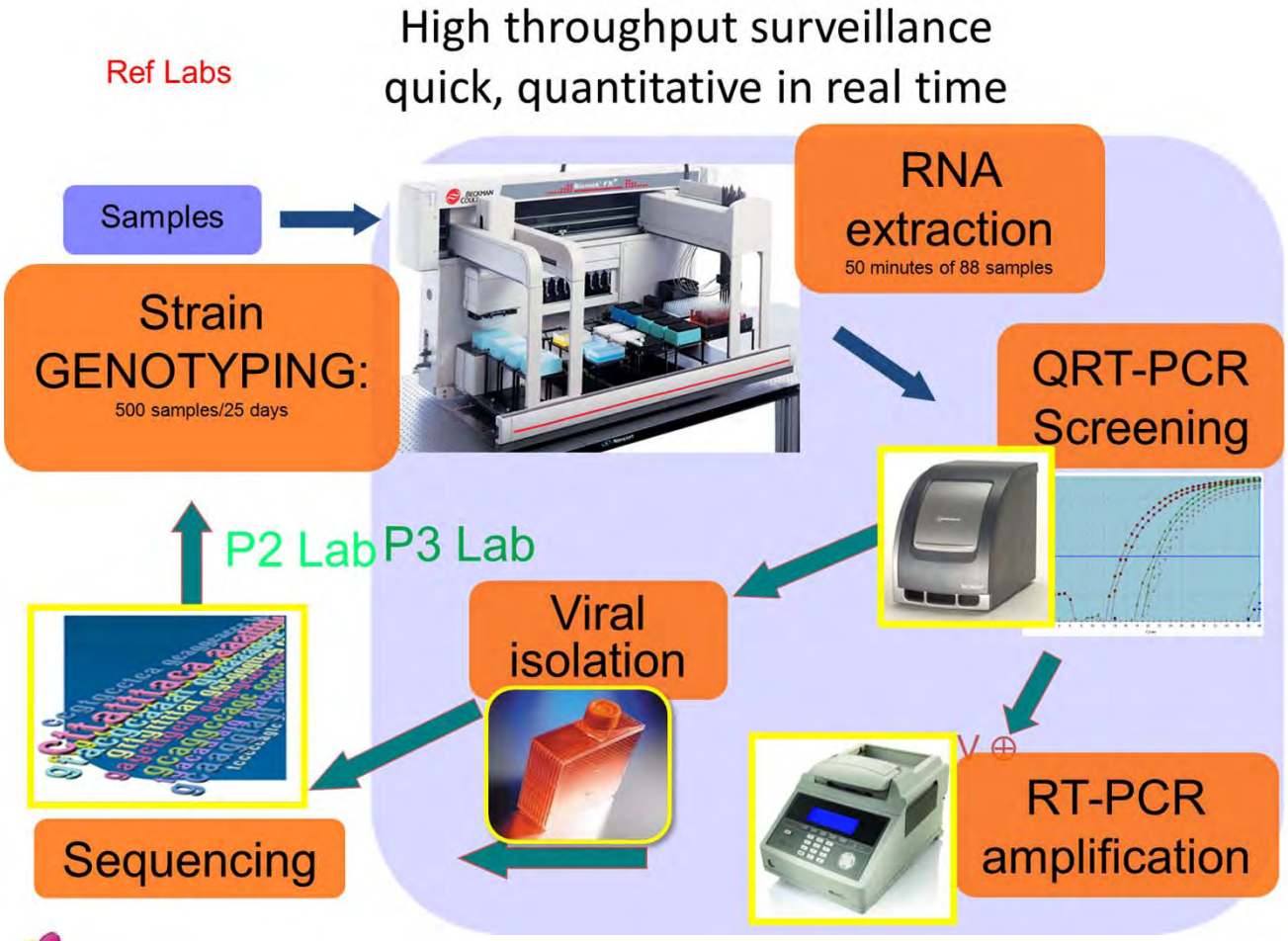
Virology tests

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 the genotyping of strains.



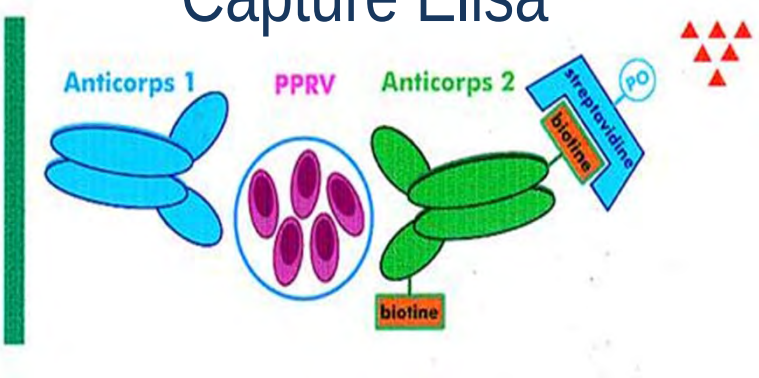
Virology tests



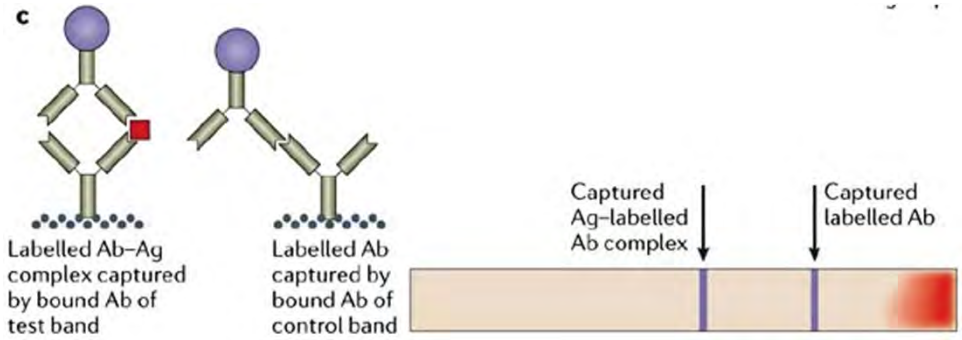
- In high capacity labs, diagnosis can be realised in real time through the use of robots. In the different steps: Real time RT-PCR is used as a screening tests and RT-PCR in association with viral isolation allows for strain genotyping.

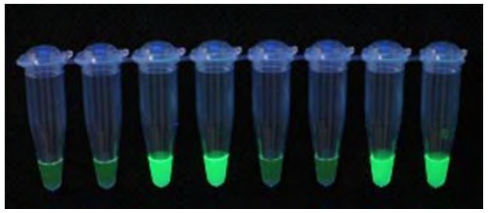
Virology tests

Capture Elisa



LFD pen-side tests





- Oppositely, simple, rapid, robust and environment friendly diagnostic assays can be adopted as routine techniques in many laboratories, able to identify the virus:
  - Antigen: such as Antigen Capture Elisa,
  - Pen-side tests: LFD
  - Gene:RT-LAMP: RT-loop-mediated isothermal amplification at **63°C**: obtained **60min**, observed by the naked eye

Antigen detection

- Antigen Capture Elisa

✓ Used on live animals or for post-mortem diagnosis

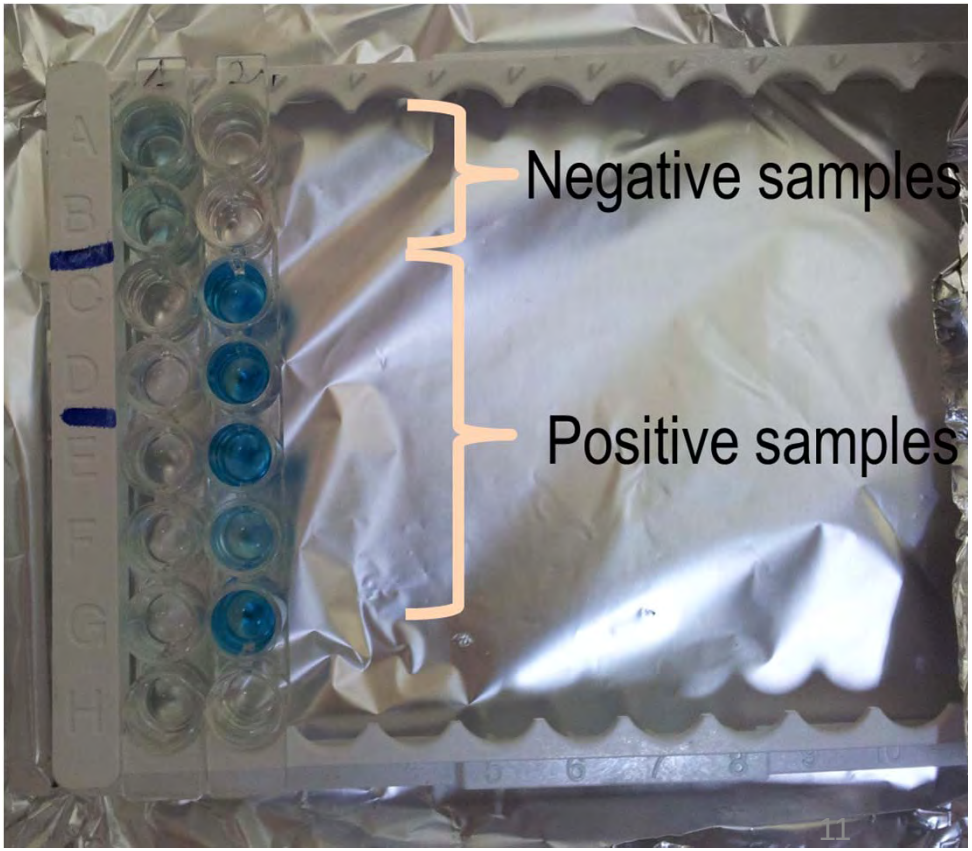
✓ Used on oral, nasal, tears swabs, tissue samples,

✓ Validated, also on rectal swabs or feces (Non invasive sampling method),

✓ Similar sensitivity to RT-PCR

✓ High specificity

✓ Takes 1h 30



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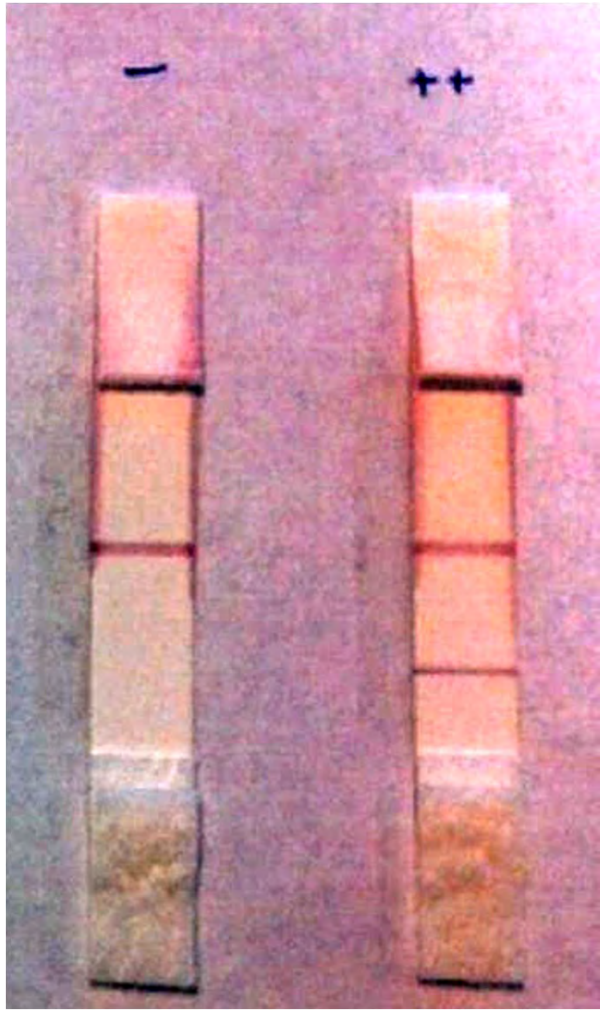
Antigen detection

- Lateral Flow Device (LFD)
- ✓ Pen-side tests
- ✓ Used on live animals
- ✓ Used on oral nasal, tears,
- ✓ Based on technology used in pregnancy test kits
- ✓ Sensitivity < Antigen Capture ELISA
- ✓ Result within minutes



Assay on PPR Prototype

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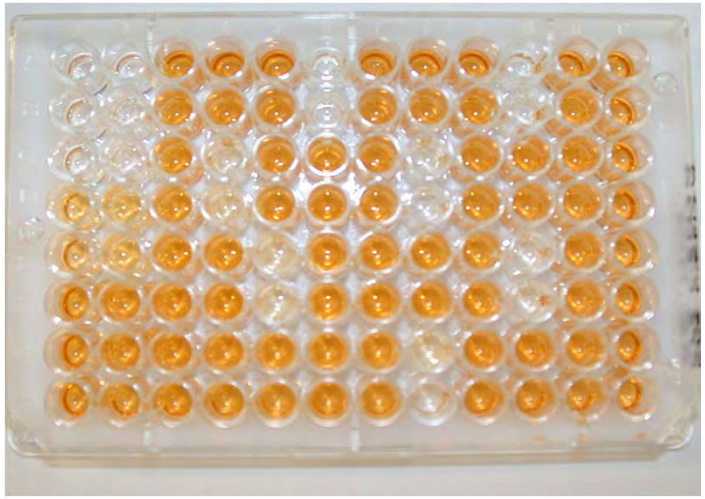
Serology tests

ELISA



ELISA (developed 30 years ago)

- These tests are able to promptly detect new outbreaks of PPRV and to produce data on the incidence and 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 well format) to large scale studies

Peste des petits ruminants

IDVET innovative diagnostics

Ruminants

ID Screen® PPR Competition

Competitive ELISA for the detection of anti-PPRV antibodies in sheep and goat serum and plasma

Peste des petits ruminants (PPR) is a contagious disease affecting goats and sheep primarily in Africa (from Tropic of Cancer to Equator), the Middle-East and the Indian subcontinent. It is caused by a species of the *Morbillivirus* genus of viruses. The disease is highly contagious, with approximately 80 percent mortality in acute cases.

In June 2008, the disease invaded Morocco crossing natural barrier of Sahara and causing concern that the disease could spread into Europe.

Serology may be used to identify and control outbreaks. The ID Screen® PPR Competition ELISA efficiently detects antibodies directed against the virus nucleoprotein.

All components are ready-to-use and each sample is deposited only once.

The test uses technology developed by a FAO reference laboratory (CIRAD-EMVT, Montpellier, France).

Product Code	PPRC-4P; Please contact IDVET for other formats
Test Principle	Competitive ELISA
Antigen	PPR recombinant nucleoprotein
Conjugate	Anti-NP-HRP concentrated conjugate (10)
Sample Type	Sheep and goat serum and plasma
Sample dilution factor	1:2
Number of tests	384 (4 plates)
Microplate format	12 x 8-well strips
Protocol	1. Sample Incubation 45 min 2. Conjugate Incubation 30 min 3. Three washes 4. Substrate Incubation 15 min
Test Interpretation	S/N < 50% = positive 50 % < S/N ≤ 60% = doubtful S/N > 60% = negative

Reference

Development of a competitive ELISA for detecting antibodies to the Peste des Petits Ruminants virus using a recombinant nucleoprotein. Libeau G, Préhaud C, Lancelot R, Colas F, Guerre L, Bishop DH, Diallo A., Res Vet Sci. 1995 Jan;58(1):50-5.

Screening format: each sample is deposited only once

Ready-to-use components, including coated plates

Simple and easy-to-use: results in 90 minutes

FAO reference lab technique

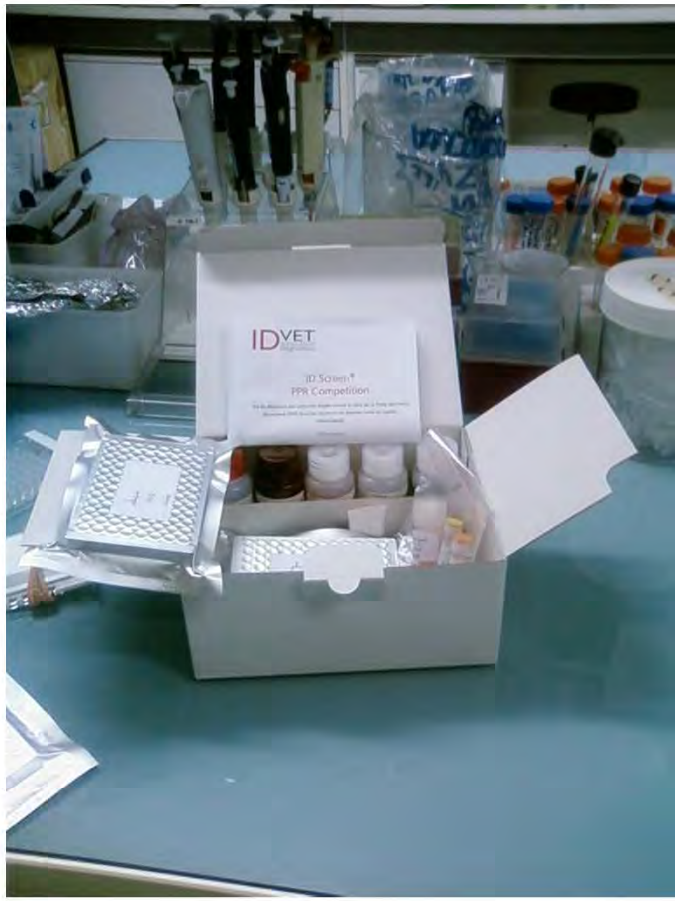
High specificity and sensitivity

AFAQ ISO 9001

Kit Contents

Coated microplates
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffers
Wash Concentrate (20X)
Substrate Solution (TMB)
Stop Solution

Facelift of supplier



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OIE manual: Purpose of the methods

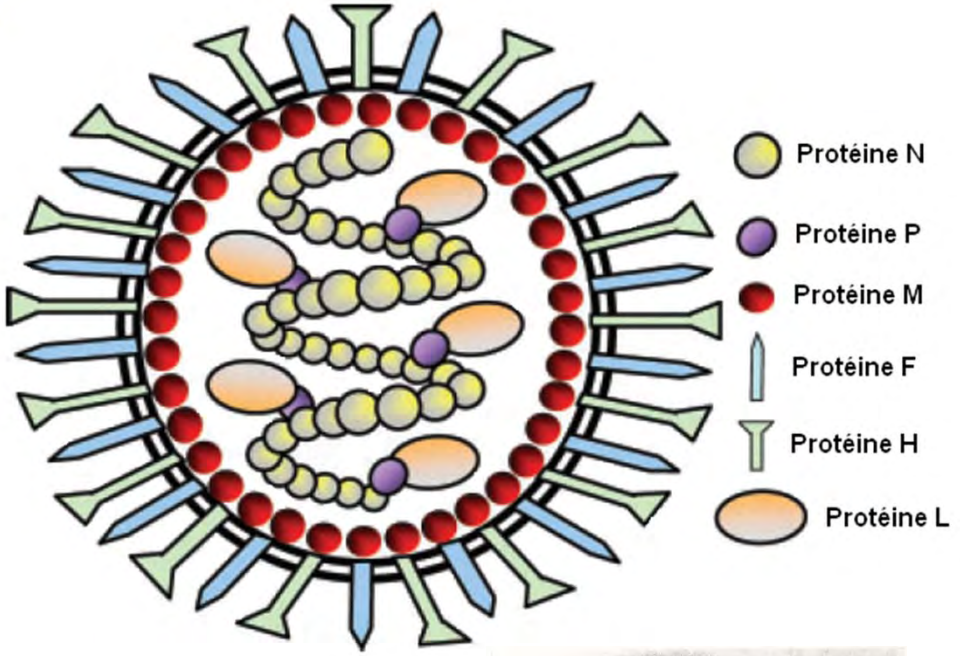
Method	Purpose						
	Target	Confirmation of clinical cases	Population freedom from infection	Individual freedom from infection	Prevalence of infection – Surveillance	Immune status in individual animals - Vaccination	
ICE- ELISA	Protein	+++					
RT-PCR	Gene	+++					
QRT-PCR	Gene	+++					
Virus isolation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

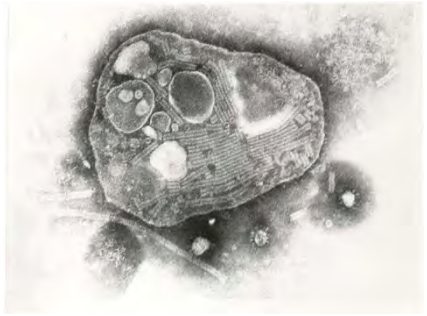
Key: +++ = recommended method;
++ = suitable method;

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

Molecular epidemiology of PPRV

- PPRV characterised by a high plasticity of its genome (ss-RNA)
- Evolution potential results in different genotypes
- Sufficient to study or anticipate the genetic diversity during the diffusion pathway





Morbillivirus

Genome structure (single stranded– RNA)

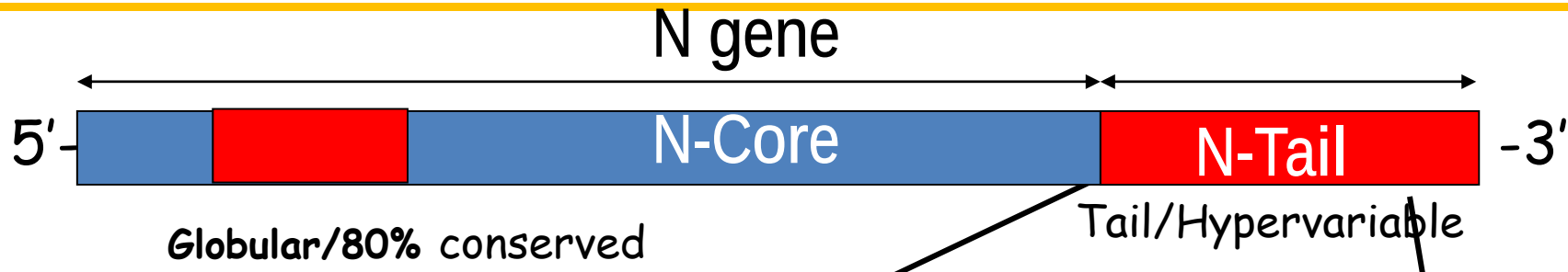
Structure du génome (ARN- simple brin) d'un Avulavirus/Morbillivirus

Molecular epidemiology of PPRV

- PPRV distribution & dynamic associated with the animal movements;
- TADs: Historical and recent events proved it can emerge in new areas: Maghreb from 2008
- PPR disease of small ruminants, “emerged” in camels;

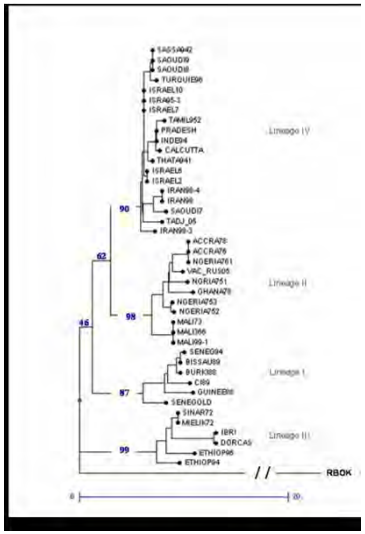
A particular focus will be made on the role of camels in the epidemiology of this diseases

Molecular epidemiology of PPRV



(Couacy-Hymann et al., 2002)

117 amino acids (351 nucleotides)



Lineage/genotypes	Strains	Sequence (421-525)
I	BISSAU89	PLLEIMPEDEASRESGQ TSREAQRSAEAL FRLQAMA
	BURKI88	PLLEIMPEDEASRESGQ TSREAQRSAEAL FRLQAMA
	CI89	PLLEIMPEDEASRESGQ TPREAQRSAEAL FRLQAMA
	SENEG94	PLLEIMPEDGASRESGQ TSREAQRSAEAL FRLQAMA
	SENEGOLD	PLLEIMPEDEVSRESGQ TPREAQRSAEAL LRLQAMA
	GHANA78	LLLEIMPEDEVSRESSQ NPREAQRSAEAL FRLQAMA
	GUINEE88	PLLEIMPEDEASGESGQ TPREAQRSAEAL FRLQAMA
	ACCRA76	LLLEIMPEDEVSRESSQ NPREAQRSAEAL FRLQAMA
	MALI	LLLEIMPEDEVSRESGQ NPREAQRSAEAL FRLQAMA
II	NGRIA75/1	LLPEIMQEDELSRESSQ NPREAQRSAEAL FRLQAMA
	NGRIA75/2	LLLEIMPEDEVSRESSQ NPREAQRSAEAL FRLQAMA
	NGRIA75/3	LLLEIMPEDEVSRESSQ NPREAQRSAEAL FRLQAMA
III	ETHIOP94	LLLEIMPEDEVPRGSGQ NPREAQRSAEAL FRLQAMA
	ETHIOP96	LLLEIMPEDEVPRGSGQ NPREAQRSAEAL FRLQAMA
	IBRI	LLLEIMPEDEVPRGPGQ TPREAQRSAEAL FRLQAMA
	SINAR72	LLLEIMPEDEVPRGSGQ NPREAQRSAEAL FRLQAMA
IV	SAOUD7	LLLEIMPEDEVSRESGQ NPREAQRSAEAL FRLQAMA
	SAOUD9	LLLEIMPEDEVSRESGQ NPREAQRSAEAL FRLQAMA
	TAMIL95/2	LLLEIMPEDEVSRESGQ NPREAQRSAEAL FRLQAMA

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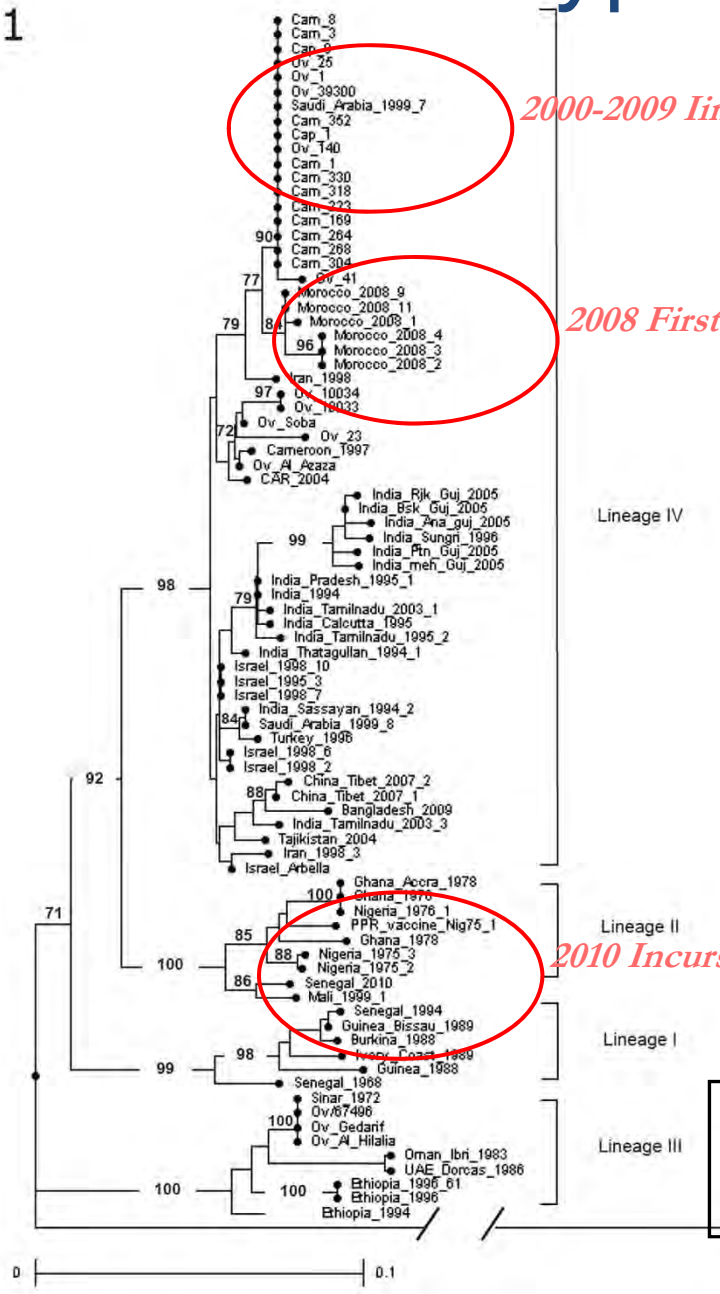
PPRV Genotyping

Fig. 1



2000-2009 lineage IV in Sudan

2008 First Incursion in Morocco

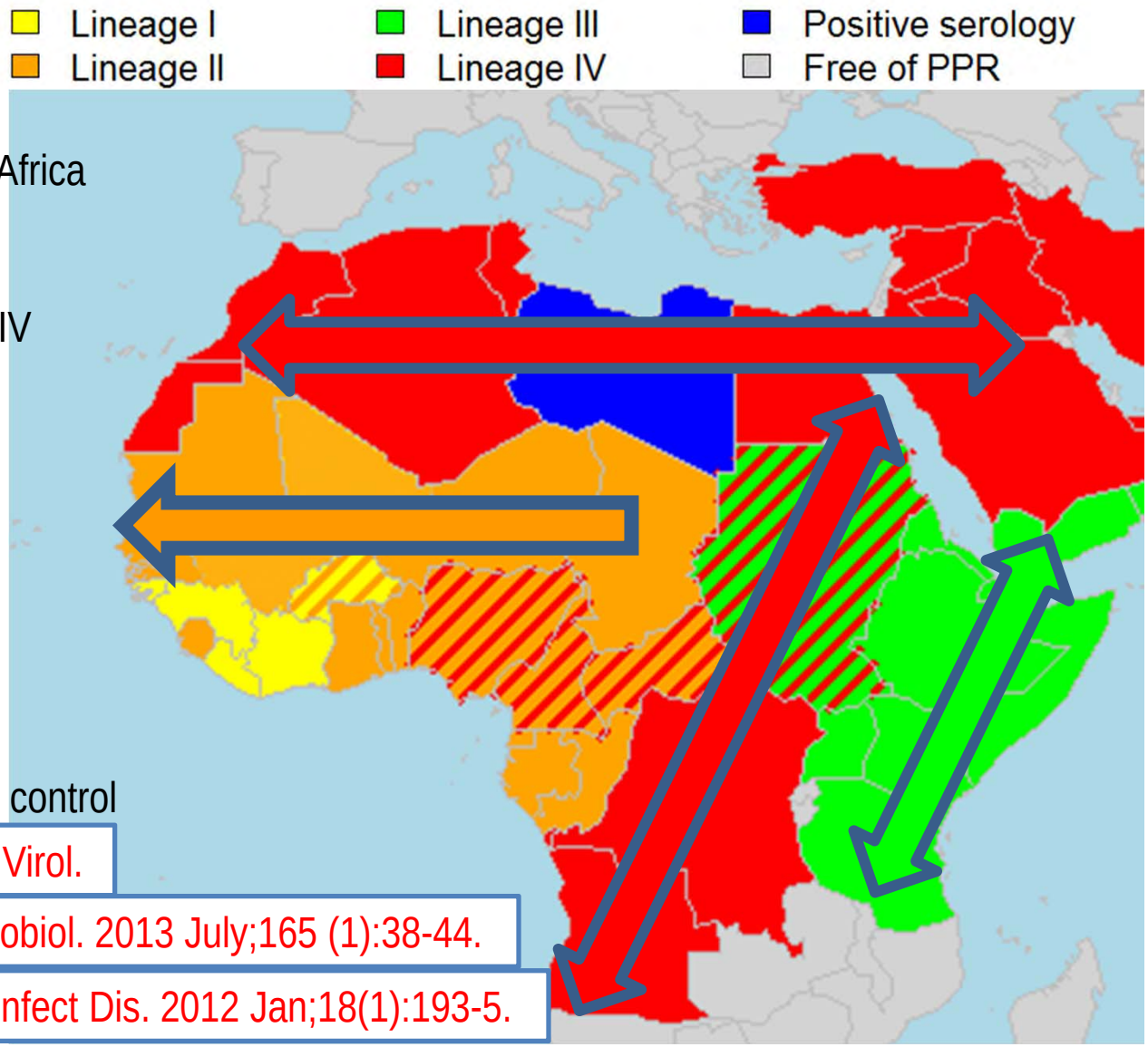
2010 Incursion of lineage II in Senegal

***Kwiatek et al**. J Comp Pathol, 2009*
***Ashley et al.** GenVirol 2010*
***Kwiatek et al**. EID, 2011*

Lineages spread at the regional scale [**]

- Extinction of lineage I, [*]
- Emergence of lineage II historically prevalent in Central Africa recently confirmed in Sierra Leone [***]).
- Extinction of lineage III, benefit IV
- Diffusion hypothesis:

  East - West
  North Africa
  Sub-Saharienne Africa
  North– South
  East Africa
  Southern Africa
- Control strategy PPR must integrate this reality for an effective control of the disease



[*] Banyard J Gen Virol.
[**] Albina Vet Microbiol. 2013 July;165 (1):38-44.
[***] Munir Emerg Infect Dis. 2012 Jan;18(1):193-5.

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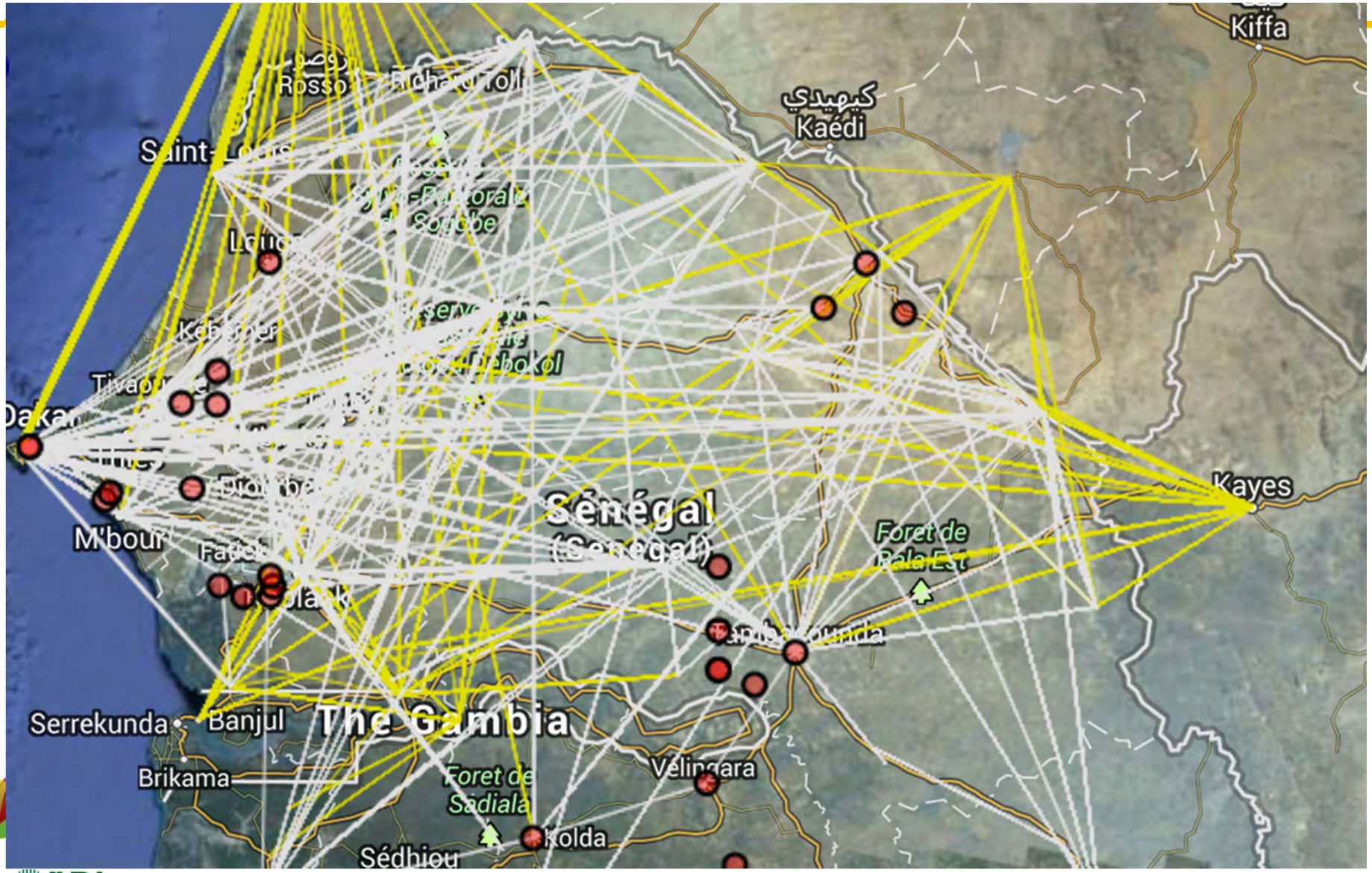
Local scale: Animal Mobility
Senegal 2012/2013



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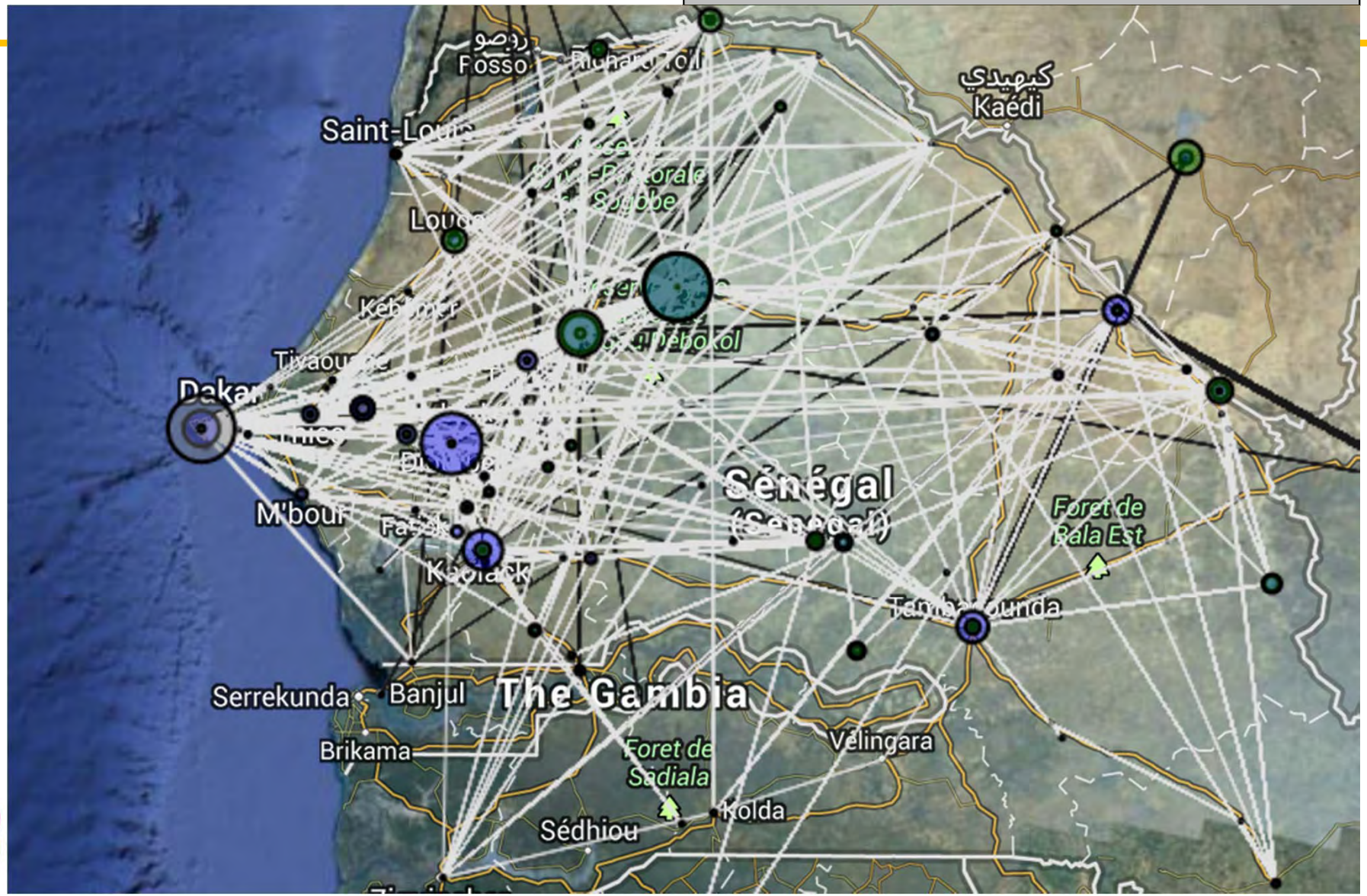
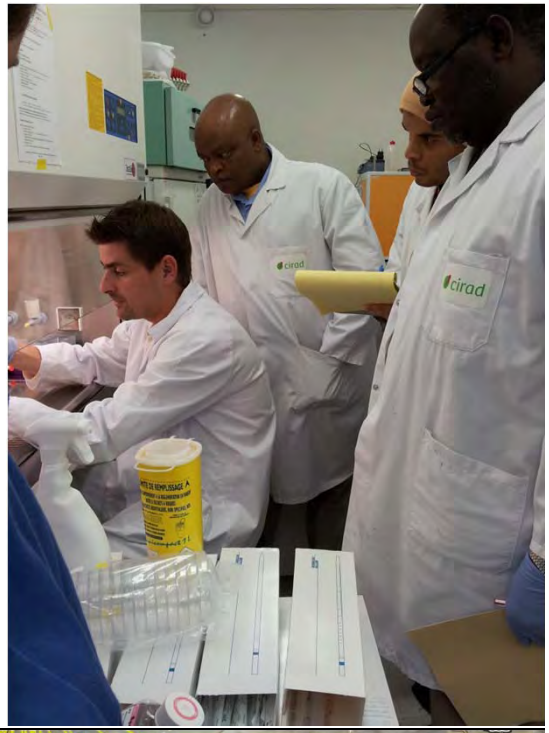
Local Scale: Animal Mobility in Senegal 2012/2013

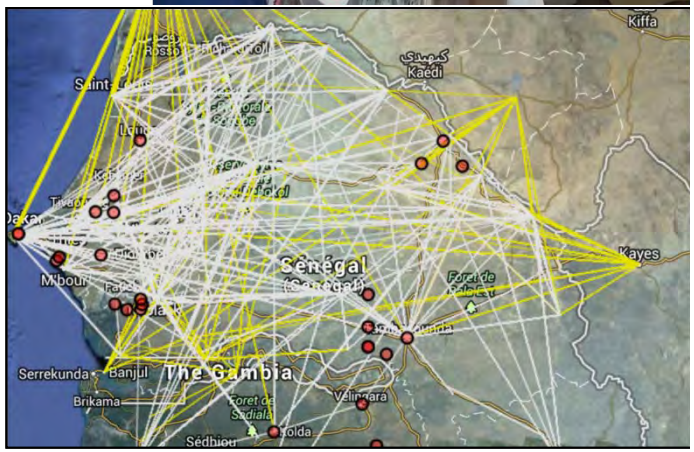


CONCLUSION 1/2

- It is crucial to provide laboratories with efficient tools allowing the early detection of PPR emergence re-emergences

- All these tests will allow to appreciate:
  - ✓ the presence/diffusion of the disease into new areas or to certify freedom from the disease
  - ✓ the origin of the virus through molecular epidemiological methods in connection knowledge of animal movements.





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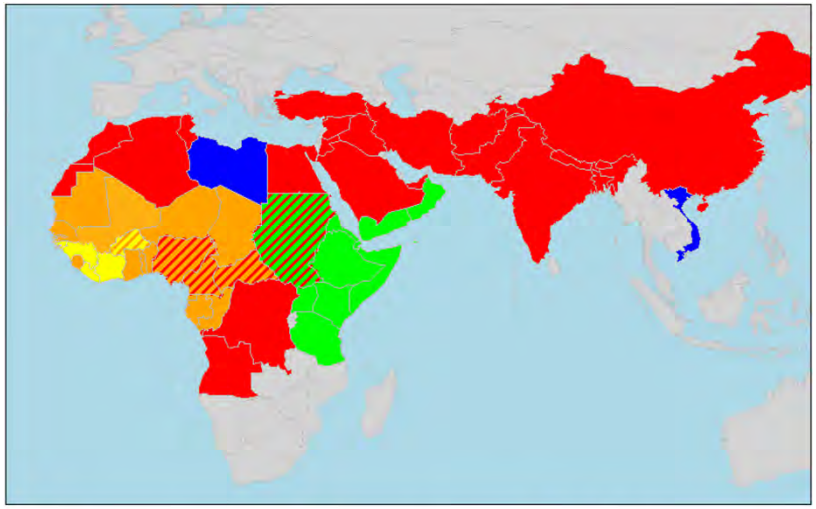
CONCLUSION 2/2

- It is important to integrate the PPRV molecular knowledge with epidemiological data (animal mobility, transhumance, trade, markets etc ...),
- Allow to :
  - ✓ Clarify the epidemiological 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



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Merci de votre attention