PPR field and laboratory diagnosis – support to national labs

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EU Reference laboratory for Peste des Petits Ruminants

Funded by the European Union

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LA RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT

WOAH
Reference Laboratory Network for PPR

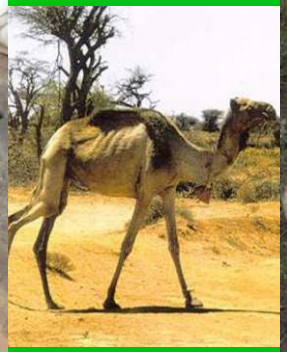
WOAH Reference Laboratory for peste des petits ruminants

Reference Centre
World Organisation for Animal Health
Founded as OIE

Peste des Petits Ruminants (PPR)

- Main host are domestic small ruminants (goats, sheep) – focus of surveillance and control efforts
- Virus of genus *Morbillivirus*, transmitted by contact (excretions, droplets)
- Mucopurulent ocular and nasal discharges, erosion of the mucosa, acute diarrhoea (mortality up to 70-90%)
- Camels, suids and some wild Artiodactyls also susceptible, but exact role in PPR epidemiology unknown (main hypothesis: spill-over from livestock)




Pictures 1-3: H. Salami

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Complexity of field diagnosis

Clinical surveillance is key for early detection, but:

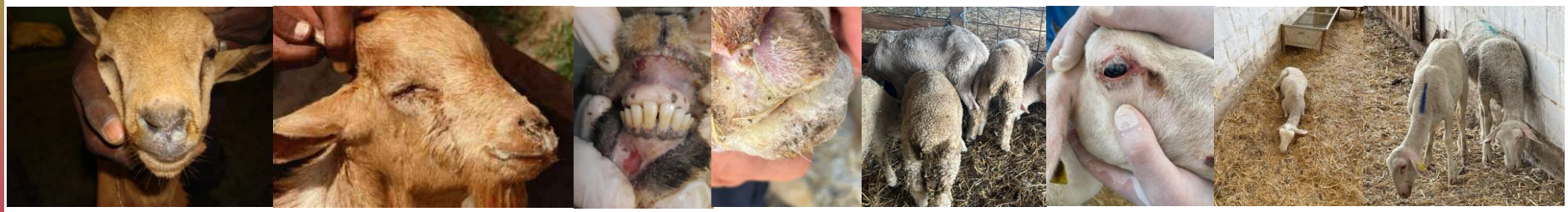
- The severity of the disease varies with species, breed, as well as the animal's immunity to PPRV
- Sheep and Goat and are not always affected to the same extent during an outbreak.

Acute form → Typical set of symptoms in herd

Peracute form → Sudden death with limited symptoms

Subacute form → Slow evolution with variable symptoms

No visible symptoms → Risk of silent spread



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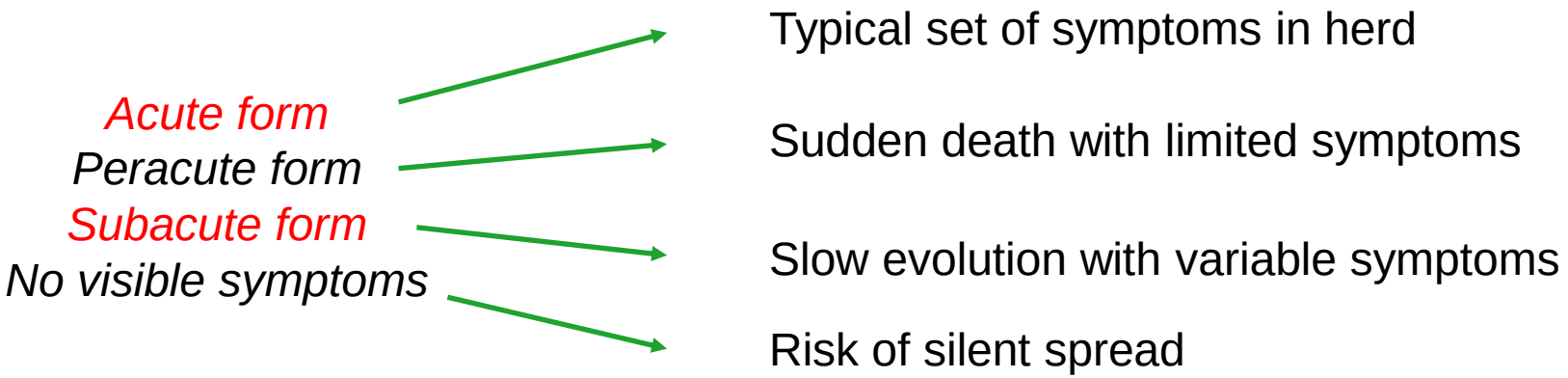
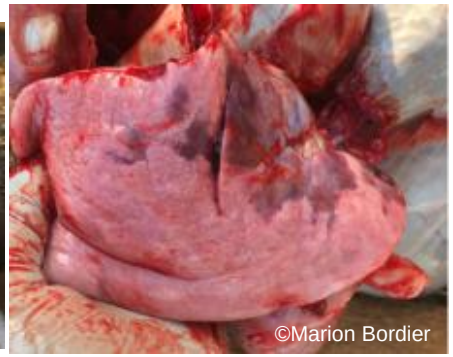


- Symptoms are often confused with, and exacerbated by, secondary infections making PPR a difficult disease to characterise and diagnose (CCPP, BTV, pasteurellosis…)

Complexity of field diagnosis

Clinical surveillance involves multiple actions:

- **Detection of clinical signs** or lesions of PPR by close physical examination or post mortem examination of animals
- **Sample collection** for serology and virology, if suspect cases detected and according to the case definition
- **Interviews** with relevant stakeholders about recent or current health issues, unusual mortality of other events, vaccination history, etc.



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Confirmation of field diagnosis

Use of point of care tests

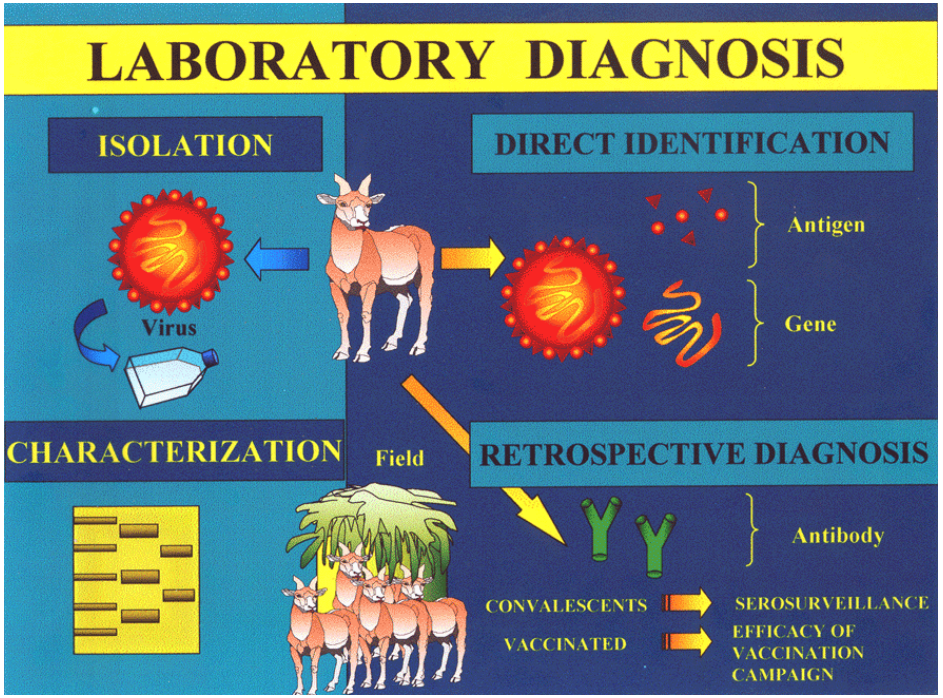
- Diagnostic tests: **Antigenic rapid test**
- Sample types: **Nasal and ocular swab** (+/- oral and rectal swab) on animals with fever and early clinical signs
- High specificity, medium sensitivity (70%)

- Recommendations for use:
  - Test up to 5 or 10 animals , stop as soon as you have a positive.
  - If positive: take a set of samples from 2-3 animals for laboratory analyses
  - If negative but strong suspicion: take a set of samples from 5-6 animals with early signs for laboratory confirmation of negativity

Interpretation: A positive test is enough to take some control measures (quarantine, ban of movement, etc.) without waiting for lab

Laboratory diagnosis of PPR

- Establishing diagnosis to complete observations of clinical symptoms- compulsory for disease confirmation
- Implementing quality diagnosis with standardised methods to deliver reliable PPR diagnosis results
- Different type of tests for different purposes



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Laboratory diagnosis of PPR

- WOAH manual : purpose and suitability of the methods

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[1]						
RT-PCR	–	++	++	+++	+	–
Real-time RT-PCR	–	++	+++	+++	+	–
Virus isolation in cell culture	–	–	–	++	–	–
Immunocapture ELISA		+	++	+++	+	–
Penside test (LFD)	–	–	++	++	–	–
Detection of immune response						
Virus neutralisation	+++	+++	–	++	++	++
Competitive ELISA	+++	+++	+++	+	+++	+++

Updating the manual is SLOW. For latest VALIDATED protocols, see WOAH/FAO ref labs: https://www.ppr-labs-oie-network.org/

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Laboratory diagnosis of PPR

Importance of sample container and storage

Type of sample	Container	Preservation medium
Swab	Viscose swab in original tube (avoid cotton swab)	No, except if no ice available
Whole blood	Blood collection tube	EDTA
Tissues from internal organs	Plastic tube with screw-cap	No, except if no ice available
Serum	Blood collection tube	No

Site	Type of sample	Storage condition	Length of storage	Packaging
From field to lab	All	On ice	<24hr	Double packaging
National lab	All	5 ± 3°C	<3 days	Double packaging
National lab	Swabs, tissues, buffy coat	≤ -65°C	No limit	Double packaging
National lab	Serum	≤ -16°C	No limit	Simple packaging

All validated protocols available at https://www.ppr-labs-oie-network.org/

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Laboratory diagnosis of PPR

For sensitive diagnosis of PPR virus genetic material: real-time quantitative PCR

Status of animal	Clinical symptoms	Type of test	Aim of test	Types of samples
Alive	with symptoms ; without symtoms if strong epi link	Real-time PCR	Detection of virus	Priority: Nasal, ocular swab AVOID whole blood
Dead/ euthanized	with symptoms	Real-time PCR	Detection of virus	Priority: Nasal, ocular swab Lymph node, Lung Optional: Spleen AVOID whole blood

Real-time PCR highly specific and sensitive, although can be affected by inhibitors (specially in blood and spleen)

Use method validated by WOAH/FAO reference laboratory:

https://www.ppr-labs-oie-network.org/

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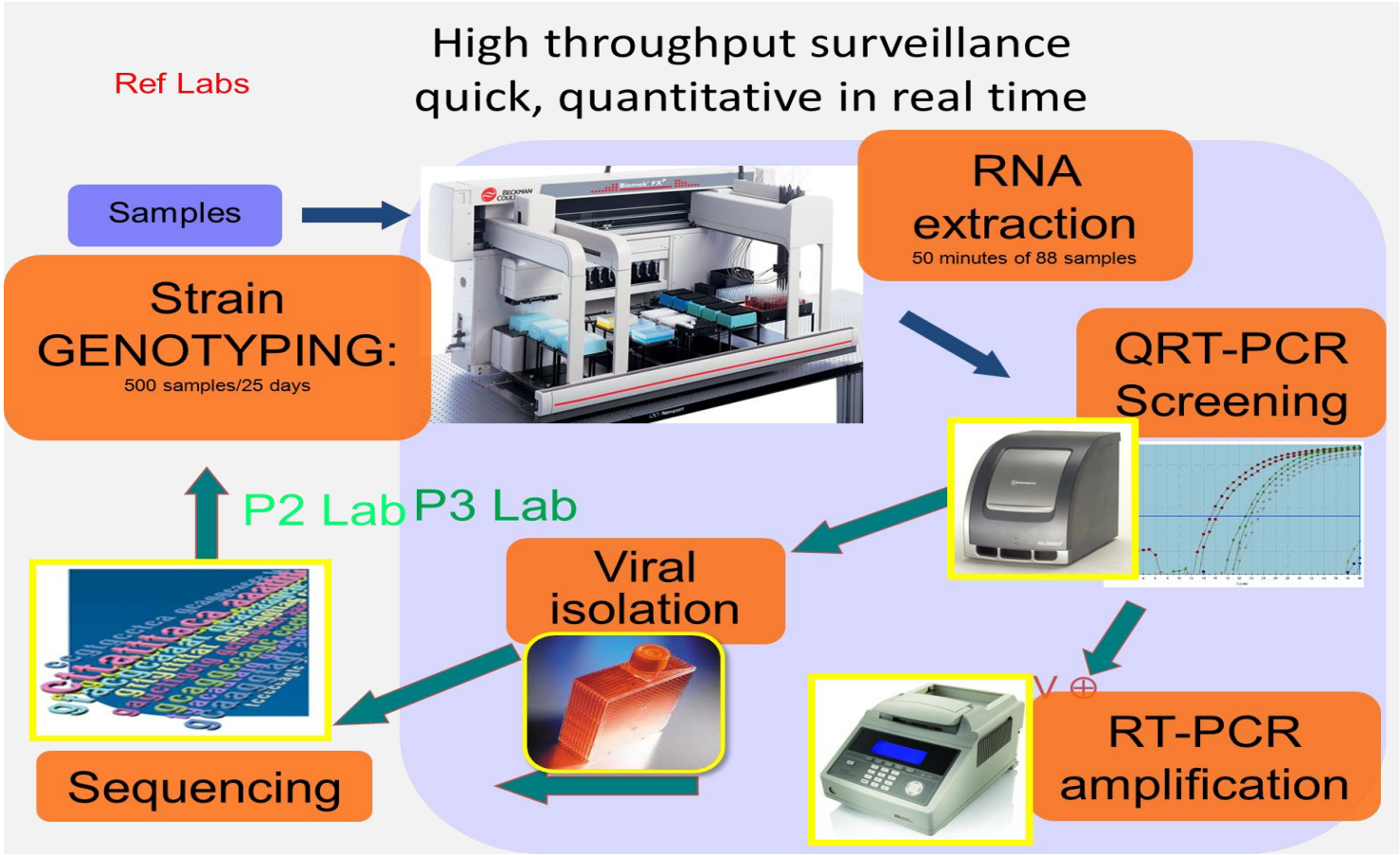
Virology tests

Reference laboratory workflow



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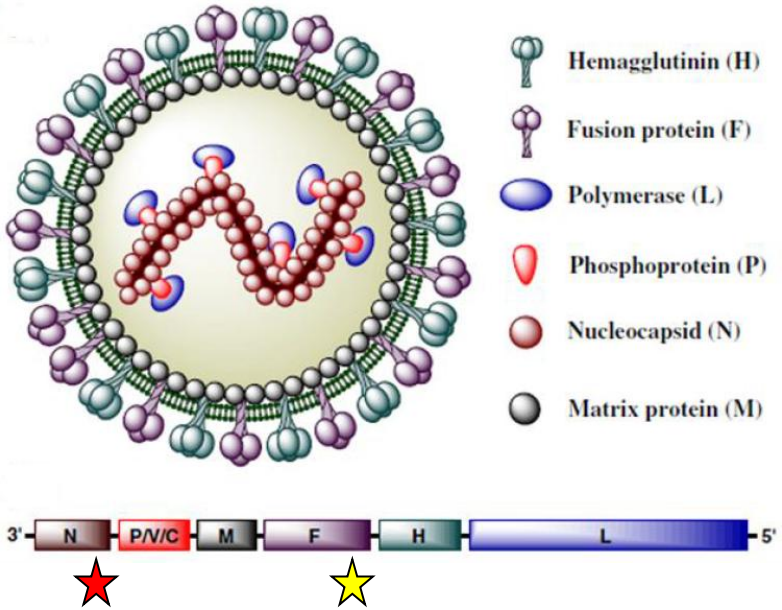
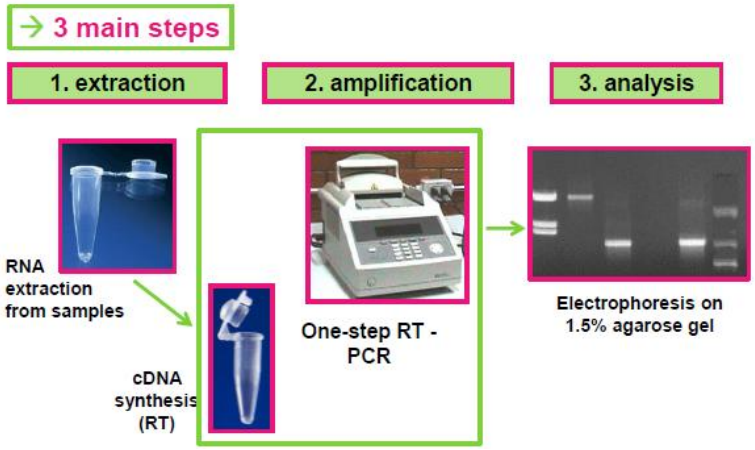
Virology tests: Conventional RT-PCR





Less sensitive than RT qPCR, and only qualitiative

★ Most commonly used test:

NP3-NP4: targeting an hypervariable region of N gene (size 351 nucleotides)

Also used:

☆ F1-F2: targeting hypervariable region of F gene (size: 370 nucleotides)

But important second step for sequencing and genotyping of strains

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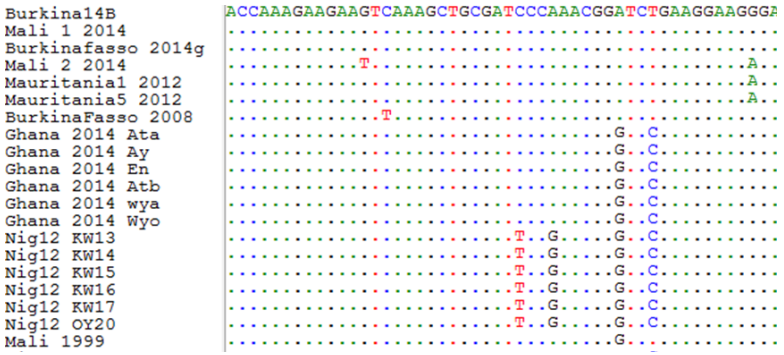
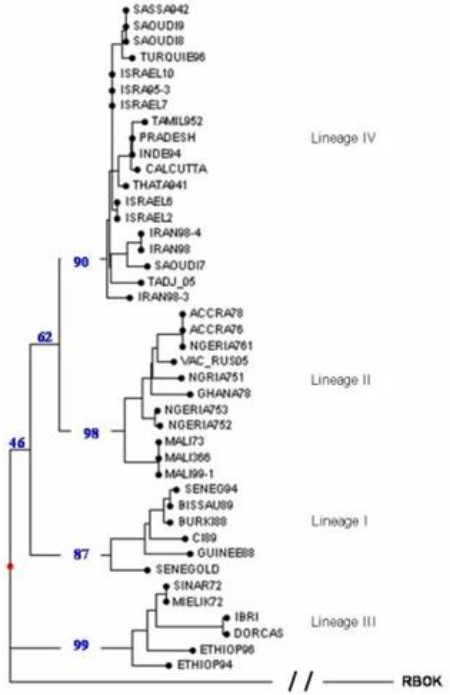
Virology tests: genotyping

Partial N gene (typically 255bp)

- Based on most used, conventional RT-PCR
- Largest dataset available publicly
- Phylogenetic analyses sufficient to identify genetic lineage and some genetic clusters regrouping strains within a lineage
- Can provide first view on the level of PPRV diversity or on connections across regions
- But some ambiguity in phylogenetic relationships between strains will remain

Main first tool to support epidemiological investigation and episystem identification

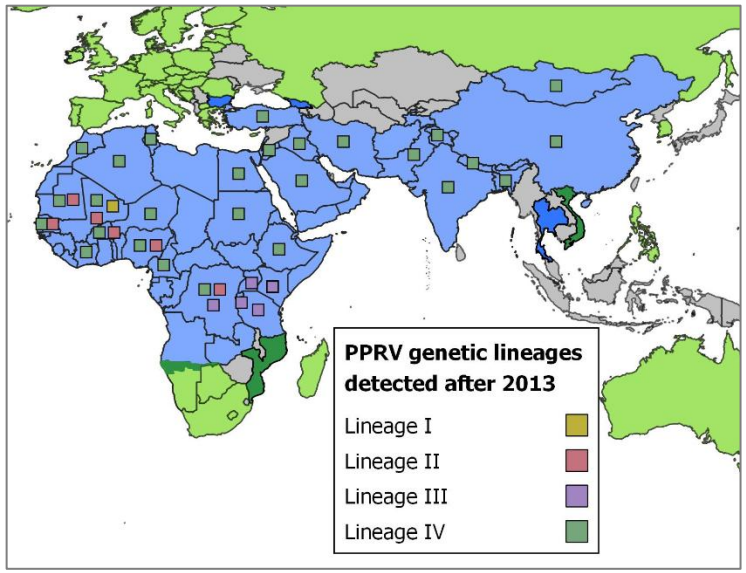


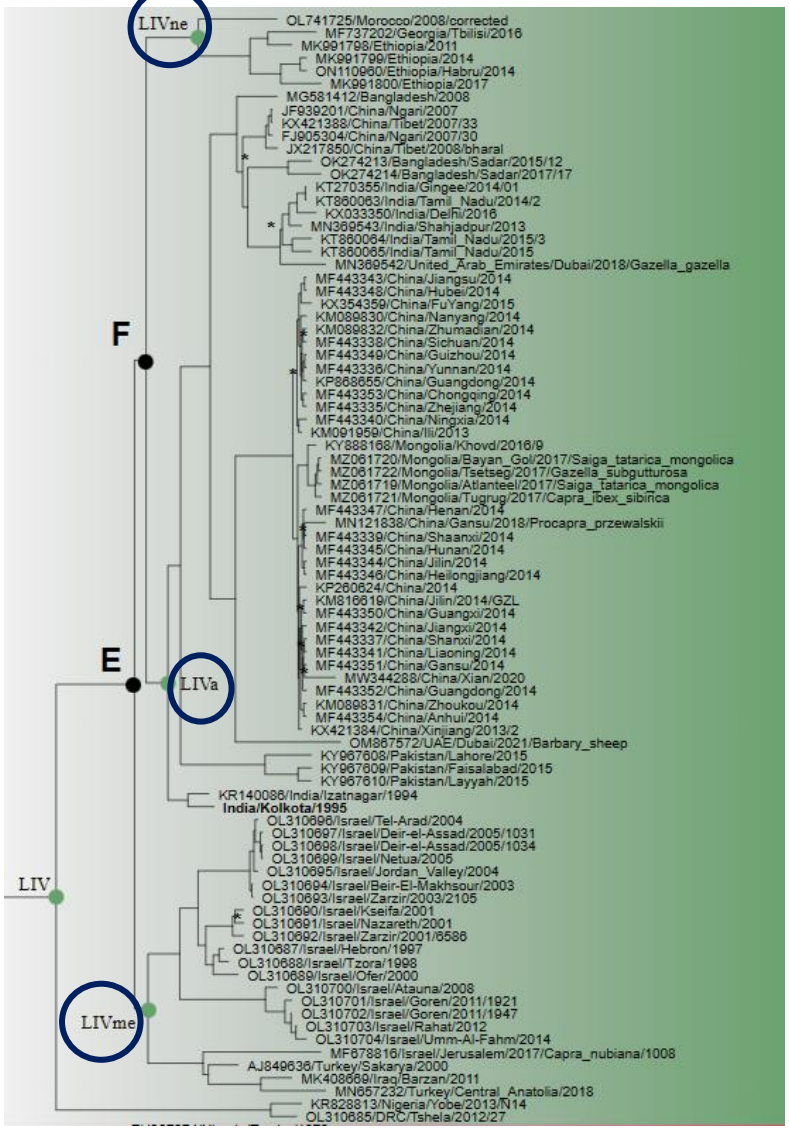


Virology tests: genotyping

Genetic diversity and distribution

- Four genetic lineages (LI-LIV)
- LIV most widely distributed, only one found in Asia and Middle East
- Identification of geographically-defined sub-lineages
- Some events of long-distance PPRV movement across regions





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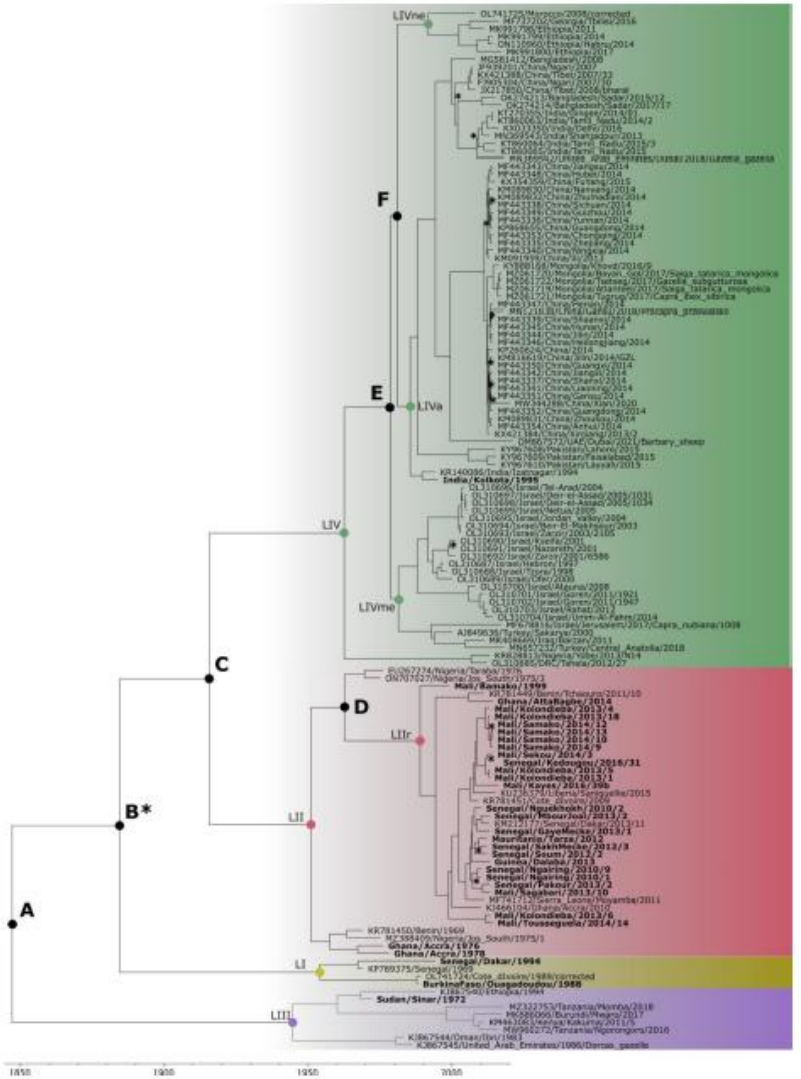
Virology tests: genotyping

Full genome sequencing

- On targeted samples, based on results from partial N gene sequencing, using NGS protocol
- Answer questions related to episystems and molecular epidemiology which are not possible to address using partial N gene sequence data
- Inform epidemiological investigation concerning PPR transmission pathways

- **Support of the WOAH reference laboratories or the joint FAO/IAEA Joint Centre possible**



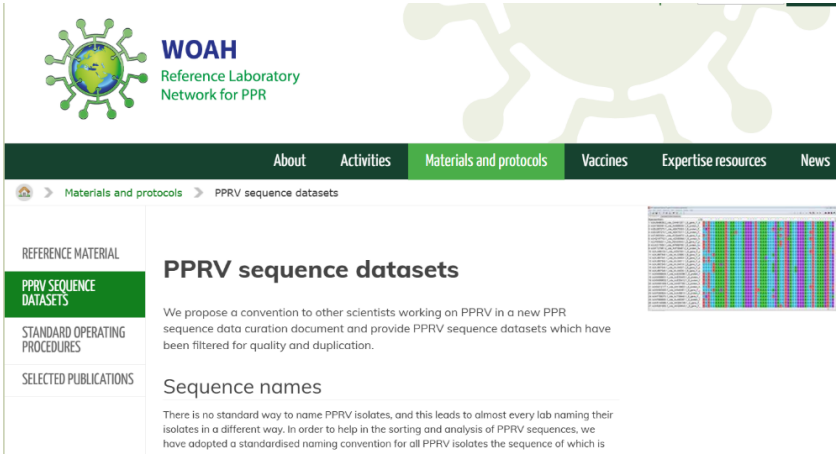
PPRV sequence curation

Should follow recommandations from WOAH ref lab network for PPR

- Use curated datasets available through the website for your analyses
- Use naming convention for PPRV sequences submitted to public databases
- Complete epidemiological data should accompany each sequence as metadata at submission
- Need careful check of sequencing results , notably for contamination

Guidelines and datasets:

https://www.ppr-labs-oie-network.org/



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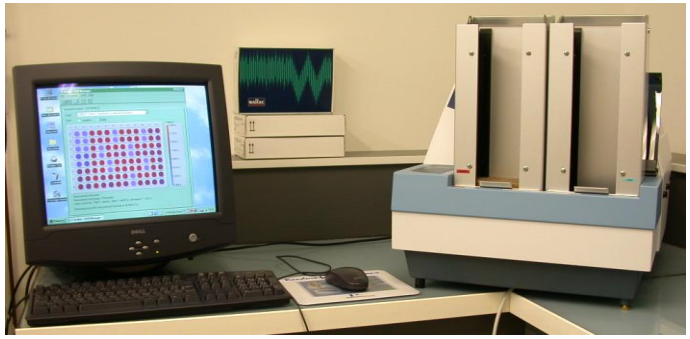
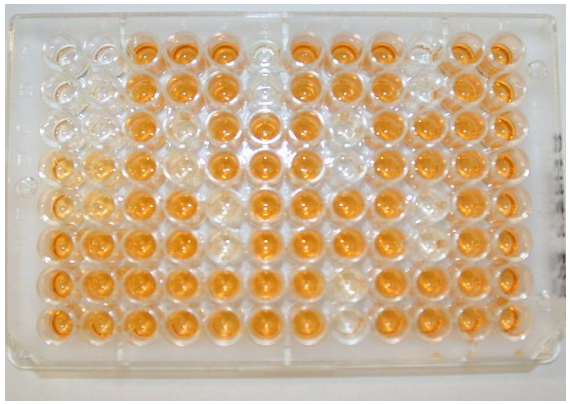
Serology tests

Serological surveillance

- cELISA test (detection of antibodies) on serum samples, with seropositivity starting 8-9 days after infection
- During 2-3 weeks, animals may be both PCR and ELISA positive
- Useful to find evidence of past PPR circulation (>10 days)
- Main tool to prove PPR freedom of a region/ country based on random or targeted sampling strategy, or to evaluate vaccination campaign
- Most commonly used kit by IDvet also validated for camelids and suids in case interest in integrating atypical hosts in surveillance





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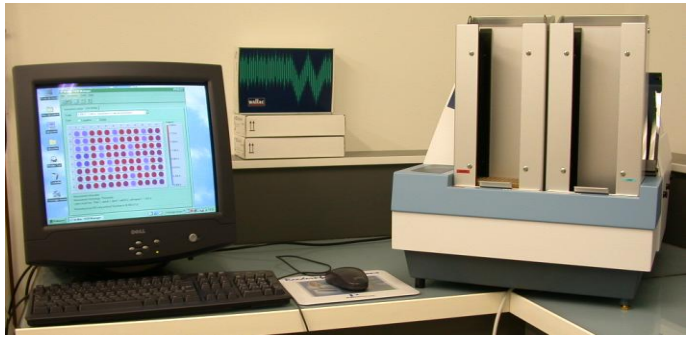
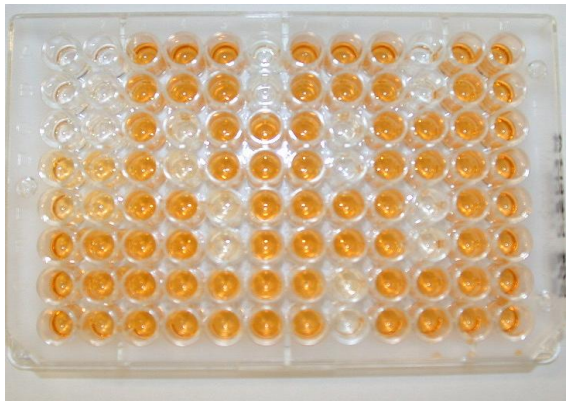
Serology tests

Serological surveillance – false positives

- Highly sensitive but validated kit (IDvet) with ~1% of false positive
- **Expect 1-2 positive results / 100 sera**
- Possible investigations on suspected false positive results
  - Favored: seroneutralisation test (gold standard)
  - Re-test on new sample from the same or more animals in the farm
  - Re-test same sample using a second validated test (AU-PANVAC test)
  - Confirmatory test by WOAH/FAO ref lab





WOAH/REF labs available to support in all cases

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Importance of ref lab networks

- The use of fit-for-purpose, validated **diagnostic methods** and of high quality **vaccines** is key to successfully control and eradicate PPR
- Diagnostic labs need to continuously **update and maintain their capacity** to detect the virus and to support vaccination and surveillance programmes
- WOAH/FAO PPR ref labs support labs involved in PPR diagnostic to be in capacity to provide **high quality diagnostic** services
- **Providing scientific and technical expertise** to organisations and countries on issues related to early preparedness and effective response

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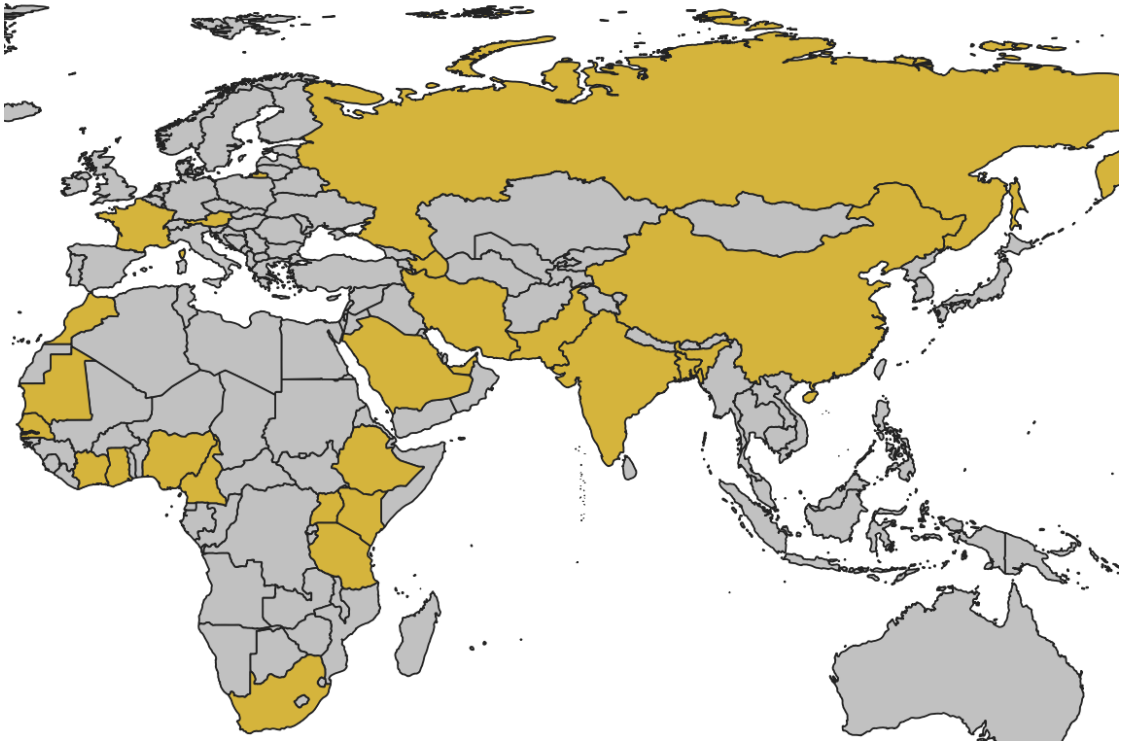
WOAH ref lab network for PPR

WOAH
Reference Laboratory
Network for PPR

Includes all WOAH ref labs, FAO/IAEA joint center, AU-PANVAC, labs from Africa (12), Middle East (3), and Asia (8); synergy with existing networks (e.g. VETLAB network)

Any lab involved in PPR diagnosis can join the network.

Contact
arnaud.bataille@cirad.fr



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中国动物卫生与流行病学中心
China Animal Health And Epidemiology Center

THE Pirbright INSTITUTE

ICAR-NIVEDI

Pan African Veterinary Vaccine Center of African Union
AU-PANVAC

The WOAH PPR ref lab network website: main tool to support laboratories

https://www.ppr-labs-oie-network.org/

Info on all protocols, reference material, training, PT, vaccines, and webinars available through the network

Annual workshop and newsletter
Email diffusion list

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Proficiency tests

Key tool to improve performance of labs in PPR diagnostics towards validated and robust results

- PT organised annually by CIRAD simultaneousy as EURL-PPR and WOAH/FAO ref lab following norm ISO17043
- Includes laboratories from Eurasia, Middle East and Africa, sometimes depending on financial support from projects (max. 50 participating laboratories)
- Participating for serology method at minimum, plus molecular biology (RT PCR and/or real time RT PCR)
- Provide follow-up support to labs that failed the PT (material, protocols, training)
- PT is key to fully validate a new method, or flag methods with major issues (participation to PT is obligatory for ISO17025)

PPR ref lab activities - CIRAD

- Standard Operating Procedures : open access on websites
- Production and supply of reference materials
- Control and maintenance of PPR reference collection
- Training course on PPR diagnostic methods
  - Proposed annually (fee required for participation)
  - Organized on-site within the frame of projects
  - Covers serology (cELISA, SNT) and molecular biology (RNA extraction, RT-PR, RT-QPCR)

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PPR ref lab activities

Active assistance in emergency situation

- Epidemiologists and virologists with PPR expertise ready to provide support to veterinary services in case of PPR emergency
- Assistance in the diagnosis of PPR outbreaks – surveillance campaigns
  - Confirmatory lab diagnosis (**under accreditation**) of field samples from suspected PPR outbreaks (often available for free)
  - Provision of reference material and lab expertise
  - Genomic and phylogenetic studies of strains isolated during outbreaks to provide information for epidemiological investigation
  - Recent support during PPR emergence in Europe




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Thank you

Any question or request:
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