

PPR field and laboratory diagnosis – support to national labs

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EU Reference laboratory for Peste des Petits Ruminants



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WOAH
Reference Laboratory
Network for PPR

WOAH Reference Laboratory
for peste des petits ruminants

Reference Centre



World Organisation
for Animal Health
Founded as OIE

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
Peracute form
Subacute form
No visible symptoms

Typical set of symptoms in herd

Sudden death with limited symptoms

Slow evolution with variable symptoms

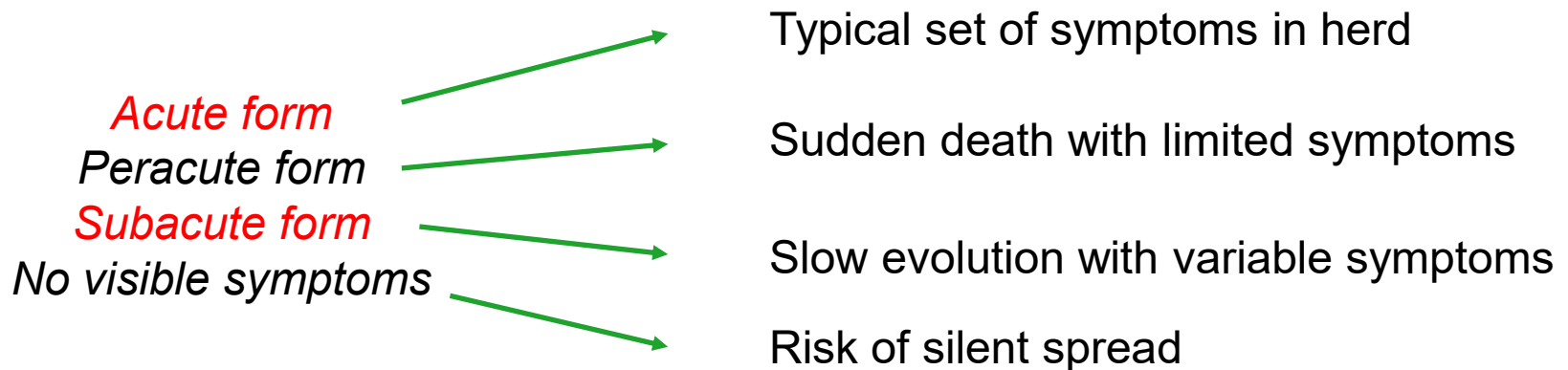
Risk of silent spread



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.



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

Confirmation of field diagnosis

Use of point of care tests

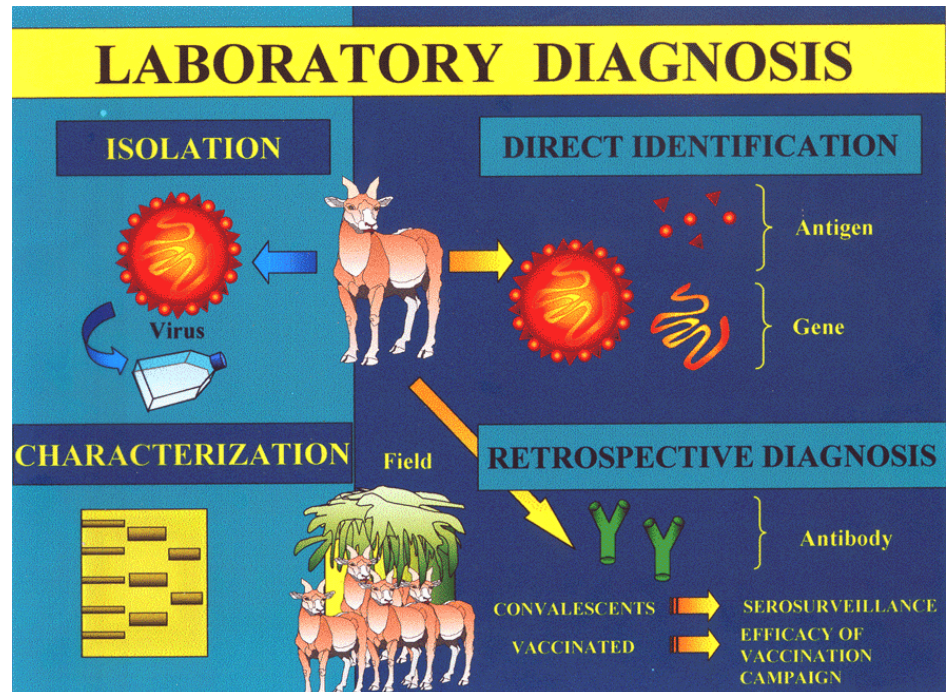
- Diagnostic tests: **Antigenic rapid test**
- Sample types: **Nasal and ocular swab** (+/- oral and rectal swab) on animals with fever and early clinical signs
- High specificity, medium sensitivity (70%)
- Recommendations for use:
 - Test up to 5 or 10 animals , stop as soon as you have a positive.
 - 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



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 WOAHA/FAO ref labs: <https://www.ppr-labs-oie-network.org/>

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	$5 \pm 3^{\circ}\text{C}$	<3 days	Double packaging
National lab	Swabs, tissues, buffy coat	$\leq -65^{\circ}\text{C}$	No limit	Double packaging
National lab	Serum	$\leq -16^{\circ}\text{C}$	No limit	Simple packaging

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

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 symptoms if strong epi link	Real-time PCR	Detection of virus	<u>Priority:</u> Nasal, ocular swab <u>AVOID whole blood</u>
Dead/ euthanized	with symptoms	Real-time PCR	Detection of virus	<u>Priority:</u> Nasal, ocular swab Lymph node, Lung <u>Optional:</u> Spleen <u>AVOID whole blood</u>

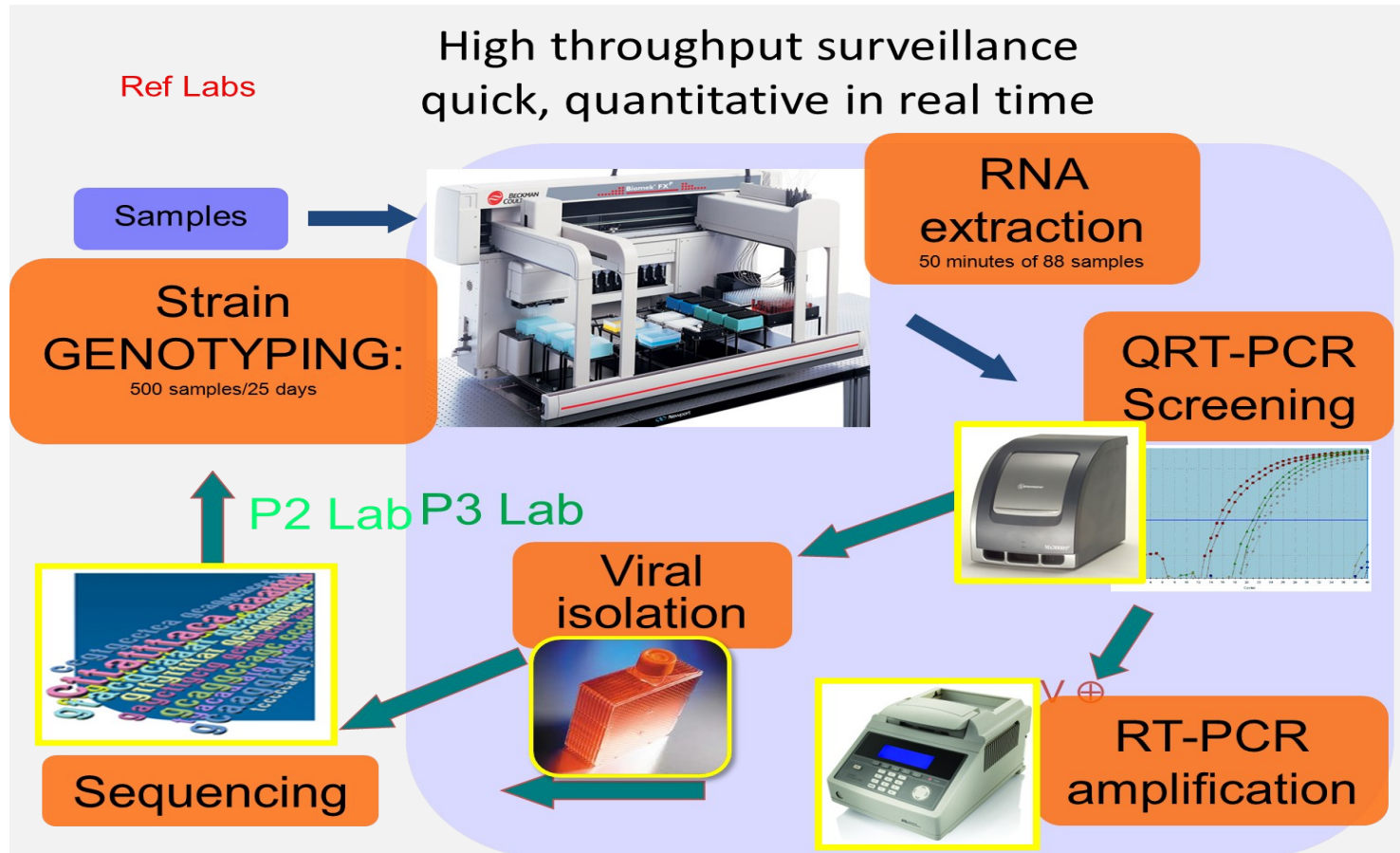
Real-time PCR highly specific and sensitive, although can be affected by inhibitors (specially in blood and spleen)

Use method validated by WOAHA/FAO reference laboratory:

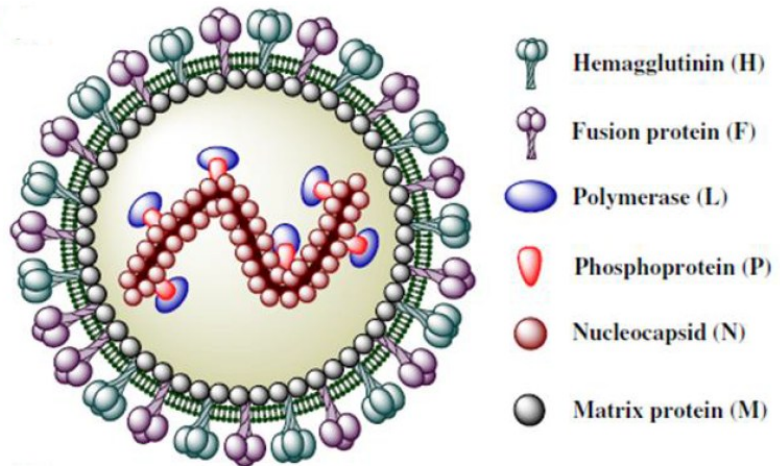
<https://www.ppr-labs-oie-network.org/>

Virology tests

Reference laboratory workflow



Virology tests: Conventional RT-PCR



→ 3 main steps

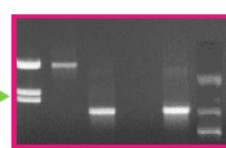
1. extraction

2. amplification

3. analysis



cDNA synthesis (RT)



Less sensitive than RT qPCR, and only qualitative

★ 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

Virology tests: genotyping

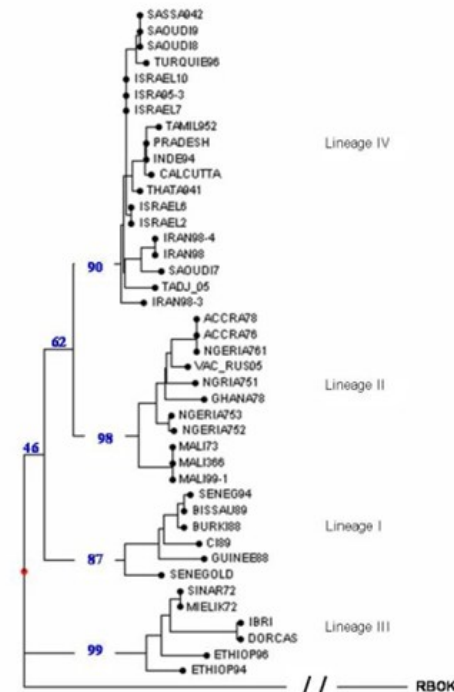
Partial N gene (typically 255bp)

- 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 epistemic identification

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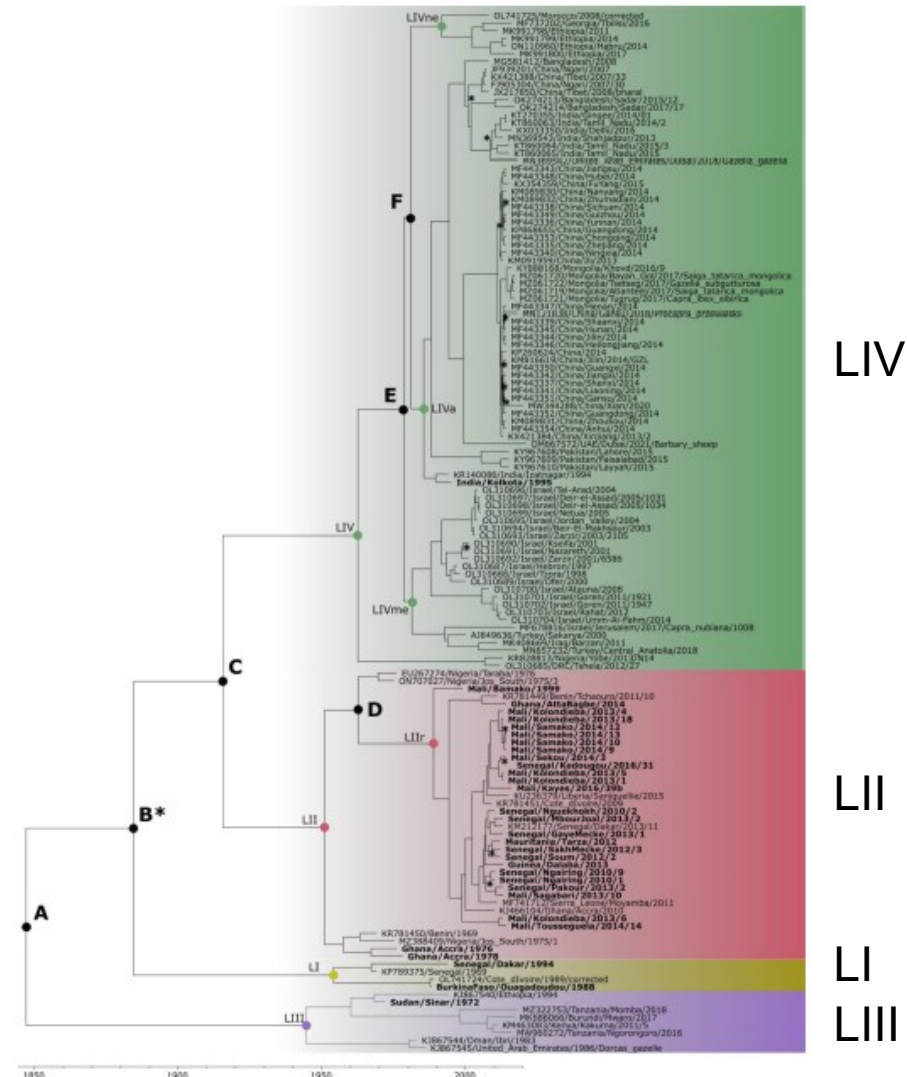
Burkina14B      ACCAAAGAAGAAAGTCAAAGCTGCGATCCCAACGGATCTGAAGGAAGGGG
Mali 1 2014     .....
BurkinaFasso 2014g .....
Mali 2 2014     .....T.....A..
Mauritania1 2012 .....A..
Mauritania5 2012 .....A..
BurkinaFasso 2008 .....T.....
Ghana 2014 Ata .....G.C
Ghana 2014 Ay .....G.C
Ghana 2014 En .....G.C
Ghana 2014 Atb .....G.C
Ghana 2014 wya .....G.C
Ghana 2014 Wyo .....G.C
Nig12 KW13      .....T.G.G.C
Nig12 KW14      .....T.G.G.C
Nig12 KW15      .....T.G.G.C
Nig12 KW16      .....T.G.G.C
Nig12 KW17      .....T.G.G.C
Nig12 OY20      .....T.G.G.C
Mali 1999       .....G..
    
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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 WOAHA reference laboratories or the joint FAO/IAEA Joint Centre possible**



PPRV sequence curation

Should follow recommendations 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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The WOAHP Reference Laboratory Network, officially launched by the WOAHP in December 2020, aims at building strong partnerships between the WOAHP Reference Laboratories and national reference laboratories throughout the world, improving links between recognised experts from national reference laboratories and from PPR diagnostic laboratories in low- and middle-income countries, in addition to the current three WOAHP

About Activities Materials and protocols Vaccines Expertise resources News

Materials and protocols > PPRV sequence datasets

REFERENCE MATERIAL
PPRV SEQUENCE DATASETS
STANDARD OPERATING PROCEDURES
SELECTED PUBLICATIONS

PPRV sequence datasets

We propose a convention to other scientists working on PPRV in a new PPR sequence data curation document and provide PPRV sequence datasets which have been filtered for quality and duplication.

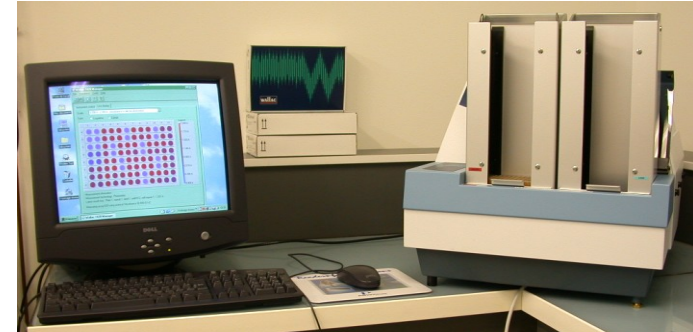
Sequence names

There is no standard way to name PPRV isolates, and this leads to almost every lab naming their isolates in a different way. In order to help in the sorting and analysis of PPRV sequences, we have adopted a standardised naming convention for all PPRV isolates the sequence of which is

Serology tests

Serological surveillance

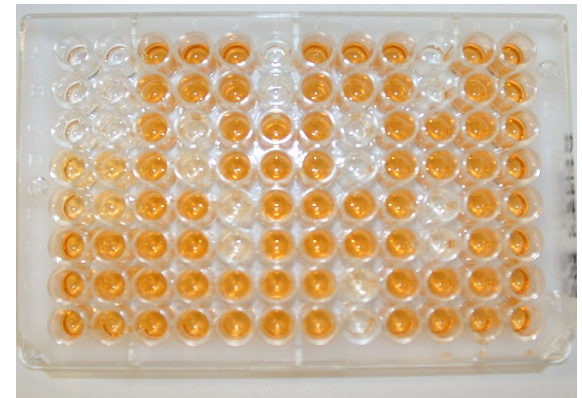
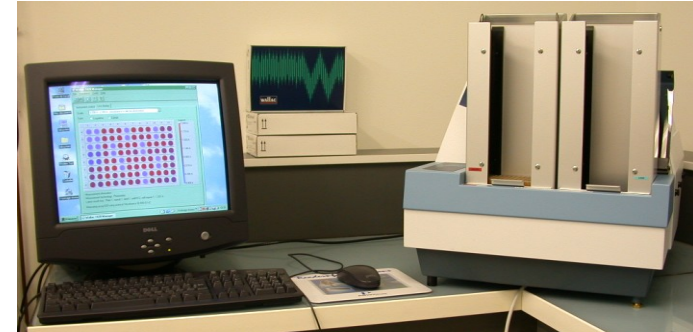
- cELISA test (detection of antibodies) on serum samples, with seropositivity starting 8-9 days after infection
- During 2-3 weeks, animals may be both PCR and ELISA positive
- Useful to find evidence of past PPR circulation (>10 days)
- Main tool to prove PPR freedom of a region/ country or to evaluate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



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

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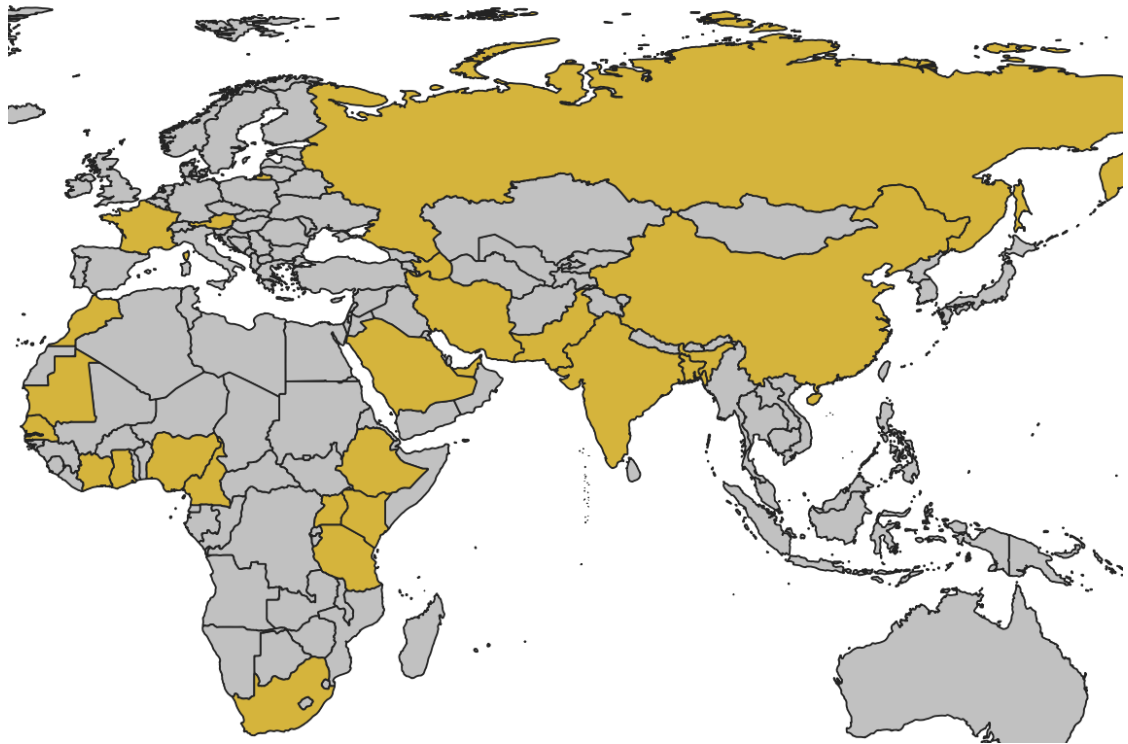
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Includes all WOAH ref labs, FAO/IAEA joint center, AU-PANVAC, labs from Africa (12), Middle East (3), and Asia (8); synergy with existing networks (e.g. VETLAB network)

Any lab involved in PPR diagnosis can join the network.

Contact

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The WOAHPPR 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

Proficiency tests

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

- PT organised annually by CIRAD simultaneously as EURL-PPR and WOAHA/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 and 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)

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Thank you

Any question or request:

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