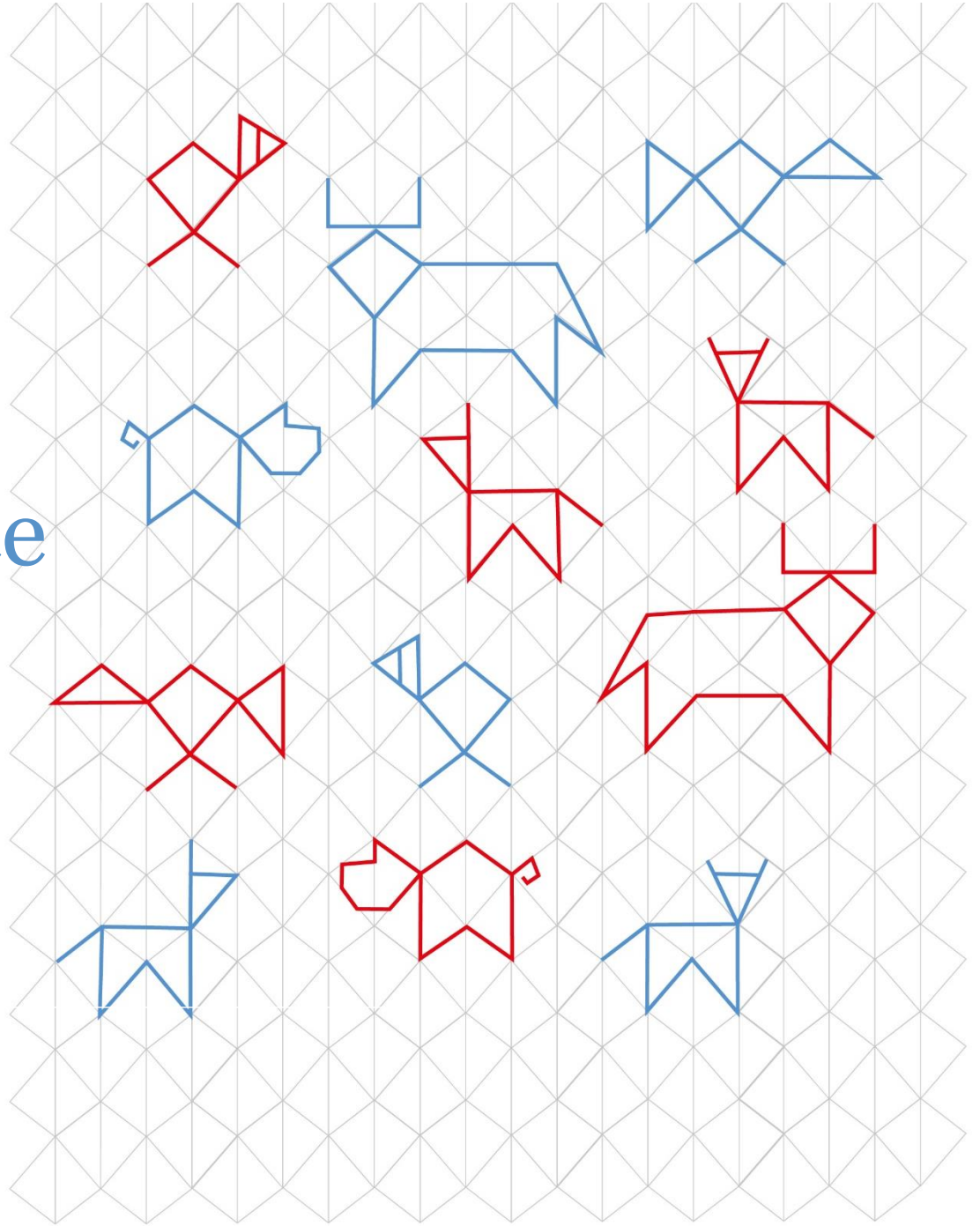
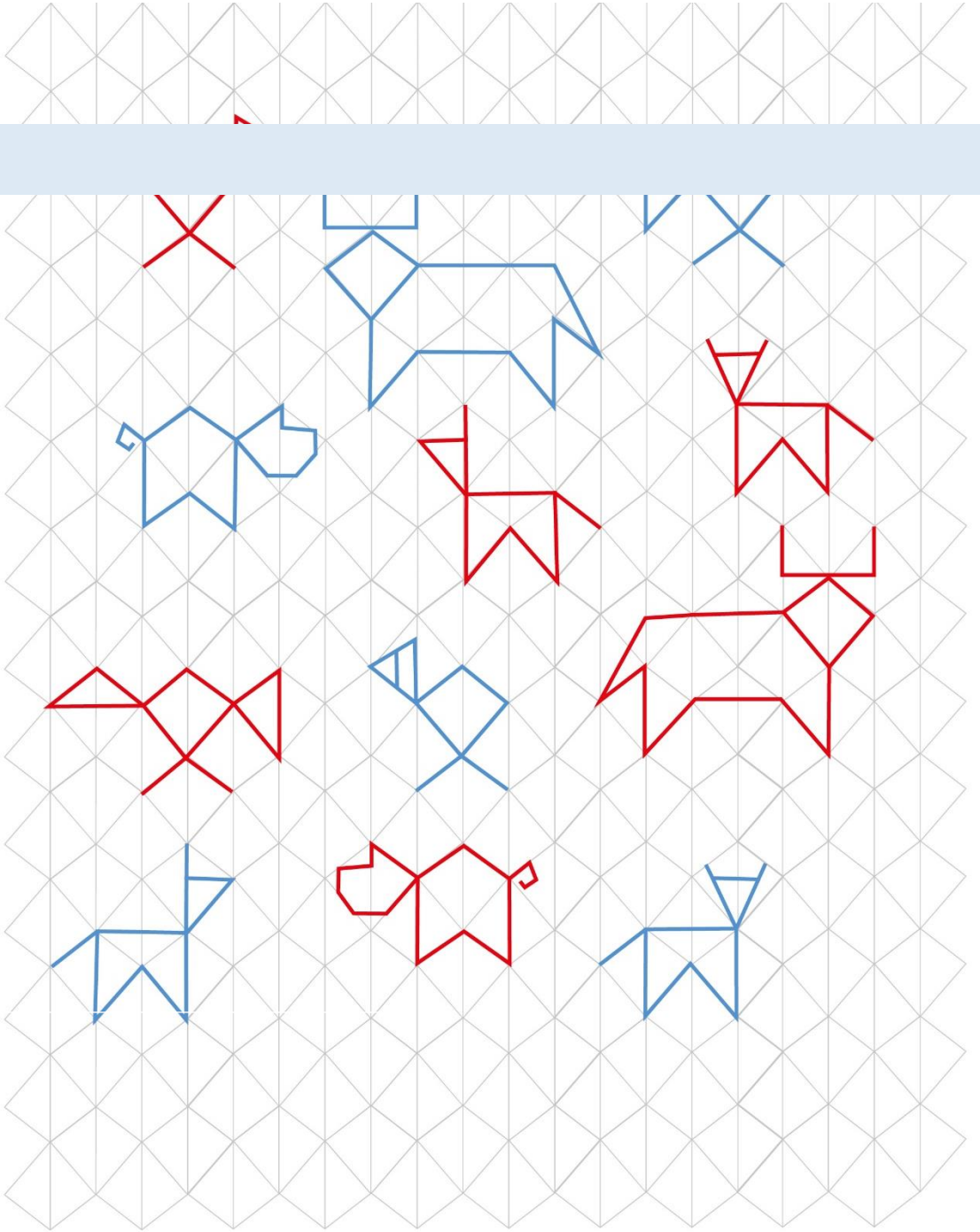
Overview of Available Serological and Molecular Diagnostic Tests Applied for the Detection and Surveillance of PPR

Jingyue Bao, PhD
China Animal Health and Epidemiology Center



Outlines

1. Background
2. Field diagnostics
3. Laboratory diagnostics
4. A brief introduction of CAHEC
5. Conclusion



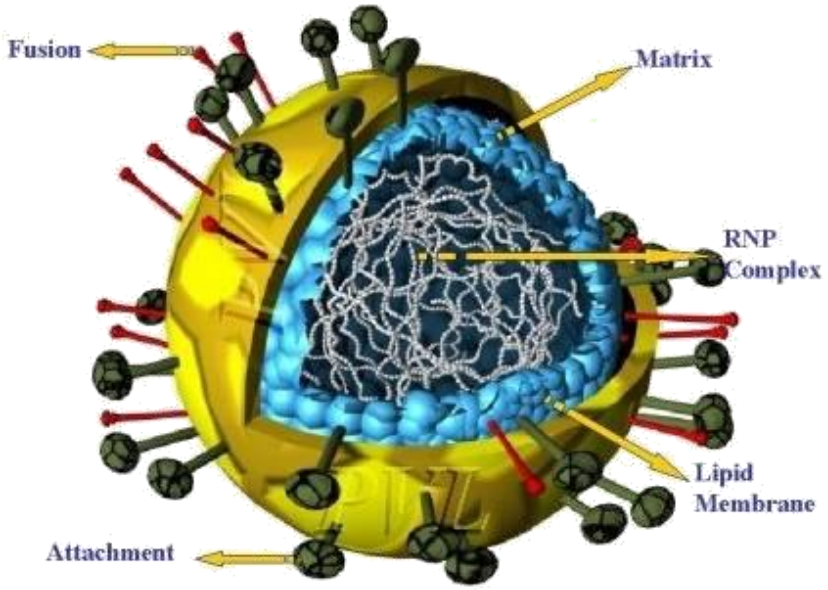
1. Background

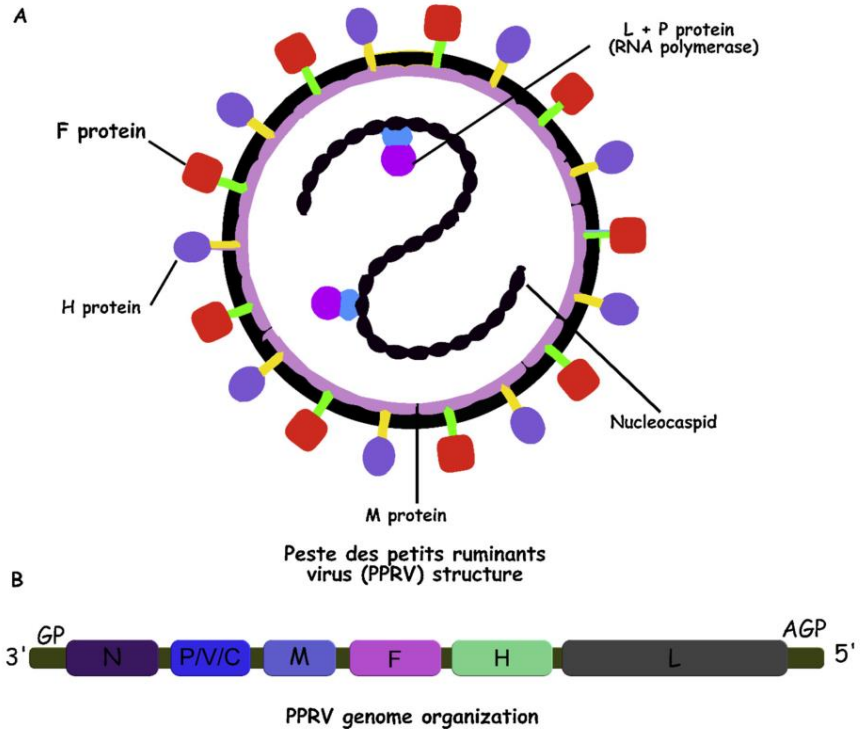
Purpose for PPR diagnostics

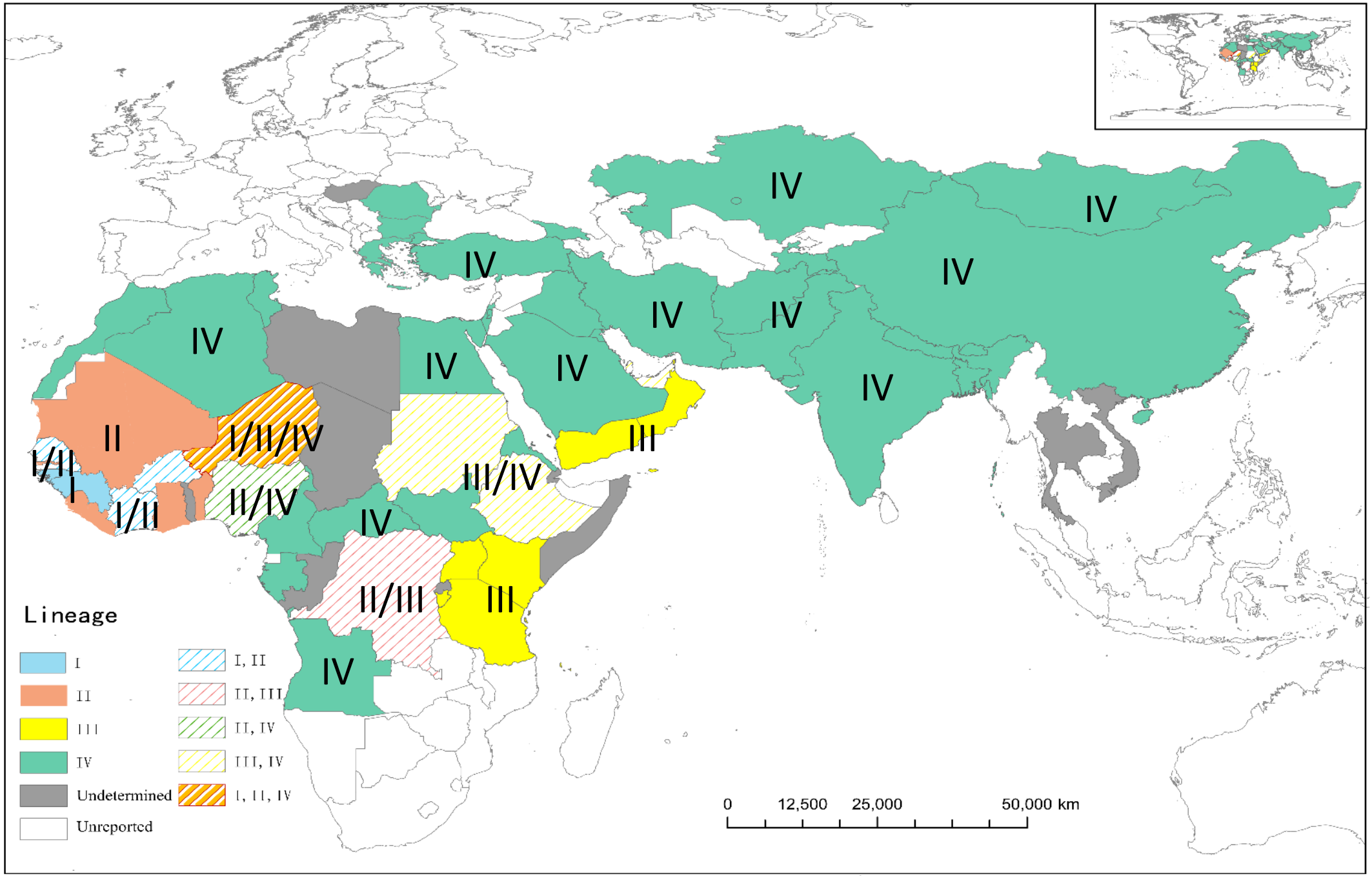
- Peste des petits ruminants (PPR) is an acute or subacute viral disease of goats and sheep
- WOAH mandatory declaration of the disease
- The presence of PPR can have a serious impact on sheep and goats industry
- Depending on the susceptibility of the population, the morbidity and mortality rates can be up to 100% in severe outbreaks

Peste des Petits Ruminants Virus

- Member of genus Morbillivirus, family Paramyxoviridae
- Enveloped virus
- Size=400~500nm






IV
IV
IV
IV
IV
IV
IV
IV
IV
IV
II
I/II/IV
I/II
I
I/II
II/IV
III
III/IV
II/III
III
IV
Lineage
I
II
III
IV
Undetermined
Unreported
I, II
II, III
II, IV
III, IV
I, II, IV
0
12,500
25,000
50,000 km

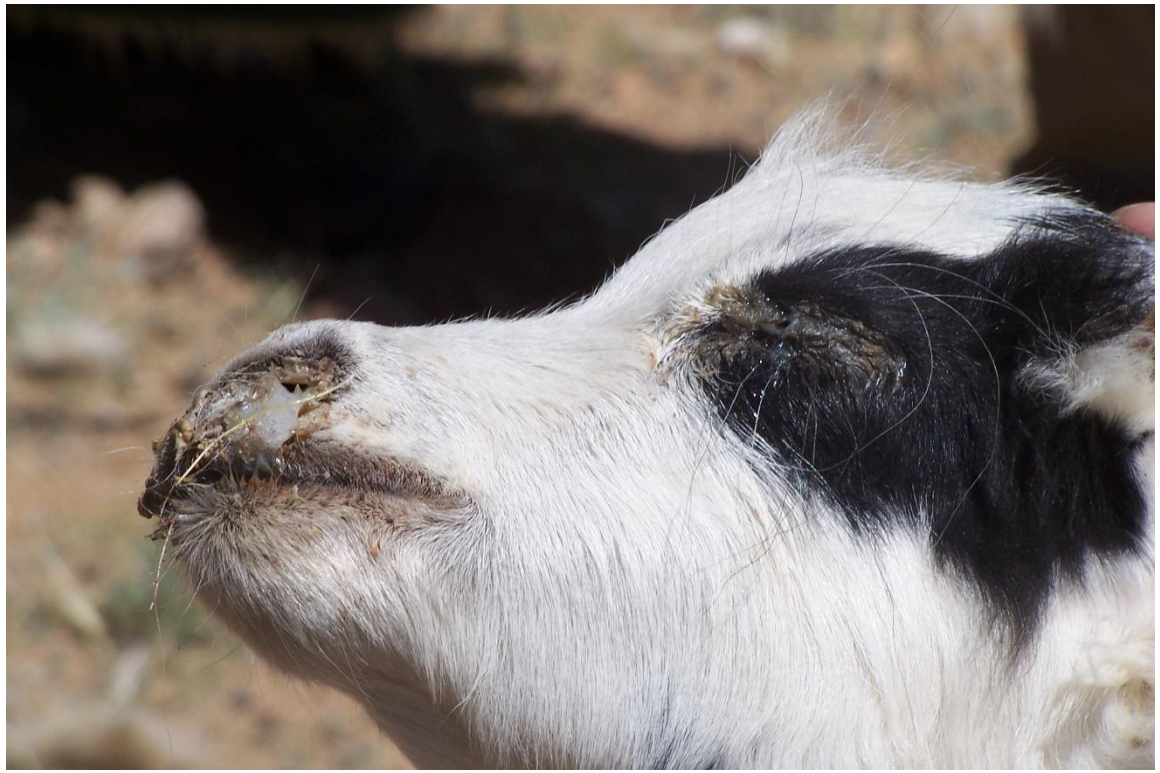
Transmission of PPR

- PPR virus is highly contagious
- It can be transmitted directly via airborne droplets, secretions, and faeces
- Sheep and goats are the primary hosts
- Wildlife species also affected

2. Field diagnostics

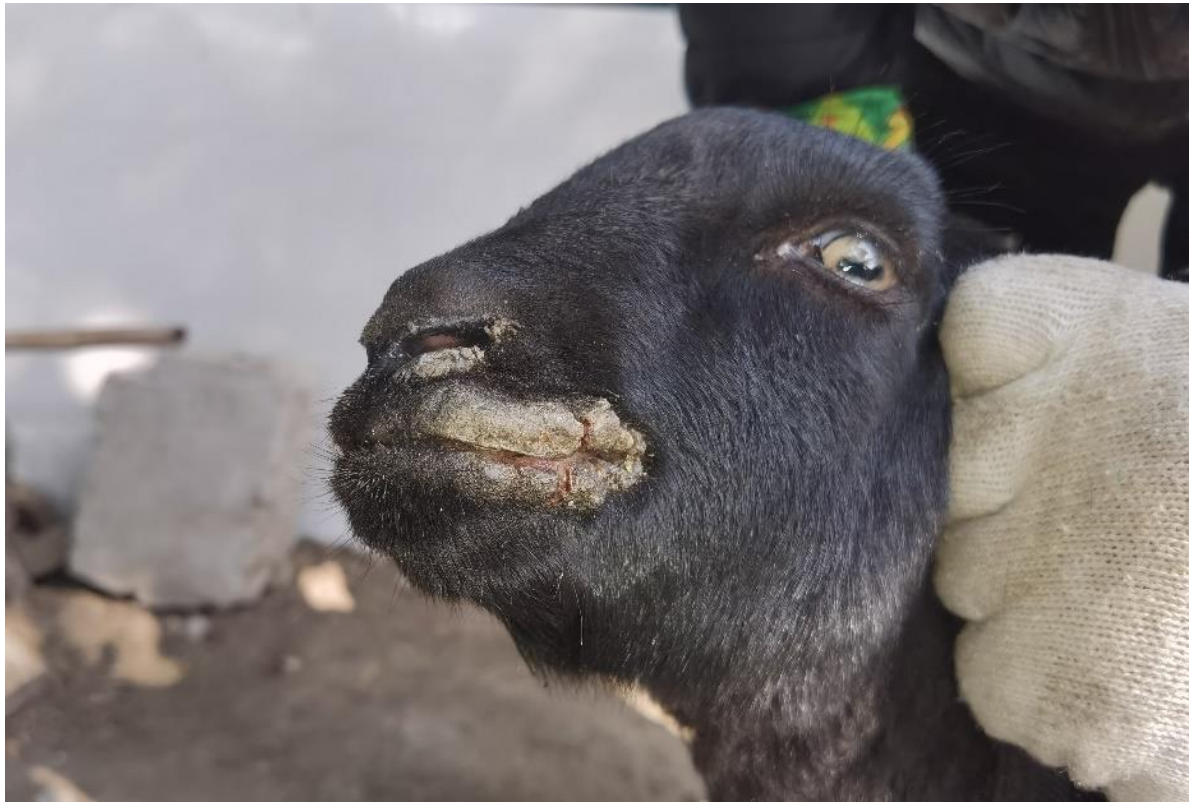
Clinical diagnostics

- Fever up to 41°C that can last for 3–5 days
- Serous oculonasal discharges become progressively mucopurulent and persist for around 14 days



Clinical diagnostics

- The gums become hyperaemic, and erosive lesions develop in the oral cavity with excessive salivation



Clinical diagnostics

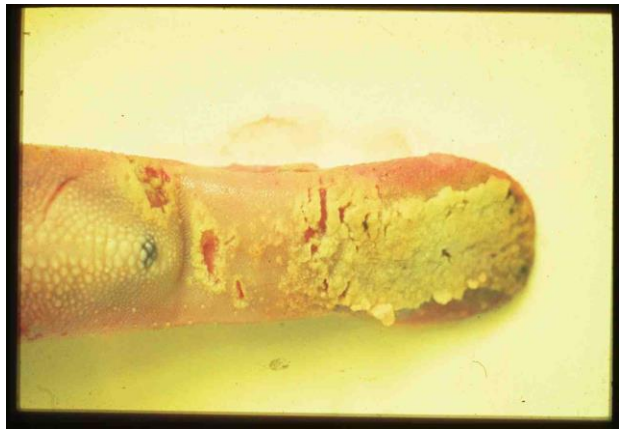
- A watery blood-stained diarrhoea is common in the later stage.
- Pneumonia, coughing, pleural rales and abdominal breathing also occur.

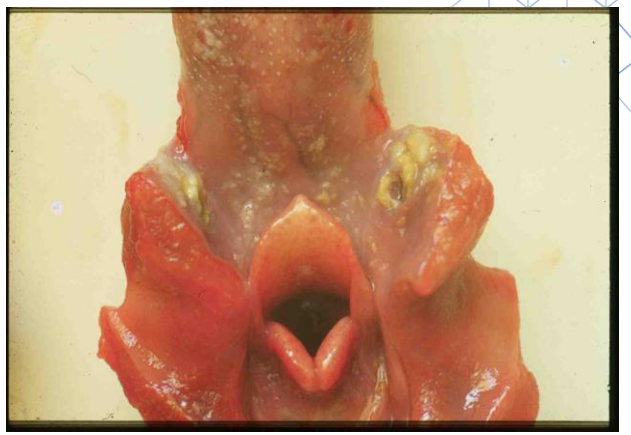
Clinical diagnostics

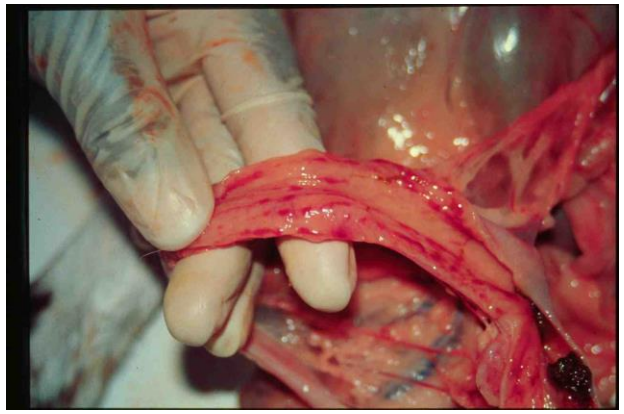
- The morbidity rate can be up to 100% with very high case fatality in severe cases.
- However, morbidity and mortality may be much lower in milder outbreaks, and the disease may be overlooked.

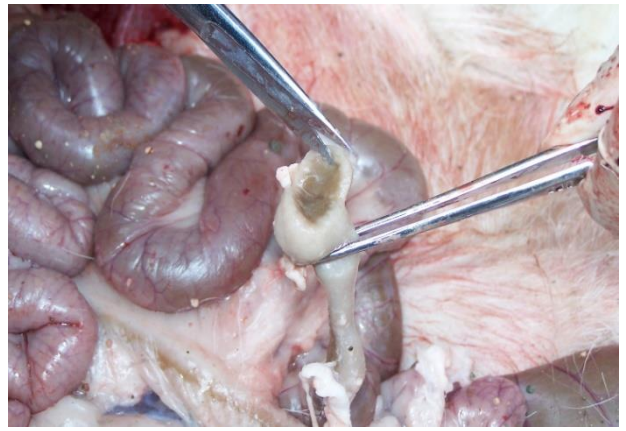
Necropsy lesions

- Erosive lesions from the mouth to the reticulo–rumen junction.
- Congestion, oedema and ulceration of digestive tract mucosa
- Enlarged lymph nodes
- Necrotic Peyer’s patches
- Necrotic spleen and liver









Field diagnostics

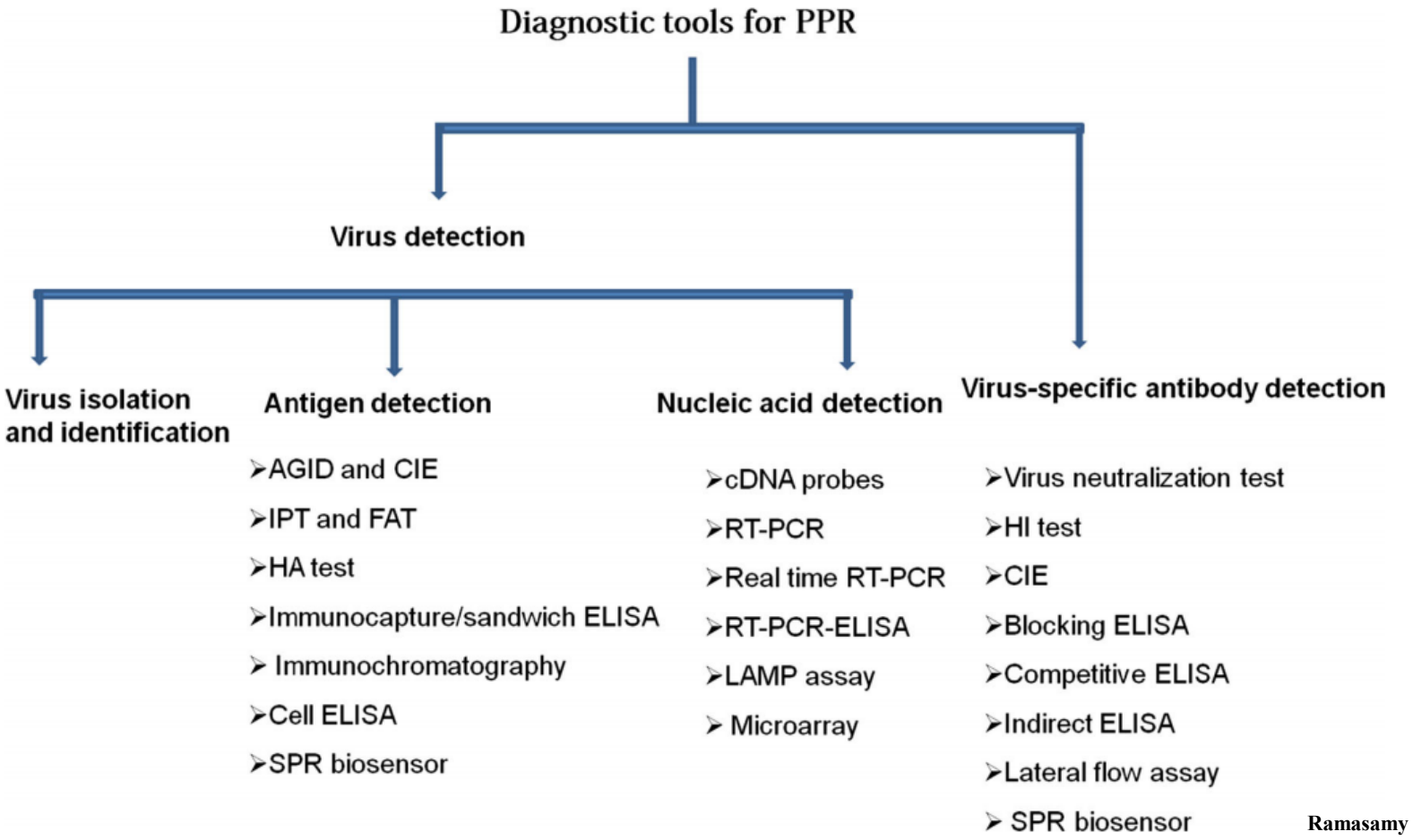
- As with many diseases, the primary diagnosis of PPR is made by field animal health workers (veterinarian, technician, etc.).
- It is important to inform them about PPR clinical and pathological findings and differential diagnosis with similar diseases.

Collection and transportation of samples

- In live animals
  - Oral, nasal, ocular or rectal swabs are collected
  - Serum
  - During the very early stage of the disease, whole blood is also collected in anticoagulant
- At necropsy, samples from 2-3 animals should be collected
  - Lymph nodes, especially the mesenteric and bronchial nodes
  - Lungs, spleen and intestinal mucosae
- Samples could be frozen and transported on ice
- Samples for histopathology test are placed in 10% formalin

3. PPR laboratory diagnostics

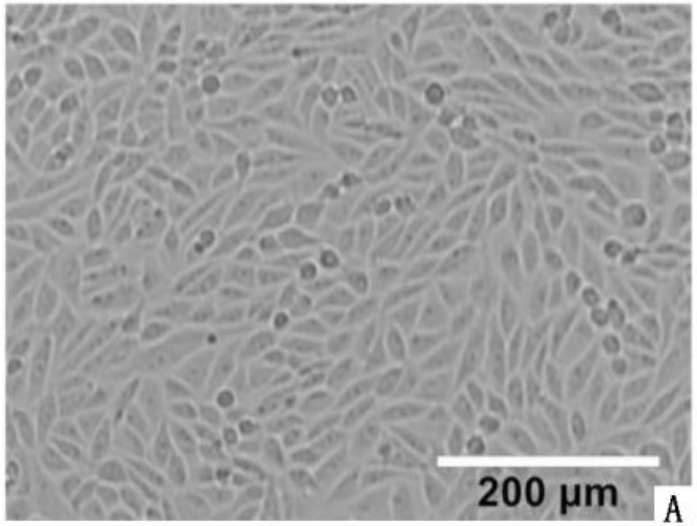
Tools for PPR diagnostics



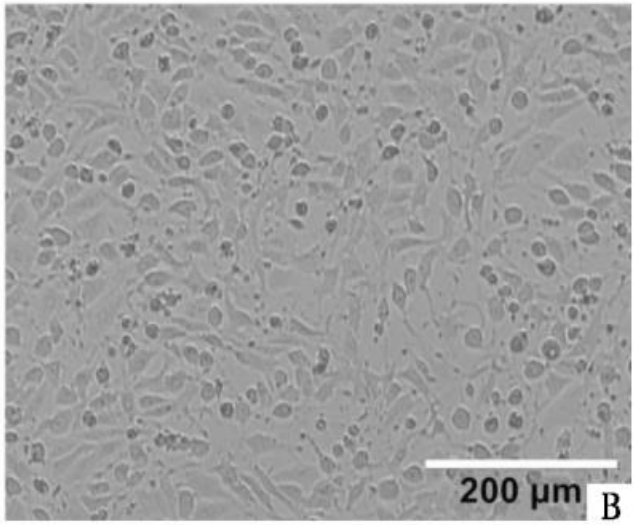
Ramasamy et al. Peste des petits ruminants diagnosis and diagnostic tools at a glance: perspectives on global control and eradication

Virus isolation

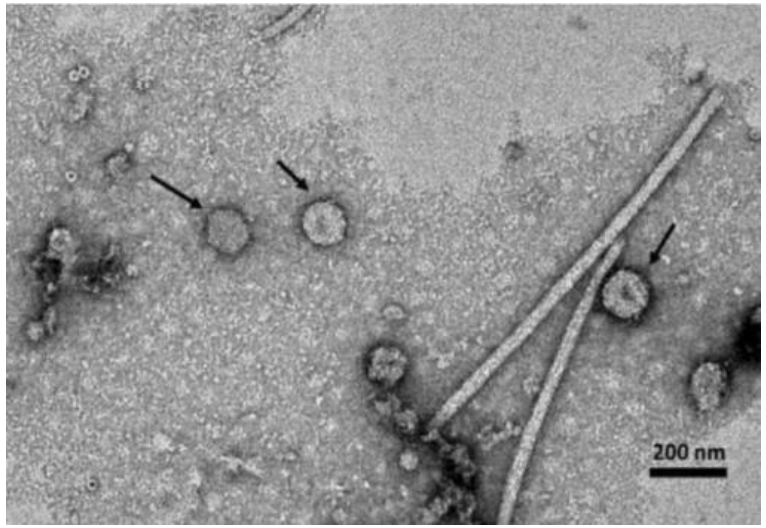
- BSL-3 laboratory needed
- Samples: ocular, nasal, oral and rectal swabs, and tissue samples exhibiting lesions
- Cells: Vero, Dog-Slam-Vero, B95a, goat/sheep kidney cells
- Less sensitive than RT-PCR
- Time consuming: 7-10 days
- Skilled technician needed



Normal Vero cells



Vero cells after the PPRV inoculation (24h)



PPRV under electron microscopy

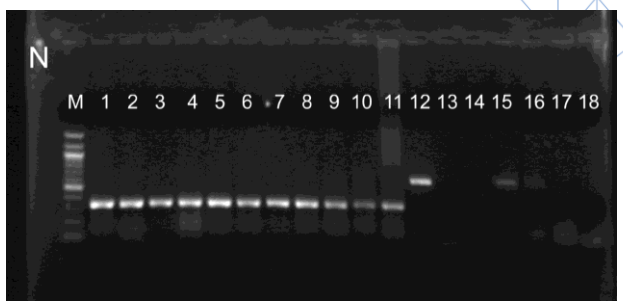
Xing et al.

RT-PCR

- N-gene based RT-PCR
  - Target: N gene
  - Primers: NP3/NP4
  - Length of product: 351bp

Primer	Sequence
NP3:	5’-GTC-TCG-GAA-ATC-GCC-TCA-CAG-ACT-3’;
NP4:	5’-CCT-CCT-CCT-GGT-CCT-CCA-GAA-TCT-3’.

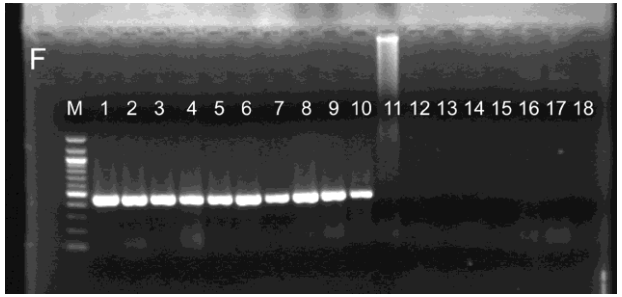
Couacy-Hymann et al. 2002



- F-gene based RT-PCR
  - Target: F gene
  - Primers: F1b/F2d
  - Length of product: 448bp

Primer	Sequence
F1b	5’-AGT-ACA-AAA-GAT-TGC-TGA-TCA-CAG-T-3’
F2d	5’-GGG-TCT-CGA-AGG-CTA-GGC-CCG-AAT-A-3’

Forsyth & Barrett, 1995



- All lineages of PPRV could be detected
- The lowest detection limit: 100 viral genome copies/reaction

Sensitivity of PPRV RT-PCR test

- The lowest detection limit: 10^{-3} $TCID_{50}$ PPRV RNA
- Detect PPRV in the swabs as early as 3 dpi

Comparison of the RT-PCR to the classical cell cytopathic (cpe) method to detect PPRV

Quantity of detected virus	PCR	cpe
100 $TCID_{50}$	+	+
10 $TCID_{50}$	+	+
1 $TCID_{50}$	+	+
0.1 $TCID_{50}$	+	−
0.01 $TCID_{50}$	+	−
0.001 $TCID_{50}$	+	−
0.0001 $TCID_{50}$	−	−
0.00001 $TCID_{50}$	−	−

Serial dilutions of the PPRV vaccine suspension were made from 10^{2} to 10^{-6} $TCID_{50}$/ml. The different diluted suspensions were submitted to either titration on Vero cells or to RT-PCR as indicated in Section 2.

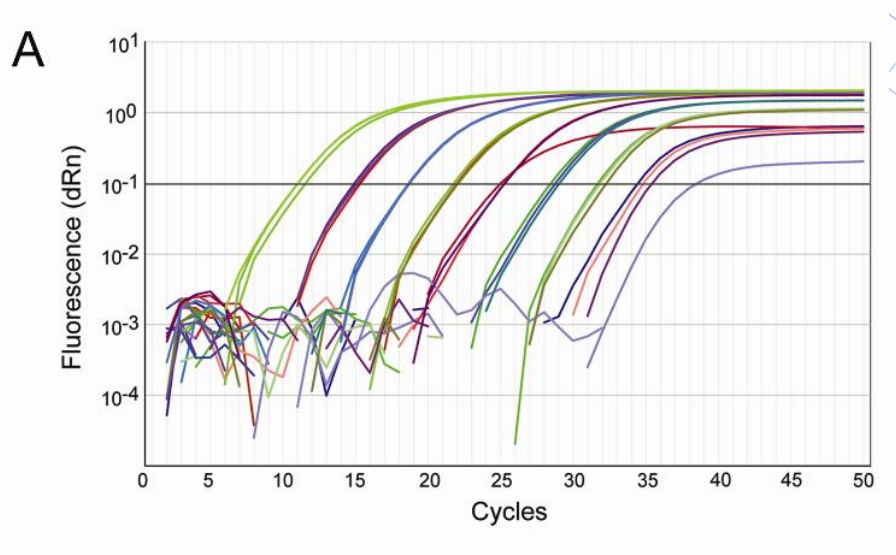
Results of RT-PCR technique along with those obtained with the hybridisation reaction on samples from infected goats.

	Days 0–2			Day 3			Day 4			Day 5			Days 6–9		
	O	N	M	O	N	M	O	N	M	O	N	M	O	N	M
Côte-d'Ivoire 89 (5)				+	+	−	+	+	+	+	+	+			
Nigeria 75/1 (5)				−	−	−	−	+	−	+	+	−			
Sudan-Sennar (5)		−		−	−	−	+	−	−	+	−	+		+	
India-Calcutta (5)				+	+	−	+	+	+	+	+	+			

O: ocular secretion; N: nasal secretion; M: mouth secretion; (5): five challenged goats; −: negative results; +: positive results.

Real-time fluorescence quantitative PCR, qRT-PCR

- Based on N gene or F gene
- All lineages of PPRV could be detected
- The lowest detection limit: 10 viral genome copies/reaction
- Quantitative detection, high sensitivity and specificity
- High cost real-time PCR machine required



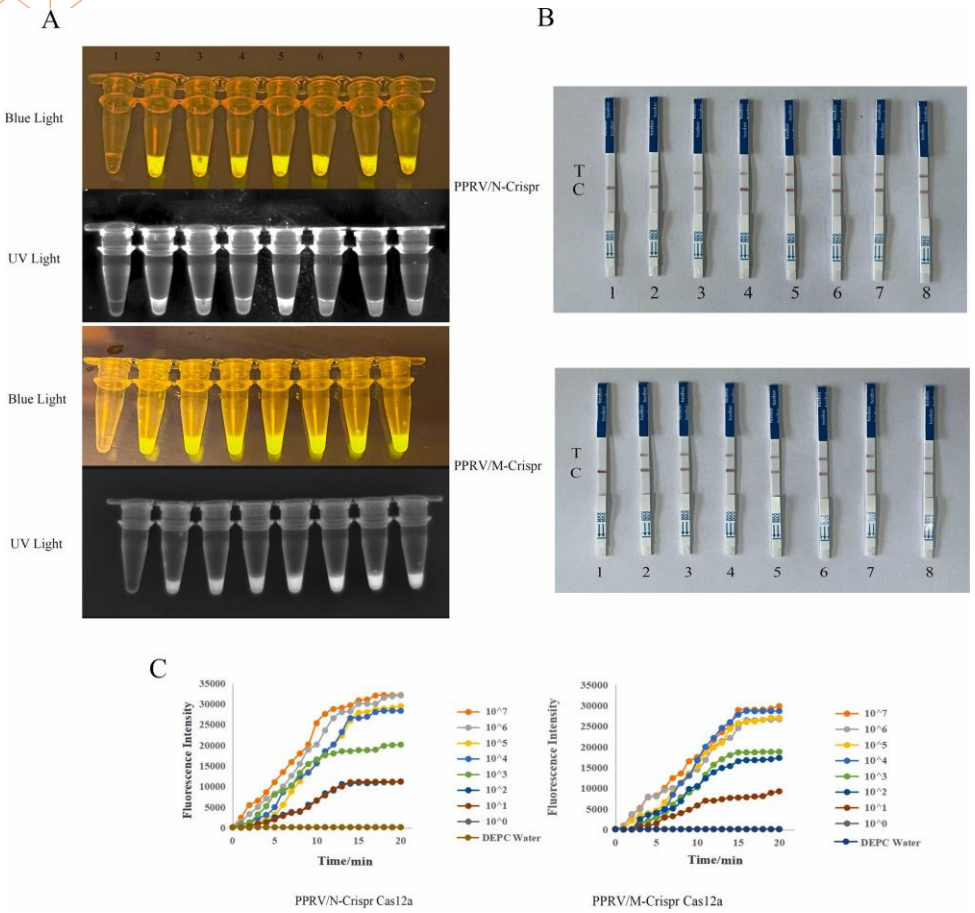
Primers and Probes

Flannery: PPRV F-gene Fwd	CAT AGS ACT GGC AGC TTG CA	20
Flannery: PPRV F-gene Rev	GAG CCC TGG GTT GAT TTT RG	20
Flannery: PPRV F-gene Probe	FAM-CTT GTC ACA TTA ATA TGC TG-MGB	10

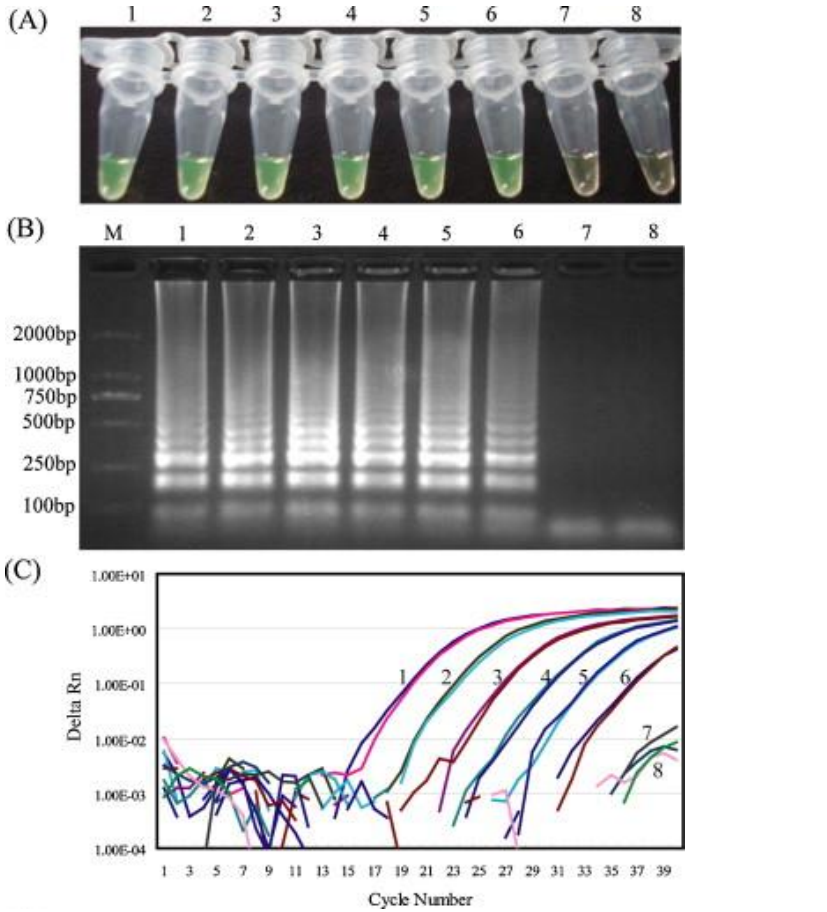
Batten PPRV N-gene Fwd	AGA GTT CAA TAT GTT RTT AGC CTC CAT	10
Batten PPRV N-gene Rev	TTC CCC ART CAC TCT YCT TTG T	10
Batten PPRV N-gene Probe	FAM-CAC CGG AYA CKG CAG CTG ACT CAG AA - QSY	5

Point-of-care diagnostic tests for PPRV RNA detection

- RAA-CRISPR Cas12a test for PPRV detection

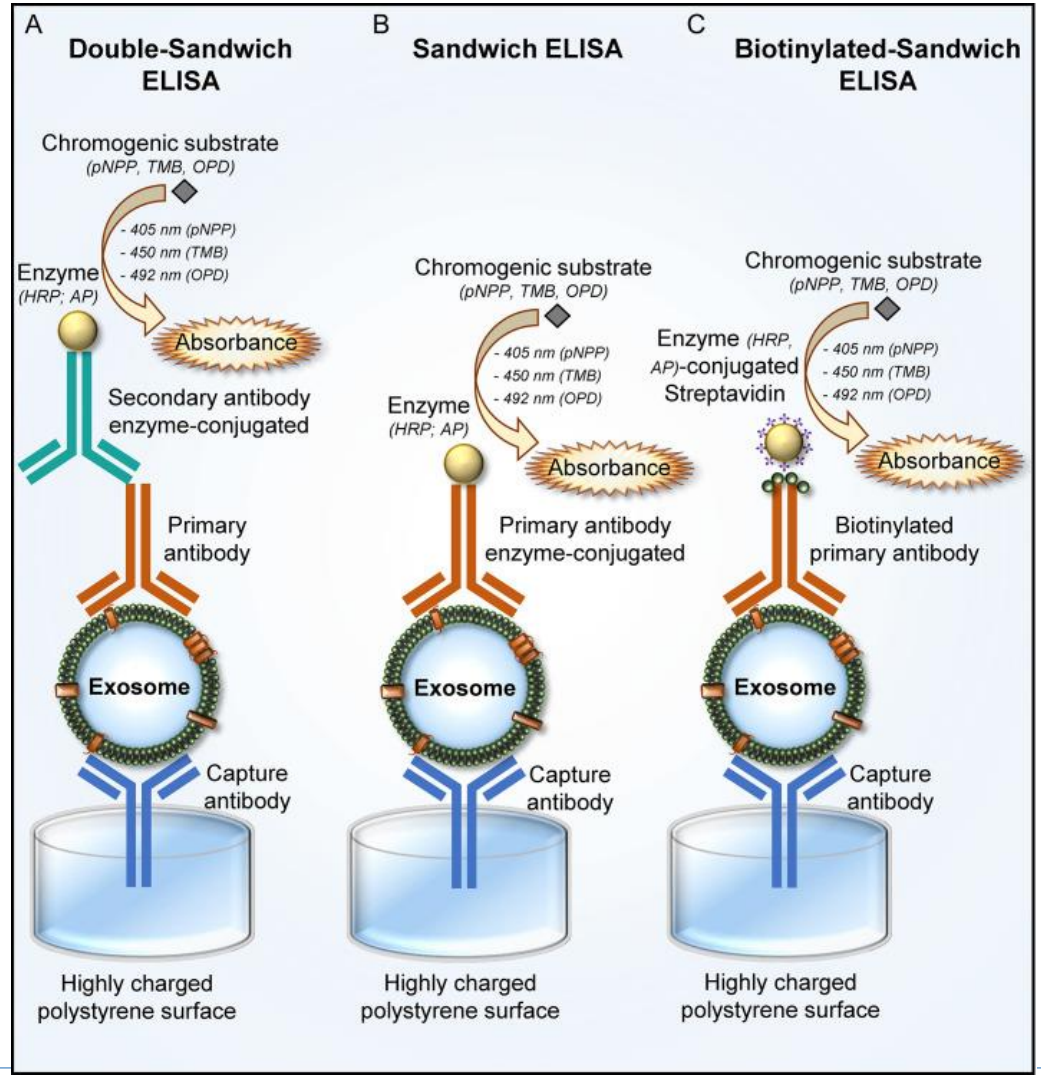


RT-LAMP test for PPRV detection



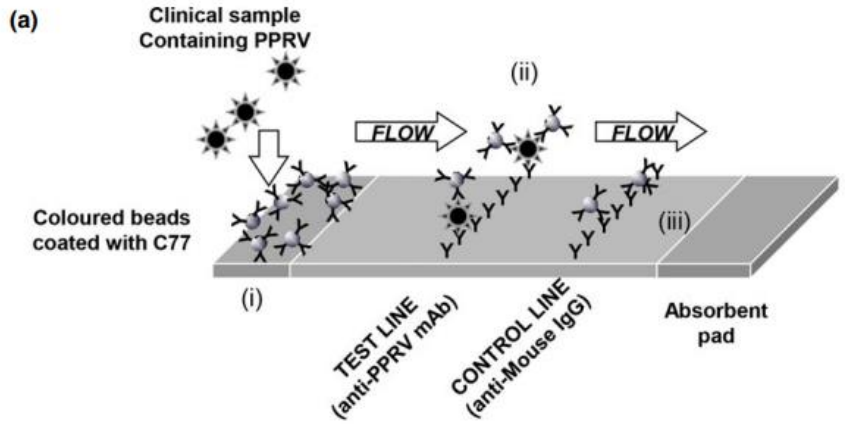
Immunocapture-ELISA

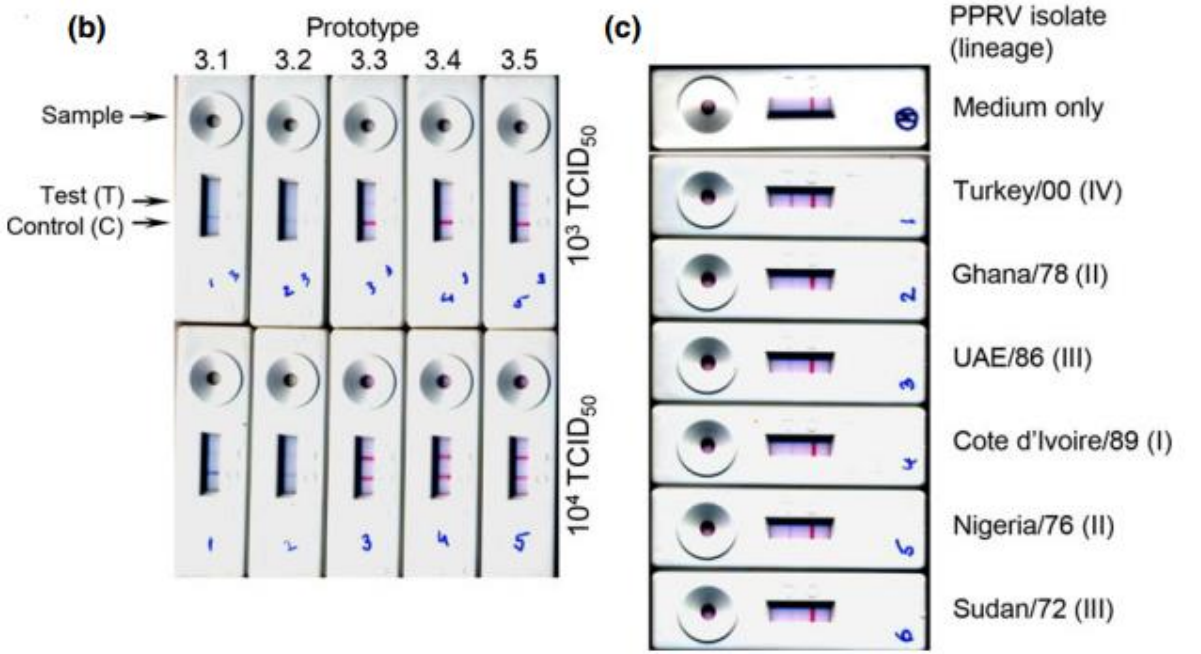
- Using PPRV-N Mab
- All lineages of PPRV could be detected
- The lowest detection limit: $10^{0.6}$ $TCID_{50}$ PPRV
- High cost



The LFD-based PPRV antigen detection test

- Using PPRV-H Mab
- All lineages of PPRV could be detected
- The lowest detection limit: 10^3 $TCID_{50}$ PPRV
- Samples: swabs, tissue extracts
- Pen-side detection

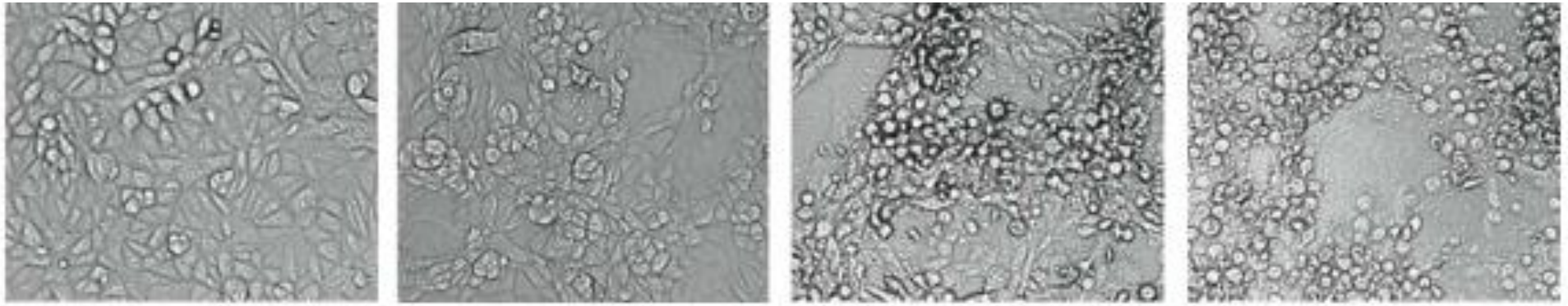




Baron et al. Development and Testing of a Field Diagnostic Assay for Peste des Petits Ruminants Virus

Virus neutralization

- Sensitive and specific for PPRV antibody detecting
- Time consuming, 7-10 days



CPE of PPRV on Vero cells

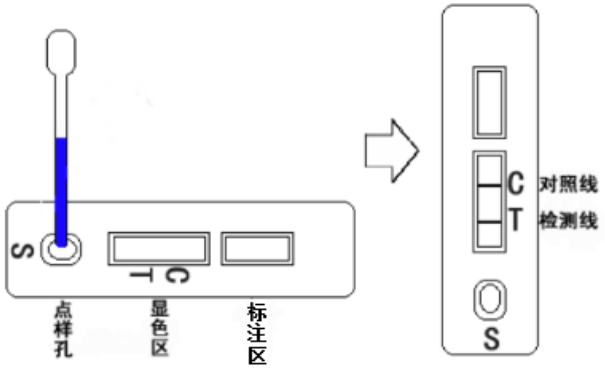
ELISA assays for antibody detection

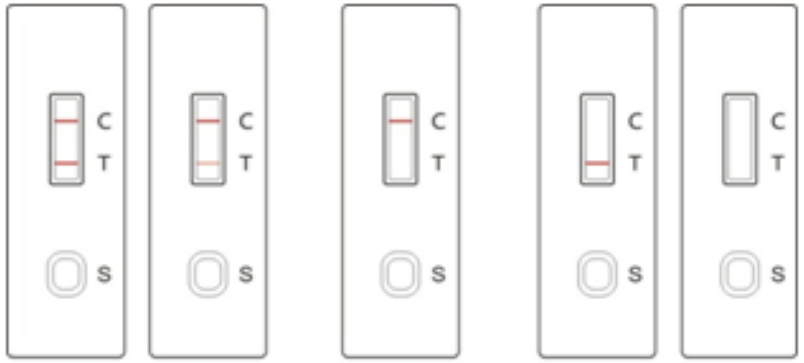
- Recognising PPRV N protein or H protein
- Competitive ELISA is recommended by WOAH

Diagnostic tests	Application	Advantages	Disadvantages	Relative sensitivity/ detection limit
Blocking ELISA	Seromonitoring and serosurveillance	User-friendly, Routine diagnosis, doesn't require sterile precautions	Less sensitive than virus neutralization test (VNT)	90.4 % compared to VNT
Competitive ELISA (Recommended by WOAH)	Seromonitoring and serosurveillance	User-friendly, Routine diagnosis, doesn't require sterile precautions	Less sensitive than VNT	90 to 95 % compared to VNT
Indirect ELISA	Alternative tool for antibody detection	Useful if hybridoma clone used for competitive ELISA is lost	Less sensitive than competitive ELISA, requires species-specific antibody conjugate	Less sensitive than competitive ELISA, requires species-specific antibody conjugate

The LFD-based PPRV antibody detection test

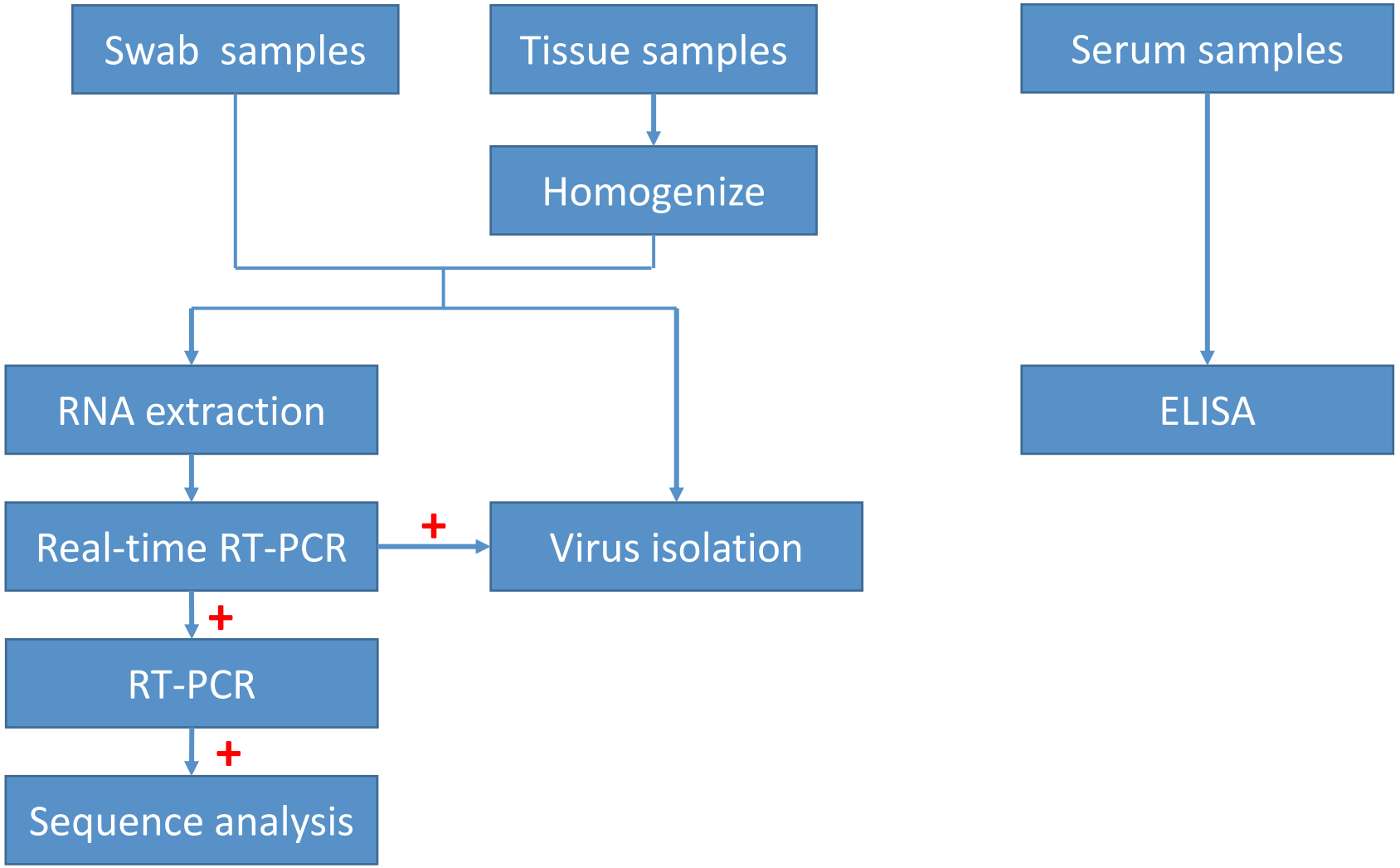
- Based on PPRV N protein Mab
- Samples: serum
- Pen-side detection
- 5-10 minutes





Positive Negative Invalid

Diagnostic workflow for the detection of PPR



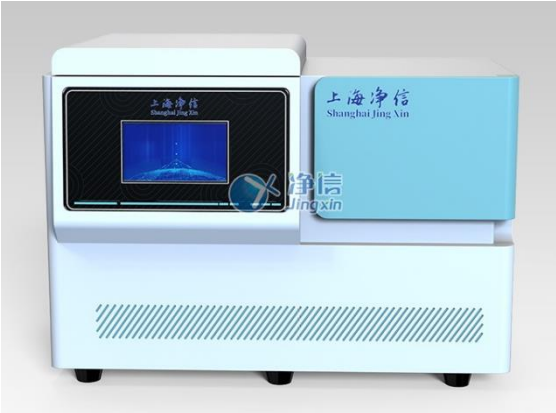
Defination of PPRV infection

- *WOAH Terrestrial Animal Health Code*, Chapter 14.7
- The following defines the occurrence of PPRV infection
- 1) PPRV, excluding vaccine strains, has been isolated and identified as such from a domestic sheep or goat or a product derived from it;
- Or 2) antigen or ribonucleic acid specific to PPRV, excluding vaccine strains, has been identified in samples from a domestic sheep or goat
  - showing clinical signs consistent with PPR, or
  - epidemiologically linked to an outbreak of PPR, or
  - giving cause for suspicion of association or contact with PPR
- Or 3) antibodies to PPRV antigens which are not the consequence of vaccination, have been detected in a domestic sheep or goat with
  - either epidemiological link to a confirmed or suspected outbreak of PPR
  - or showing clinical signs consistent with recent infection of PPRV

Automatic instruments for highthrougput detection of PPRV

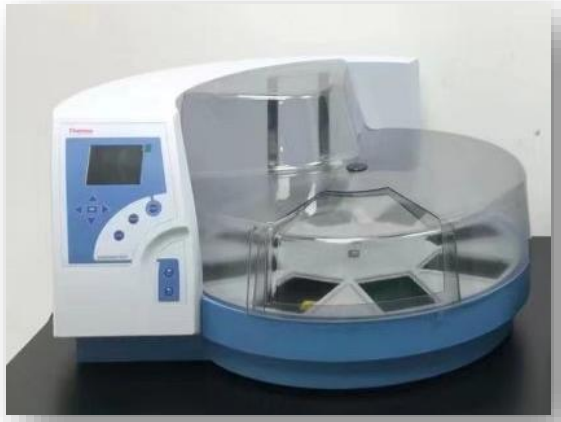
Choice of diagnostic tools depending on the skill levels of the diagnostician and the equipment available in the test laboratory

Homogenize 24 samples in 5 minutes



Homogenizer

RNA extraction of 96 samples in 30 minutes



NA extraction machine

Real-time RT-PCR for 96/384 samples in 1.5 hours

Real-time PCR machine

Qualification of the diagnostic laboratory

- ISO 17025 certification is recommended for a diagnostic laboratory for PPR detection
- Virus isolation of PPRV should be conducted in a BSL-3 laboratory

Case or outbreak confirmation

- When the disease was first introduced into a country, clinical samples could be sent to an international reference laboratory for confirmation

No: PPRL20160010

检 验 报 告

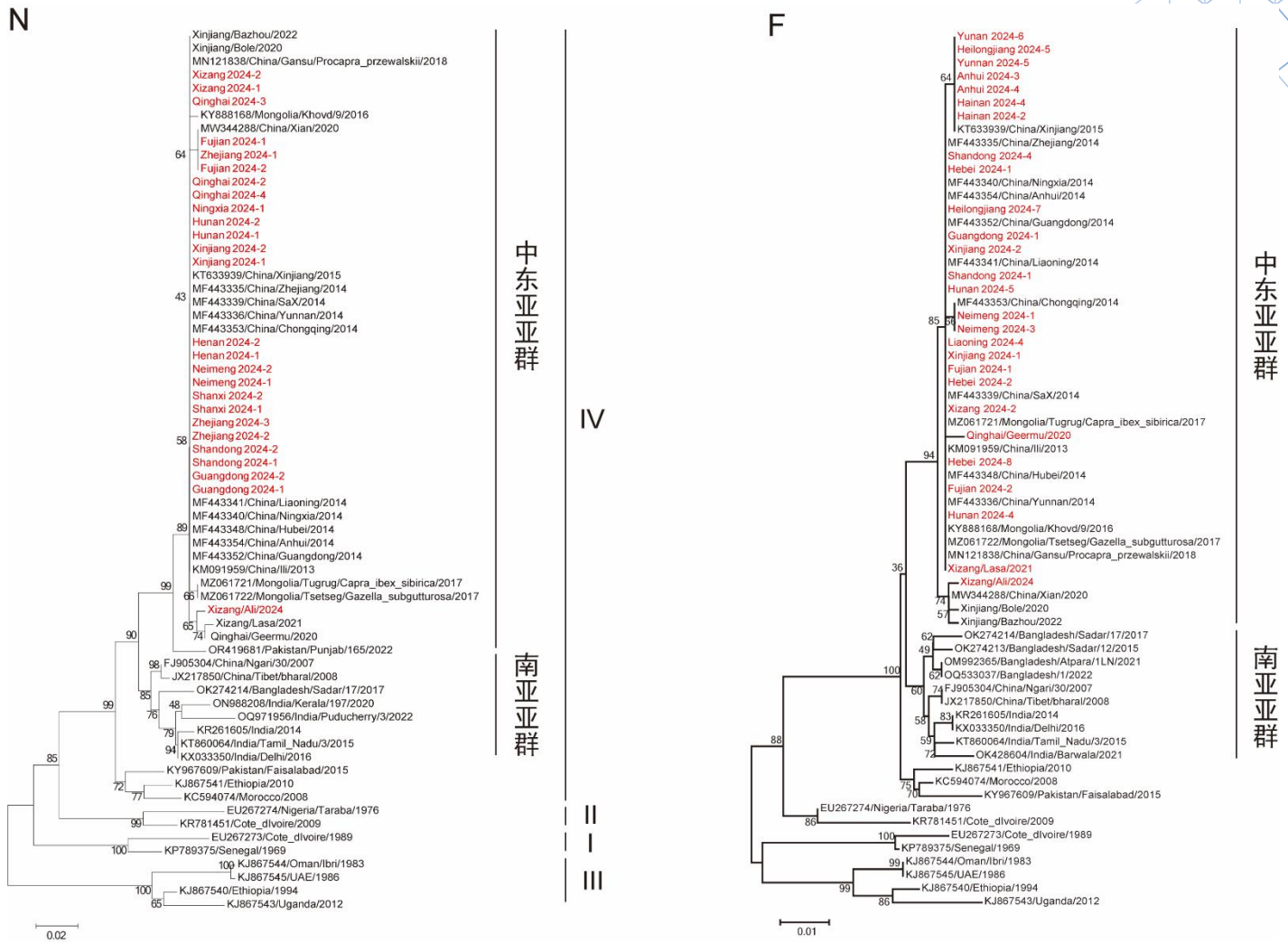
TEST REPORT

样品名称	羊血清、组织、分泌物
Name of Product	Serum, Tissue and Discharge of *Caprina*
受检单位	蒙古国中央兽医实验室
Testee	State Central Veterinary Laboratory, Mongolia
检验类别	委托检验
Test Category	Entrusted inspection

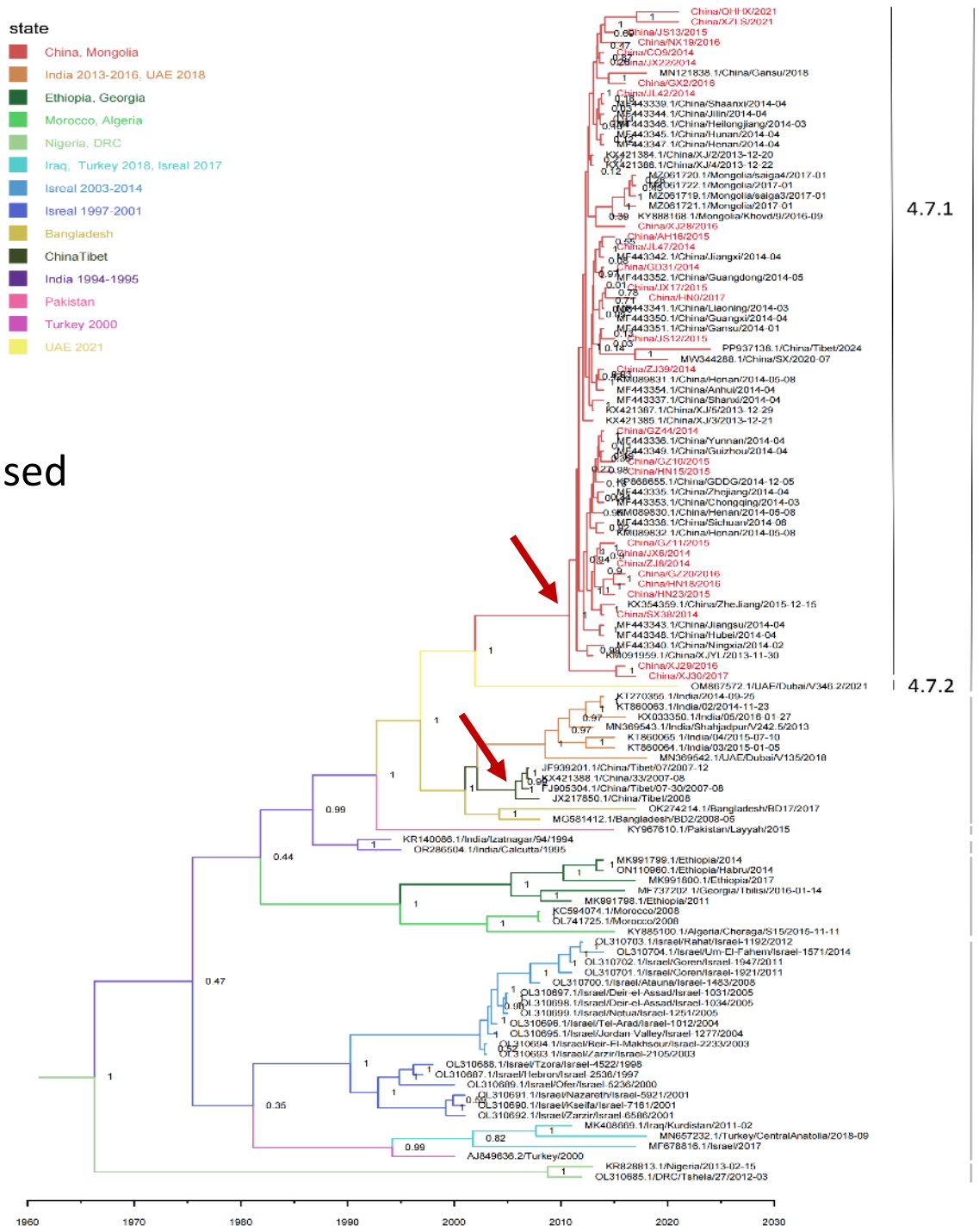
中国动物卫生与流行病学中心
国家外来动物疫病诊断中心
OIE 小反刍兽疫参考实验室
China Animal Health and Epidemiology Center
National Diagnostic Center for Exotic Animal Diseases
OIE Reference Laboratory for *Peste des Petits Ruminants*

Virus characterization and clustering

- N and F partial gene sequence could be used for lineage identification for circulating virus strain



- Genome sequences could be used for tracing the origin of PPRV circulating strain
- PPRV LIV have evolved into 7 clades with distinct spatial correlation



4.7.1

4.7 East Asia & UAE

4.7.2

4.6 South Asia & UAE

4.5 Pakistan

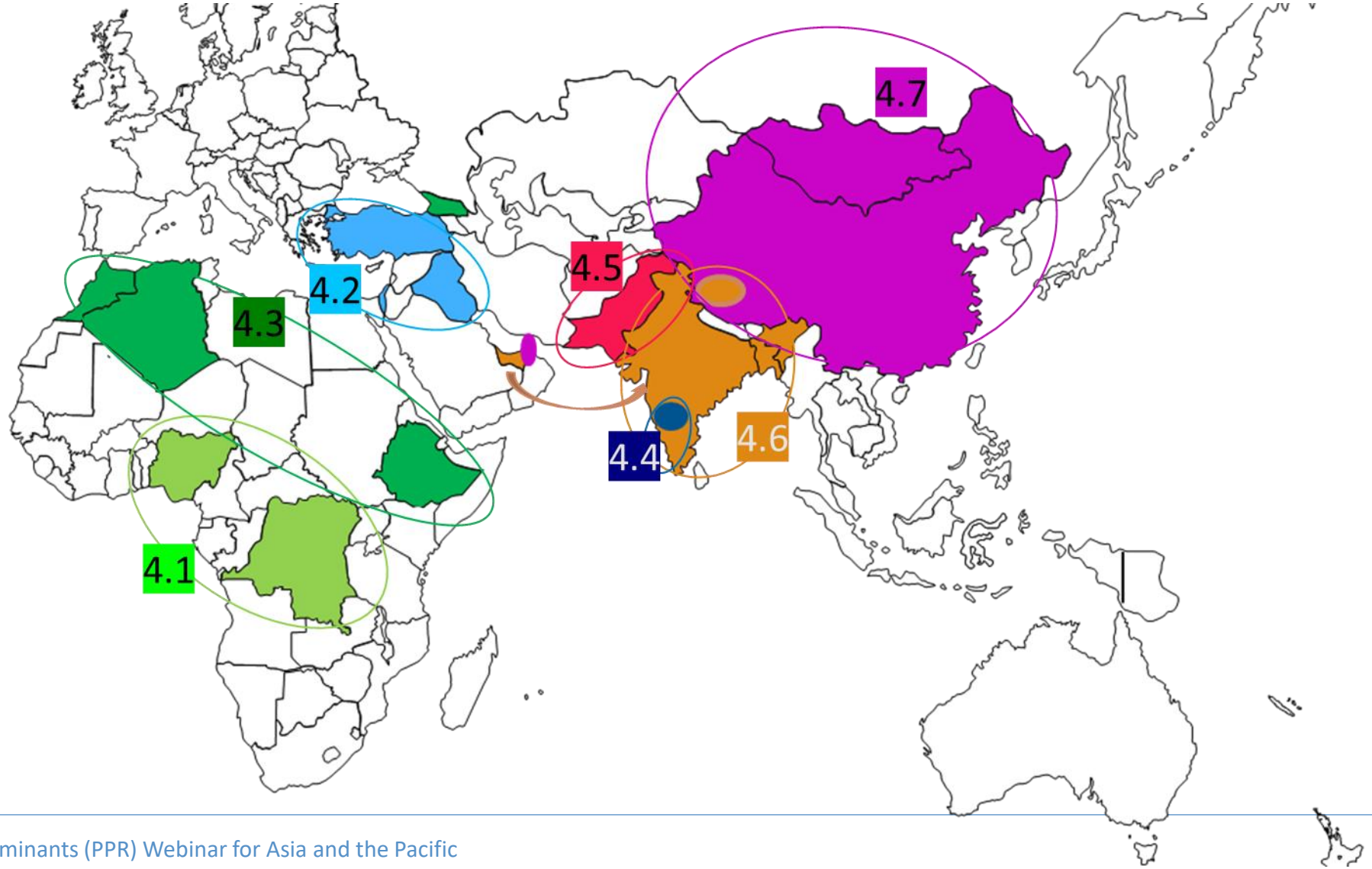
4.4 Early India

4.3 North Africa & Geo

4.2 Middle East

4.1 Central-East Africa

Distribution of 7 clades of lineage IV



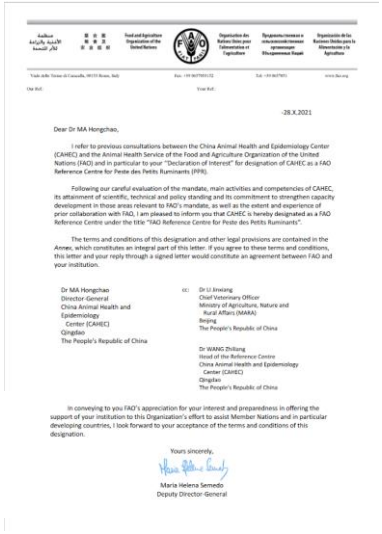
4. A brief introduction of CAHEC

China Animal Health and Epidemiology Center (CAHEC)

- A national animal health institution directly subordinated to the Ministry of Agriculture and Rural Affairs (MARA) of People's Republic of China
- The mandate of the institute
  - Epidemiological investigation and surveillance for major animal diseases
  - Veterinary health evaluation on animal and animal products,
  - Research on animal health laws and regulations
  - Technical measures of prevention and control of major exotic animal diseases

WOAH reference laboratory for Peste des petitis ruminants in CAHEC

- PPR Expert: Dr Zhiliang Wang, Deputy Director-in-General of CAHEC
- ABSL-3 certificated
- ISO 17025 certificated
- WOAH reference laboratory for Peste des petitis ruminants (2004)
- FAO reference center for Peste des petitis ruminants (2021)
- Secretary of the WOAH reference laboratory network (launched 2020)
- China national reference laboratory for Peste des petitis ruminants (2025)



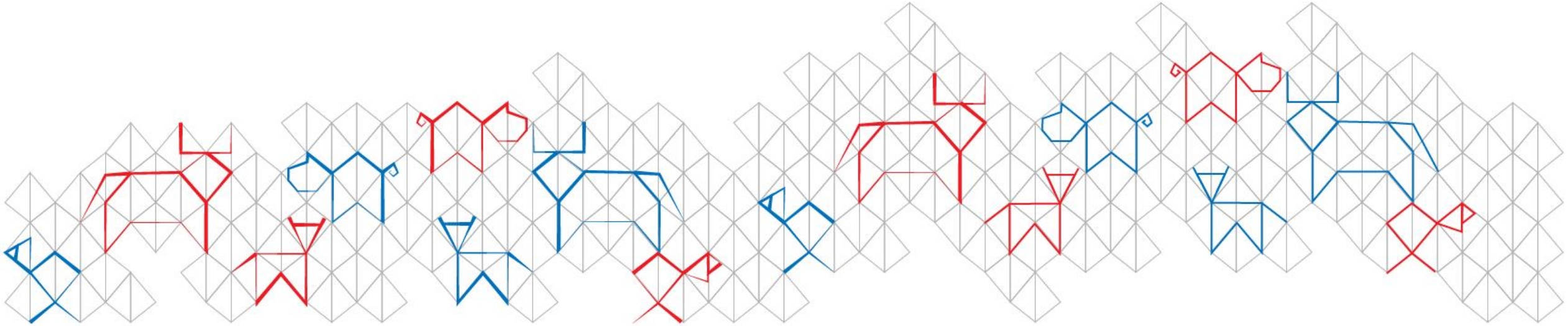
WOAH
Reference Laboratory
Network for PPR

WOAH Asia Regional Training on Peste des Petits Ruminants

Join us this year

Conclusion

- Various serological and molecular diagnostic tests are now available for the detection and surveillance of PPR
- Choice of diagnostic tools depending on the skill levels of the diagnostician and the equipment available in the test laboratory
- Inter-laboratory Proficiency Testing (PT) should be organized to strengthen and harmonize the ability of the laboratories
- Regional laboratory network should be established to harmonize diagnostics techniques



Jingyue Bao, baojingyue@cahec.cn

Thank You!