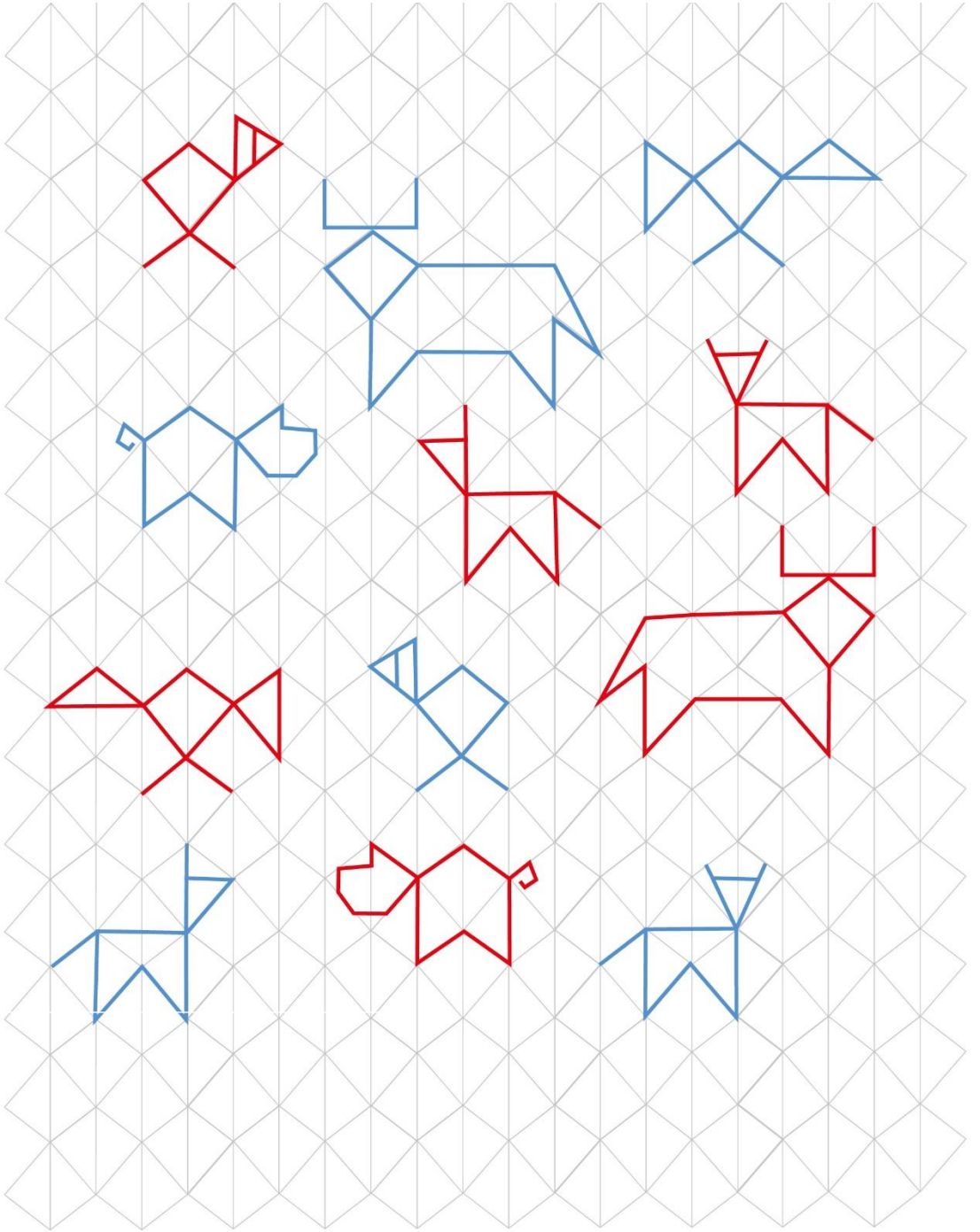
China, Mongolia, and Central Asia Episystem Workshop for Peste des petits ruminants (PPR) eradication

Ulaanbaatar, Mongolia, 1-3 April 2025



With support from:

中华人民共和国农业农村部
Ministry of Agriculture and Rural Affairs of the People's Republic of China

Co-funded by the European Union

PPR molecular epidemiology: Role of molecular epidemiology in identifying Episystem

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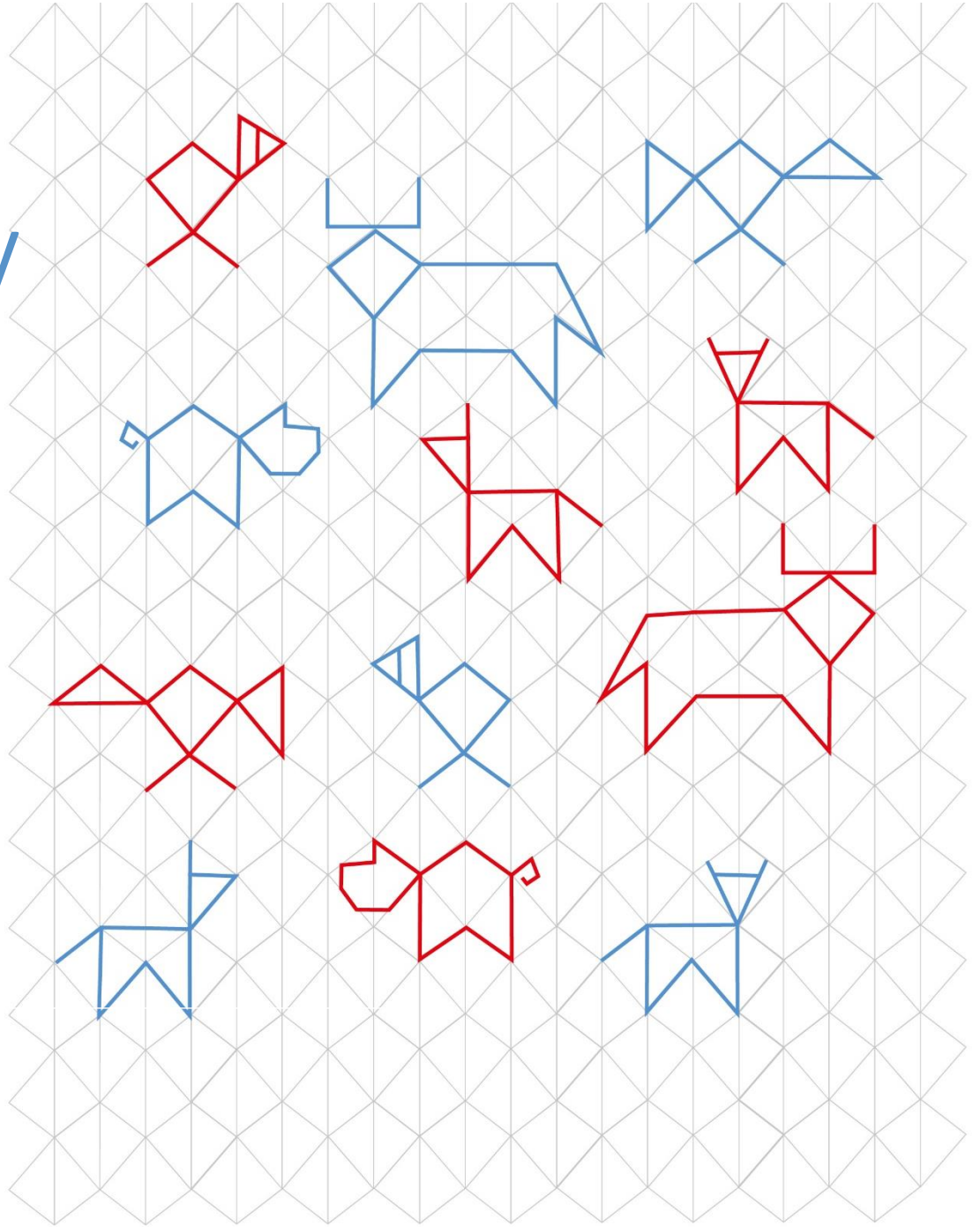
WOAH/FAO/EU reference laboratory for PPR
PPR GREN episystem Working Group leader



cirad
AGRICULTURAL RESEARCH FOR DEVELOPMENT

WOAH
Reference Laboratory Network for PPR

WOAH Reference Laboratory for peste des petits ruminants
Reference Centre
World Organisation for Animal Health
Founded as OIE

With support from:

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Molecular epidemiology in the context of episystem approach

In this context:

Molecular epidemiology

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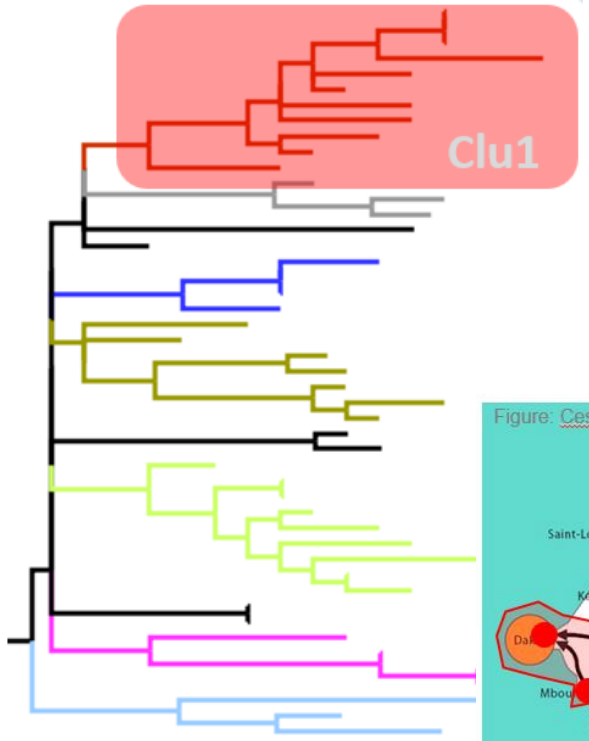
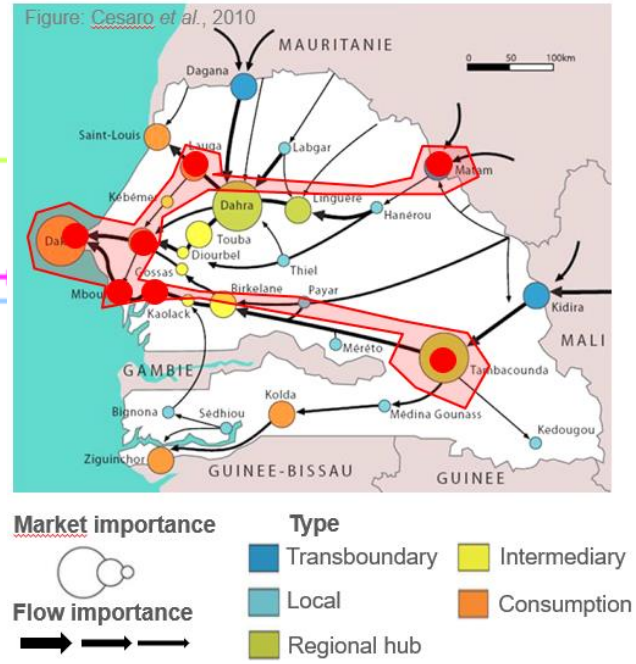
Using virus genetic data to investigate epidemiological links between infected region and disease transmission pathways

Mainly through phylogenetic analyses

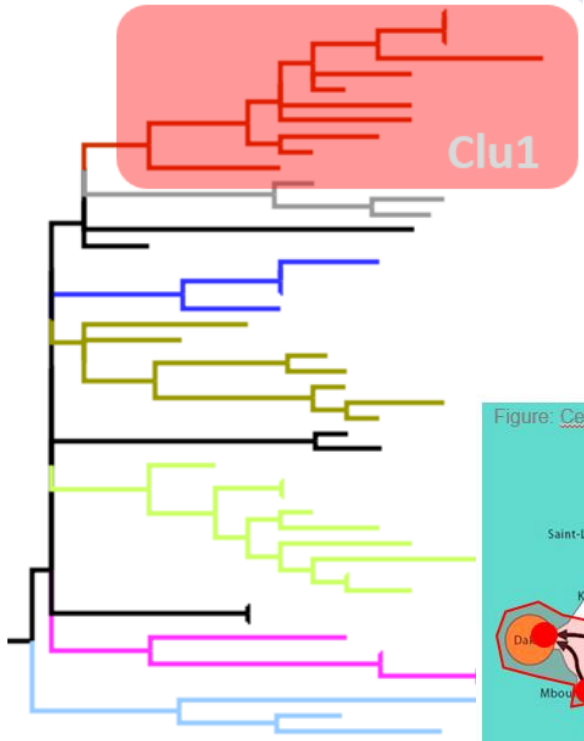
Guidelines available to all through a sharepoint

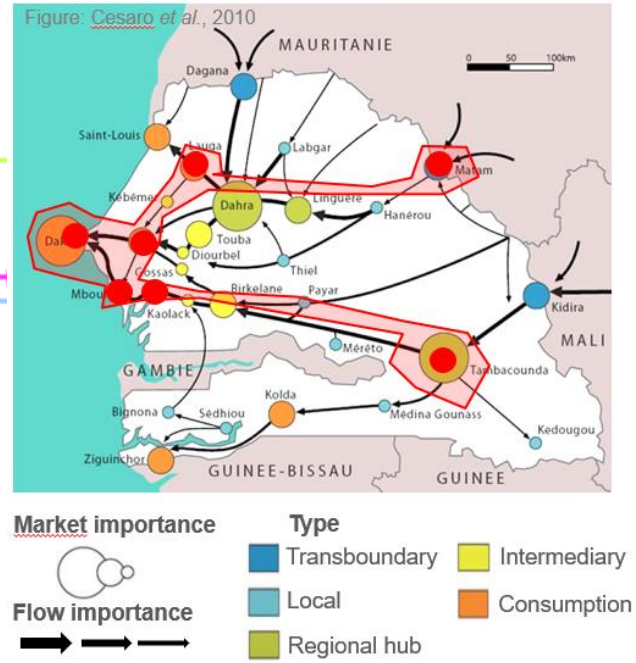




General vision for the use of molecular epidemiology to identify episystems

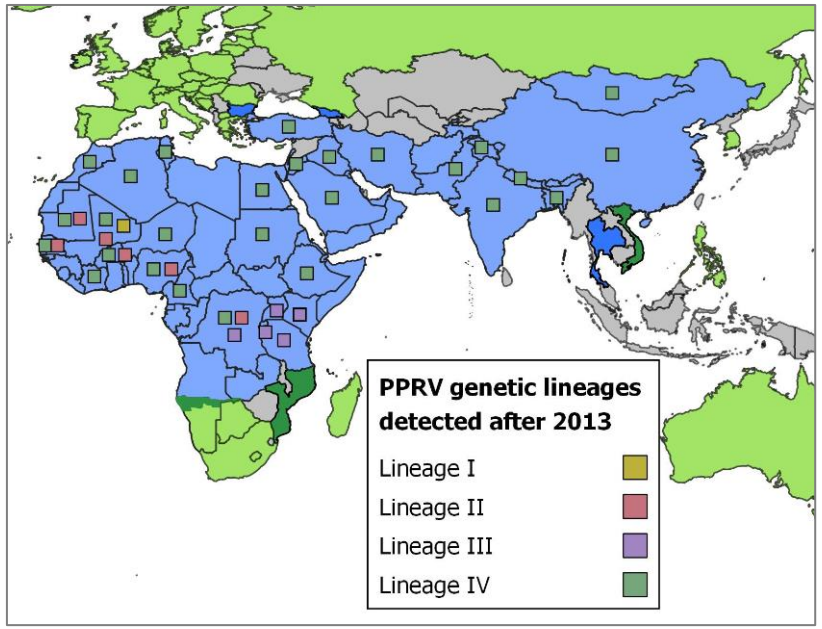
- ***Targeted sampling and sequencing to support*** *analyses performed using animal mobility and epidemiological data*
- ***Confirming / completing*** *episystem identified with other data OR point towards* ***unknown*** *episystem*
- *Using* ***homogenized practices*** *to facilitate data sharing and analyses*
- ***Sharing data*** *through publicly available platform*
- ***Understanding the limitation*** *of the phylogenetic analyses performed*

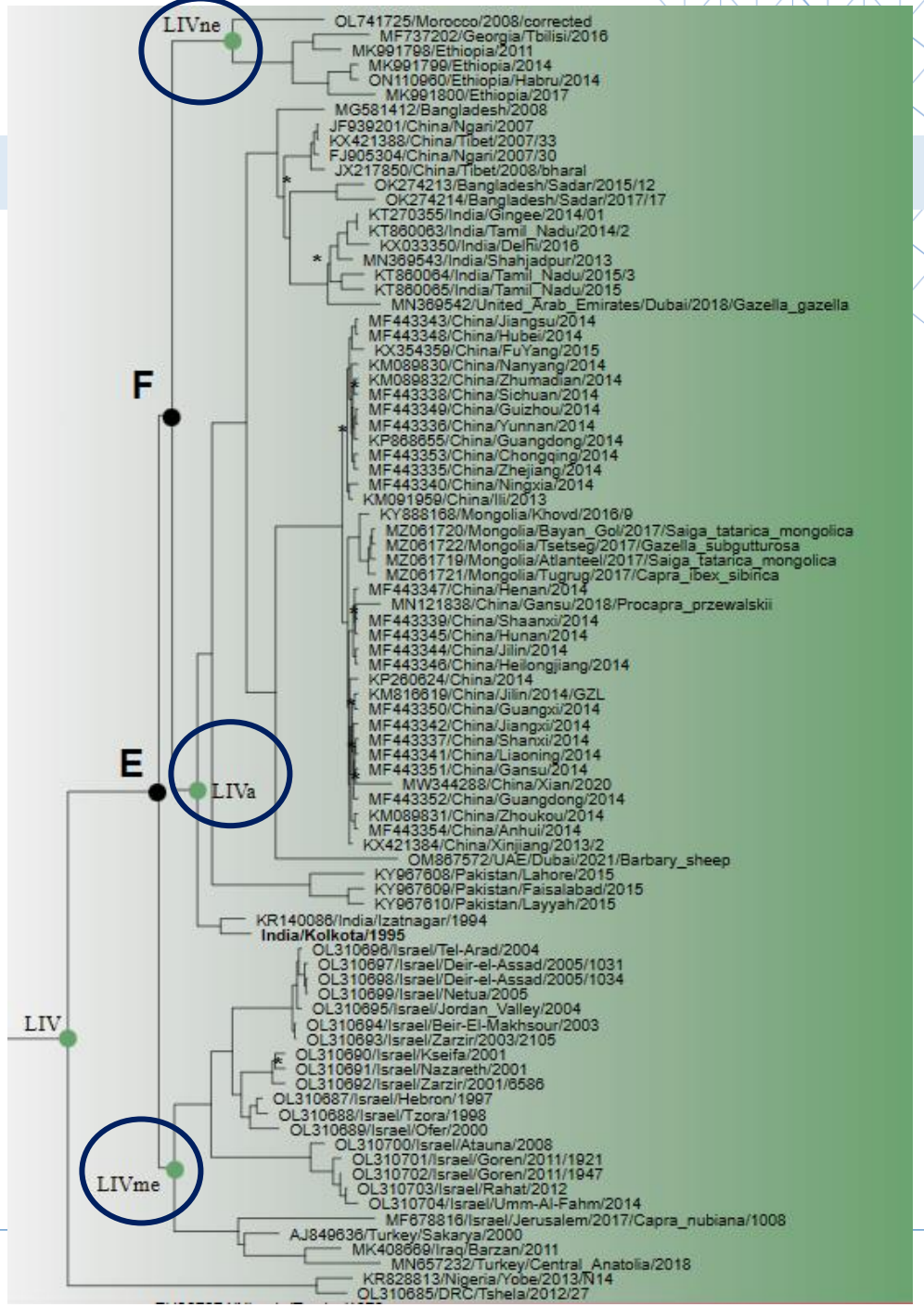




Genetic diversity and distribution of PPRV

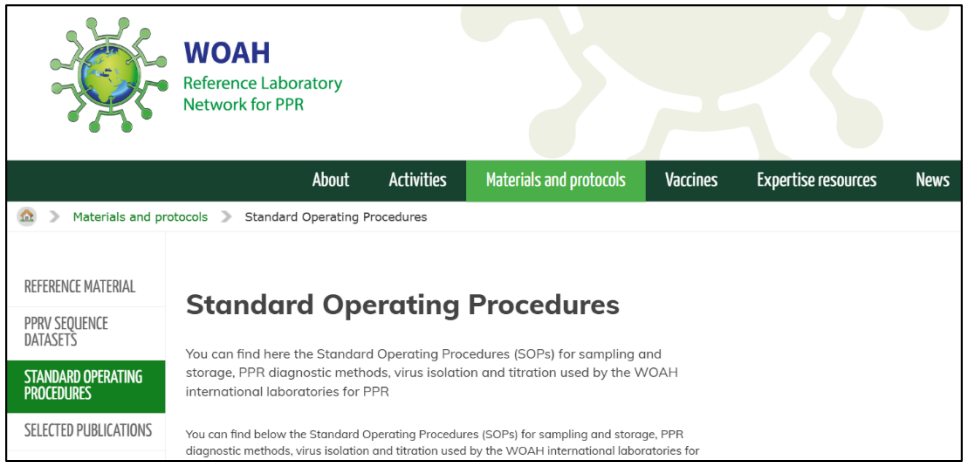
- Four genetic lineages (LI-LIV)
- LIV most widely distributed, only one found in Asia and Middle East
- Identification of geographically-defined sub-lineages
- Some events of long-distance PPRV movement across regions





Sampling collection strategy

- Need personal trained in taking appropriate samples for PCR and PPRV sequencing analyses
- Laboratory analyses should be done in capacitated laboratories (e.g. National ref lab)
- Standard protocols and guidelines available



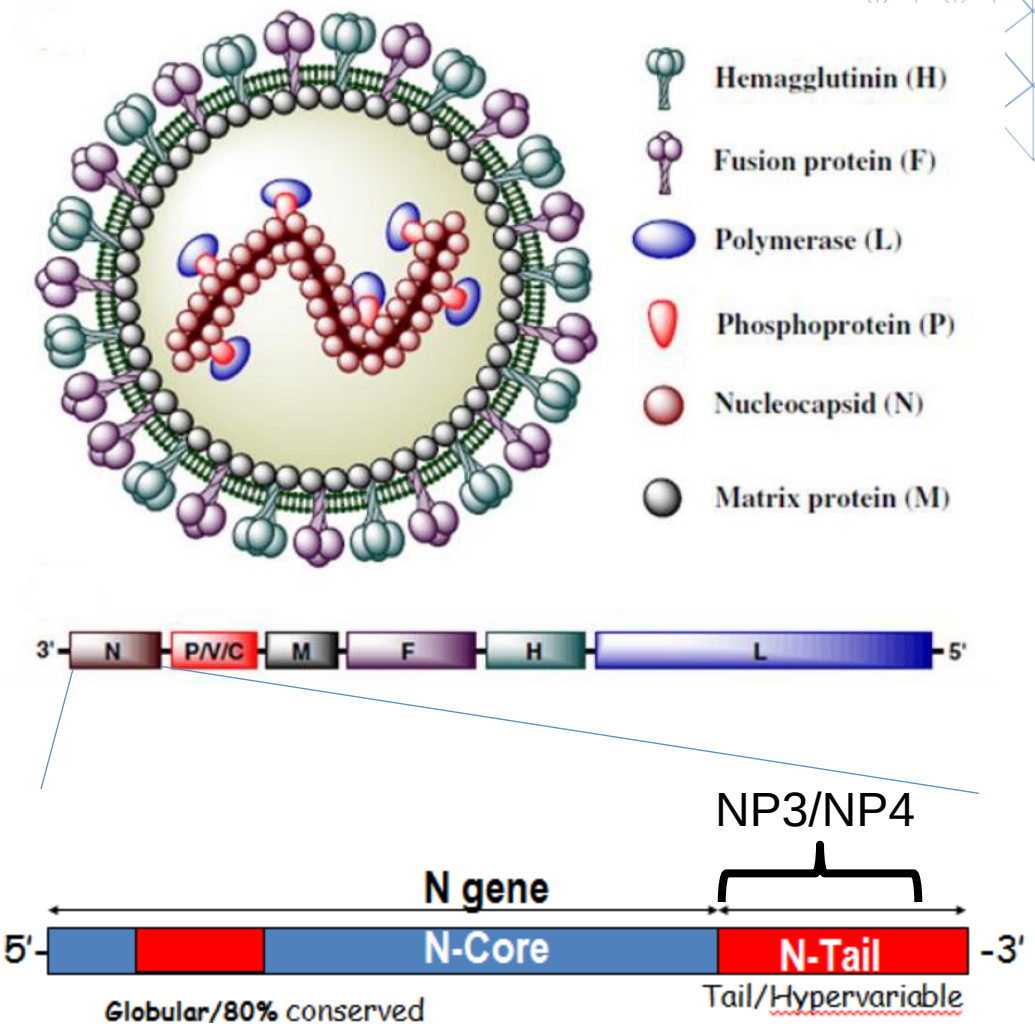
Sequencing strategy

PPRV genome length = 15,948- 15,954 nucleotides

Different **sequencing approaches** can be chosen depending on the epidemiological question and the financial and technical resources available

Main factor: ‘resolution’ for distinguishing the genetic relationships between different sampled sequences

With full genome providing highest genetic information

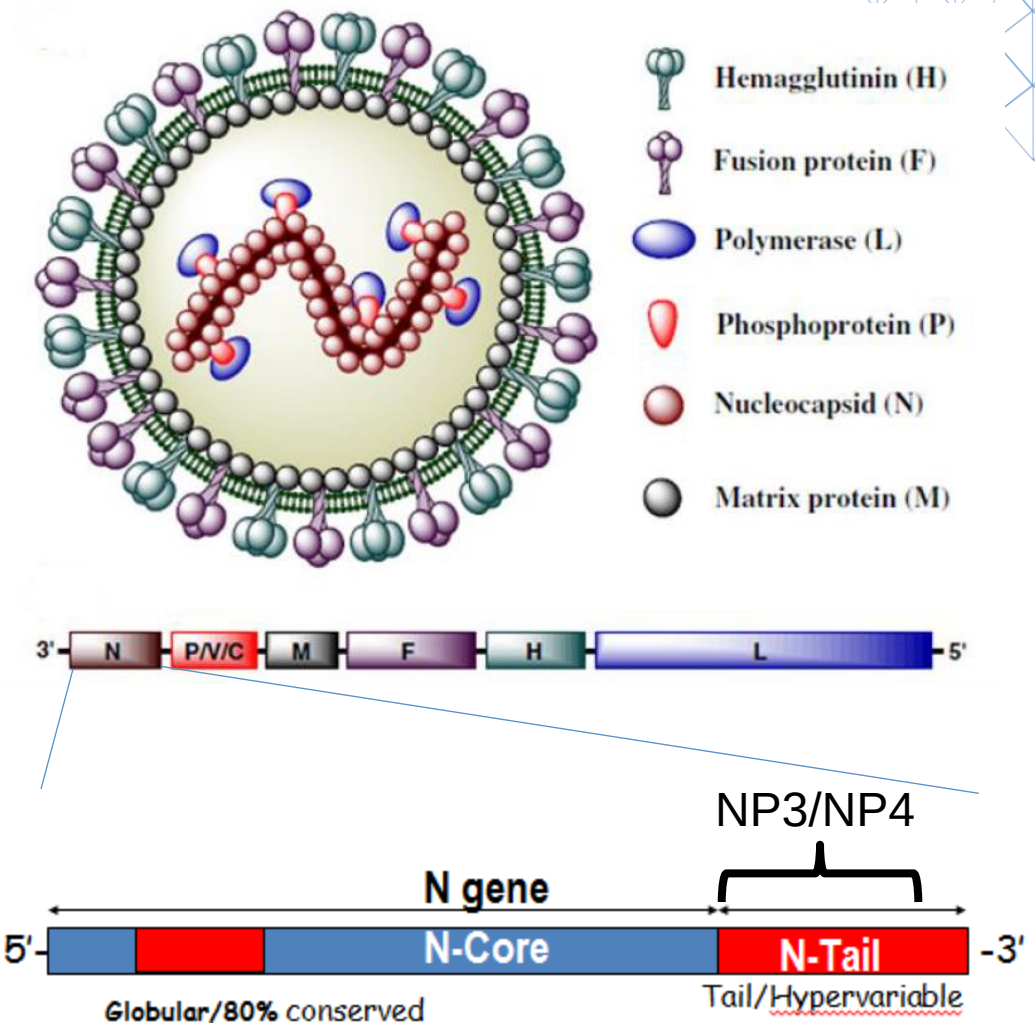


Sequencing strategy

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/hubs
- But some ambiguity in phylogenetic relationships between strains will remain

Main important tool to support episystem identification



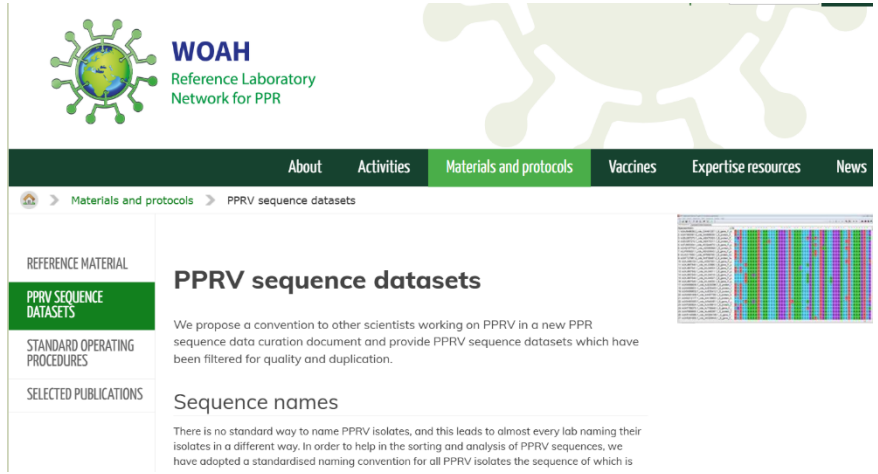
Sequencing strategy

Partial N gene (typically 255bp)

Suggested procedure:

1. Use curated dataset of unique N gene sequences available on website of WOAH network of PPR ref labs
2. Create a custom dataset with all sequences from region targeted and 1-5 sequences from other regions across lineages
3. Based on first phylogenetic tree, remove or add sequences until most informative tree obtained

If new sequence obtained is identical to a sequence already published, keep in the analysis if date and/or location is different



Sequencing strategy

Partial N gene (typically 255bp) – **example**

- Diversity of PPRV in Nigeria, studied by sampling in markets
- sequence was obtained from 99 positive samples. A total of 33 unique sequences were identified and used in phylogenetic analysis (Maximum likelihood)

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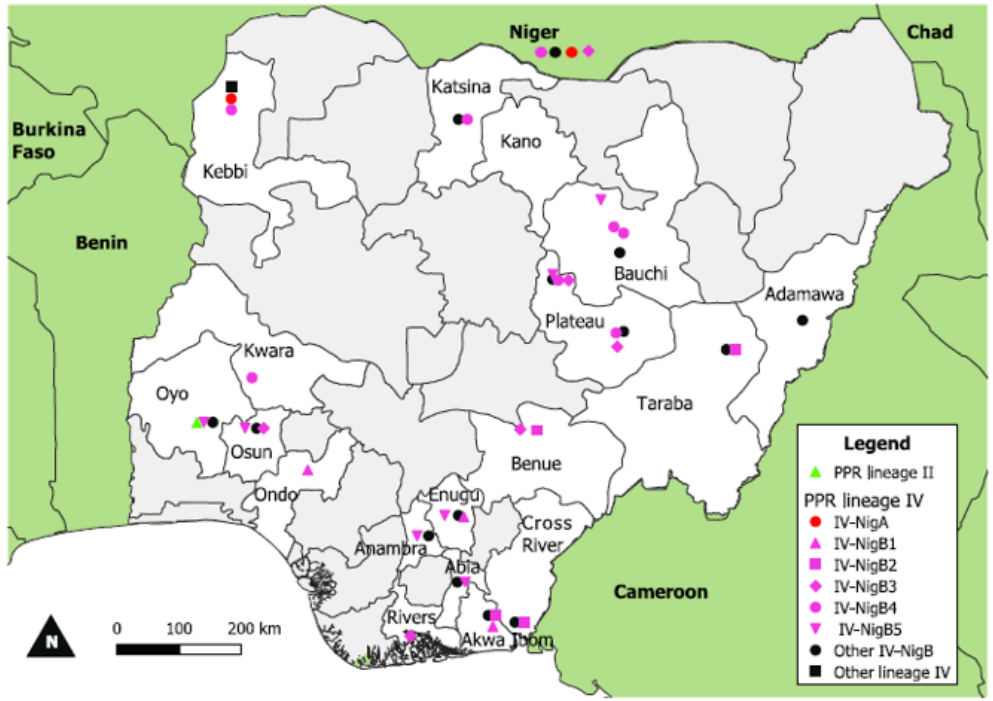
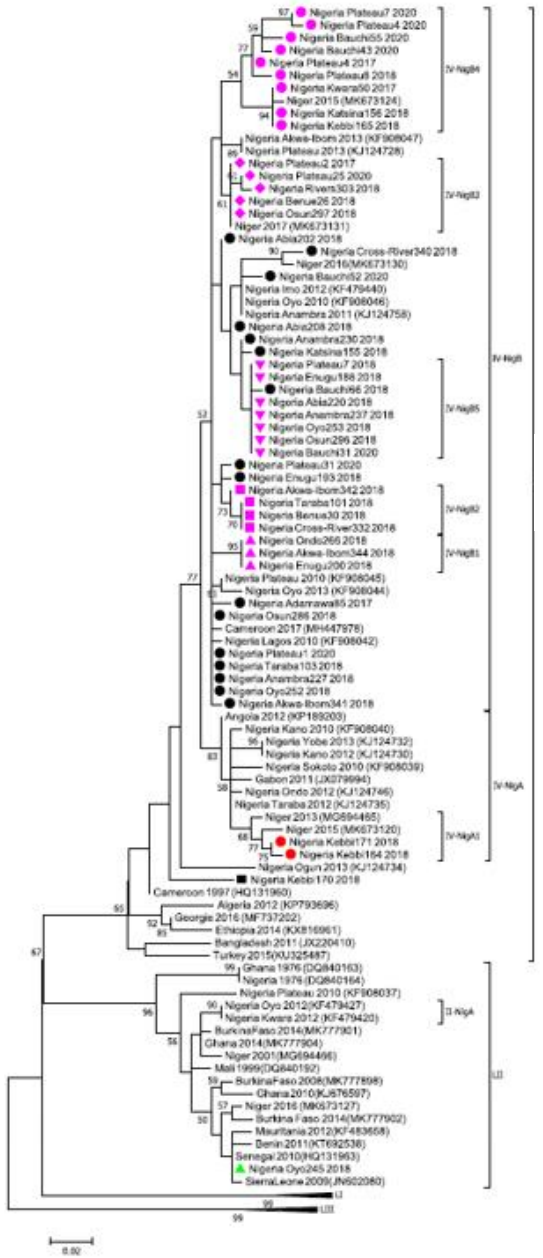
- Clusters with sequences from states all across Nigeria, separated by hundreds of kilometers
- Connection with neighbouring countries
- Extensive circulation, need vaccination strategy based on animal movement





LIV

Sequencing strategy

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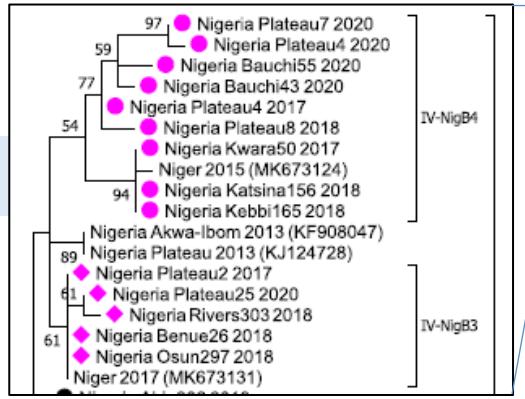

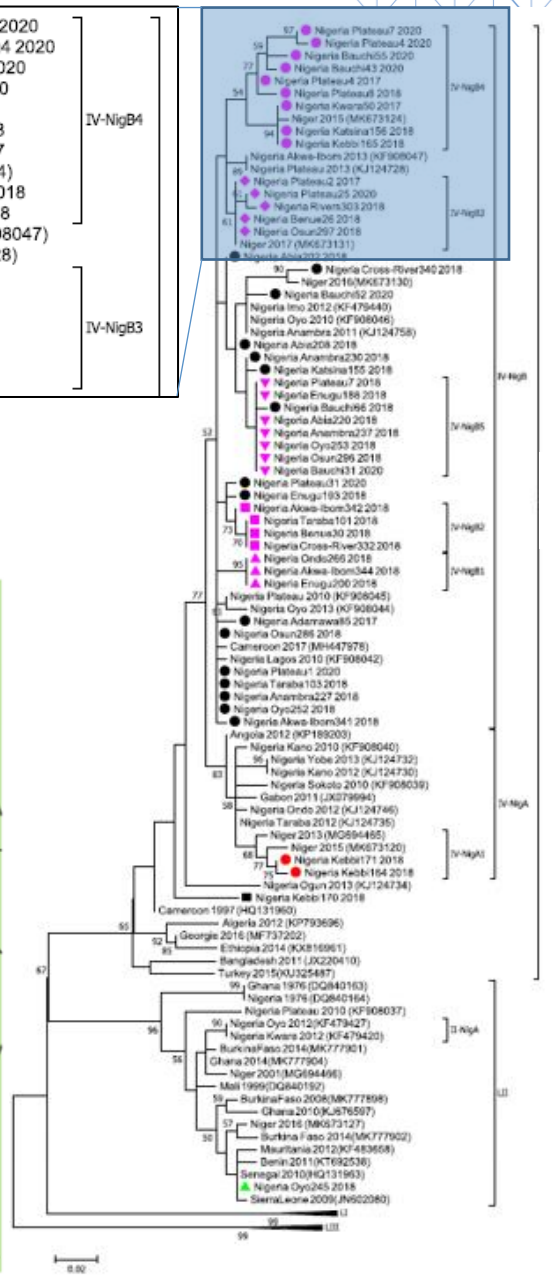

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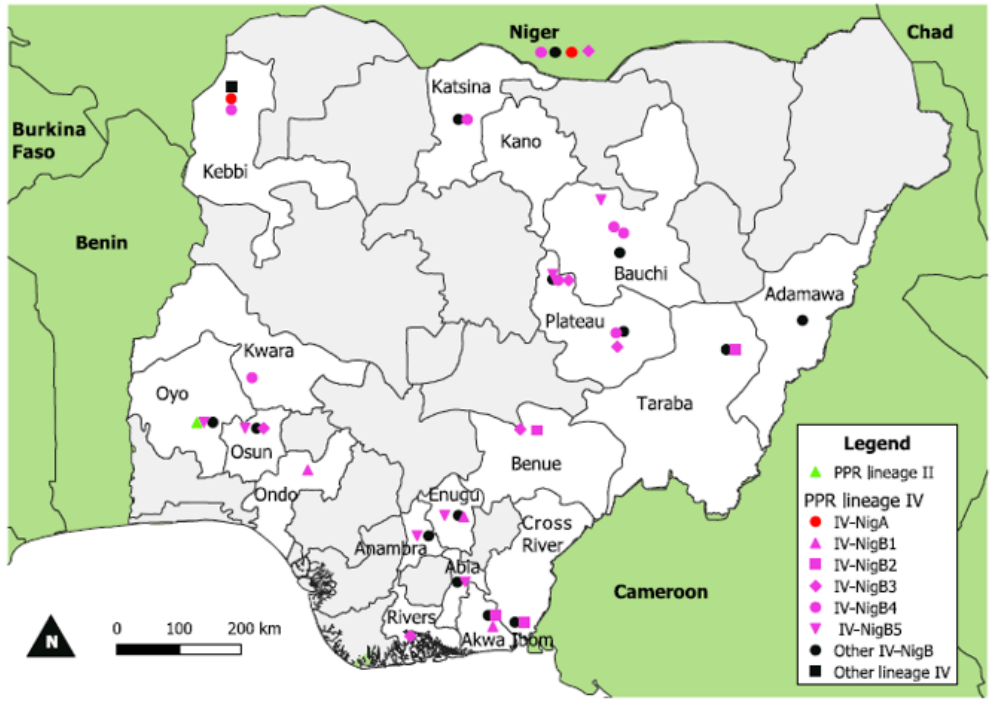


Sequencing strategy

Sequencing a **genome region** with higher resolution

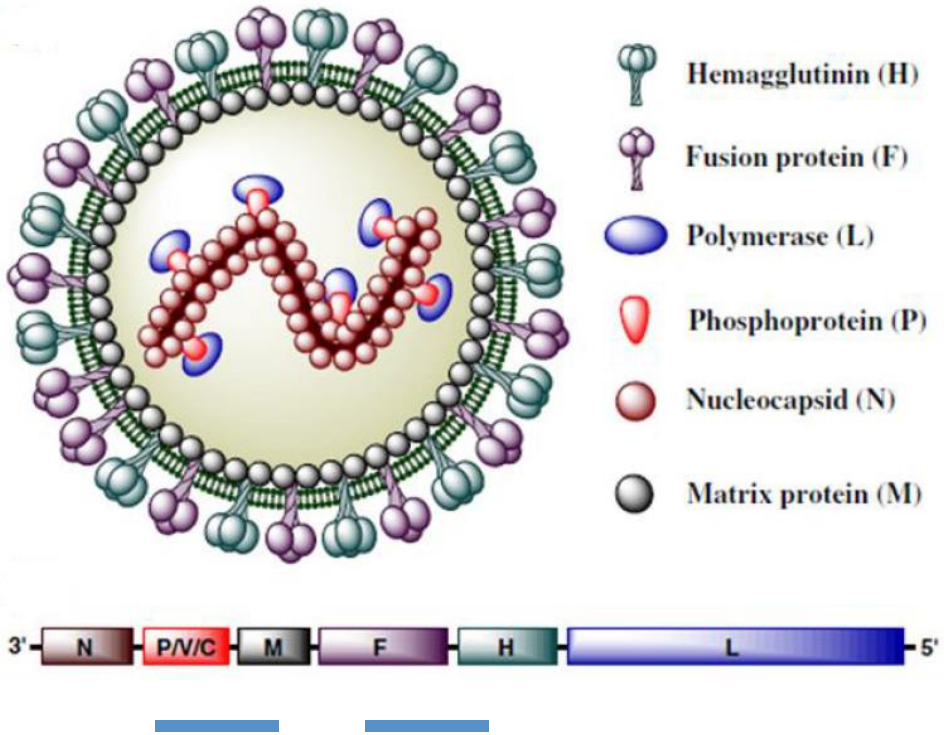
- Based on one or two PCR (not NGS)
- can provide almost the same level of phylogenetic resolution as full PPRV genome sequences
- answer questions related to episystems which may not be possible to address using partial N gene sequence data

Two regions of interest (using published long fragment PCRs):

Region 3451-4250 [+ 6951-7750]

Region 6100-7500 (good for East Asia cluster)

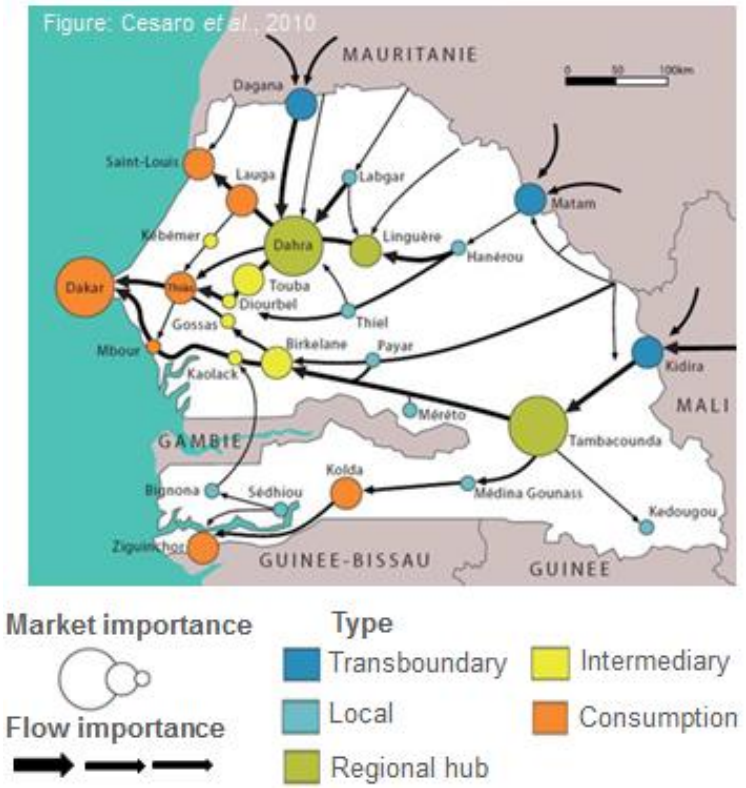
Can be a solution when partial N gene not enough and full genome sequencing not possible

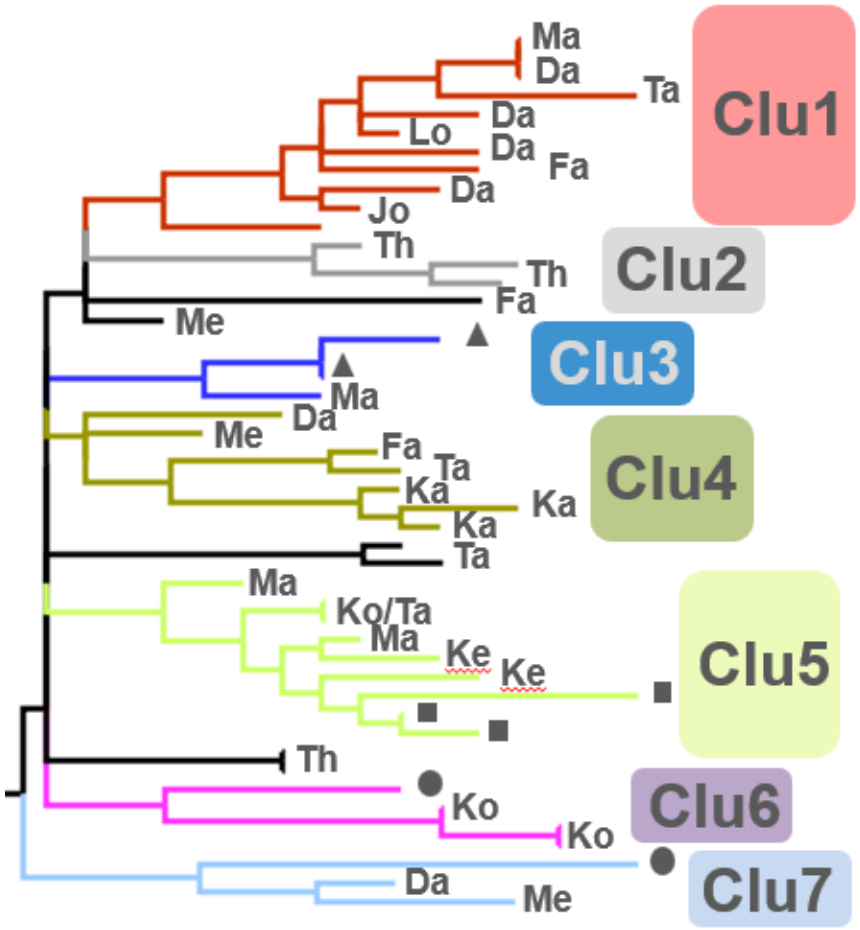


Sequencing strategy

Sequencing a **genome region** with higher resolution – **example**

N and F gene sequencing and compare with animal network data in Senegal





Sequencing strategy

Sequencing a **genome region** with higher resolution – **example**

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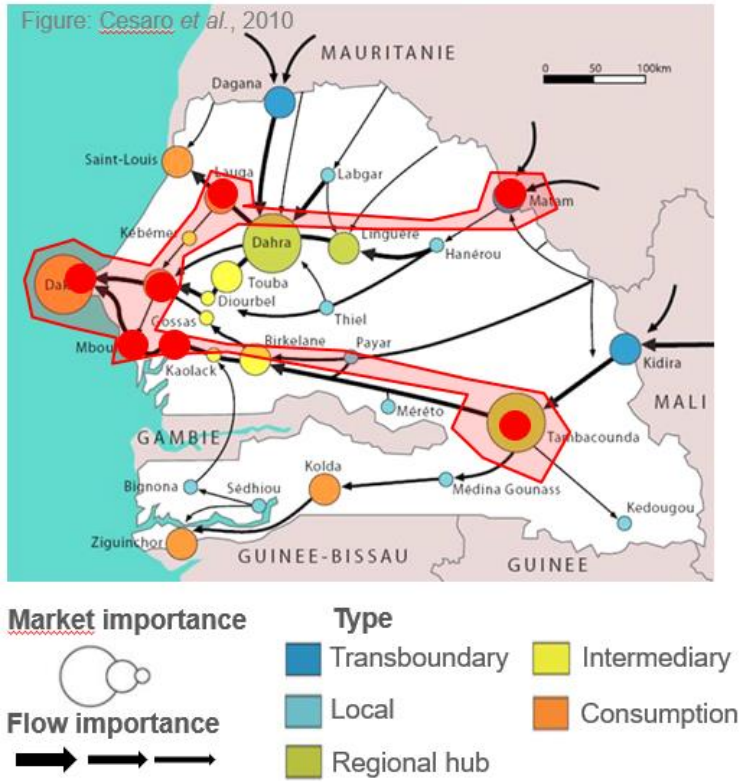
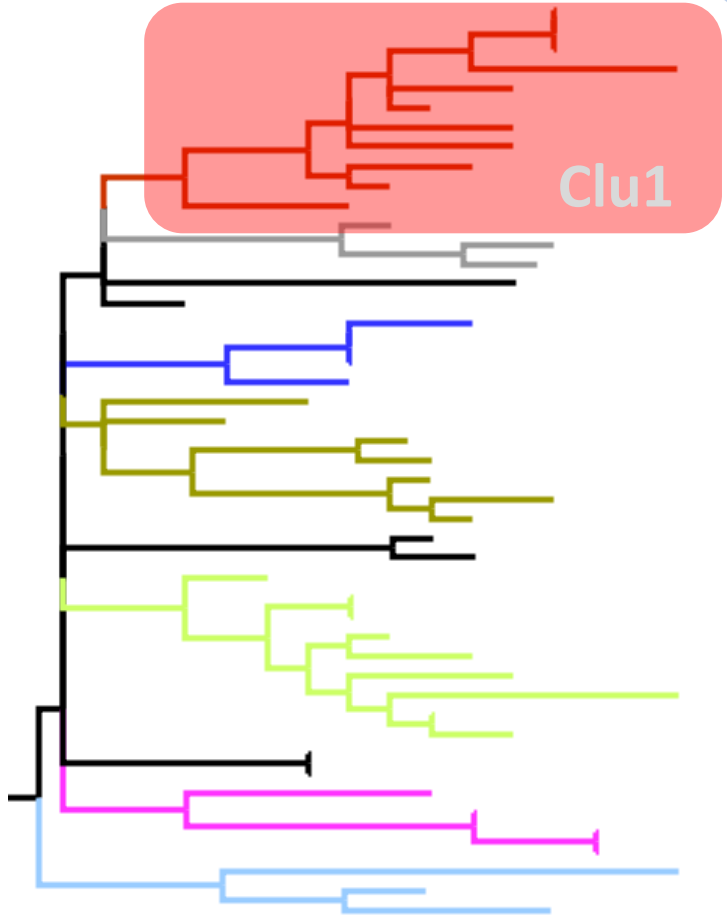
Good fit between genetic data and animal trade data





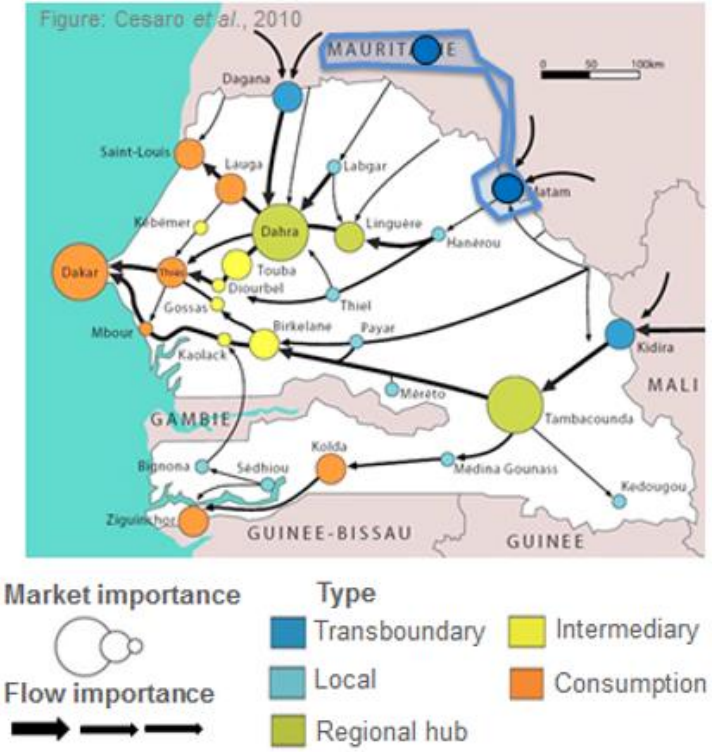
Sequencing strategy

Sequencing a **genome region** with higher resolution – **example**

N and F gene sequencing and compare with animal network data in Senegal

Good fit between genetic data and animal trade data

Confirmation of entry points



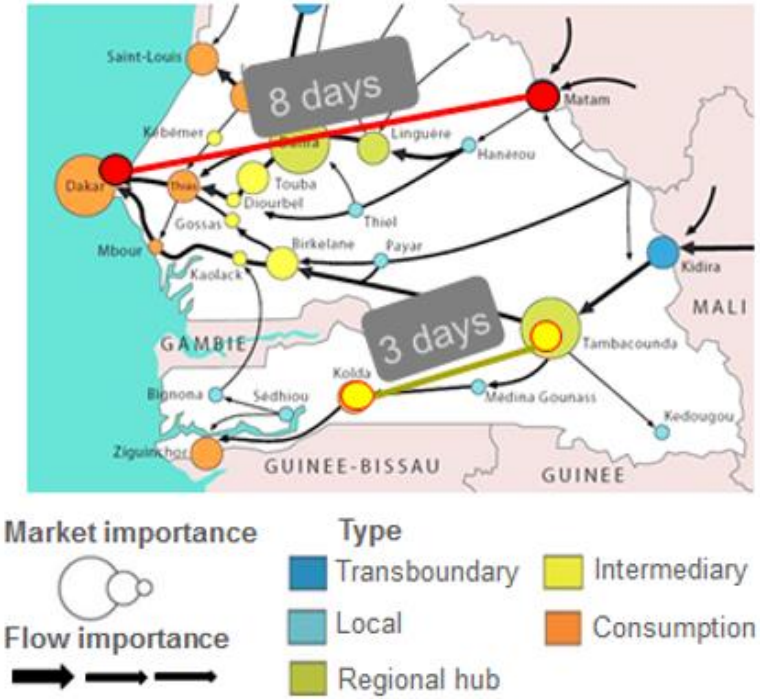
Sequencing strategy

Sequencing a **genome region** with higher resolution – **example**

N and F gene sequencing and compare with animal network data in Senegal

Good fit between genetic data and animal trade data

Identical sequences showing long distance movements



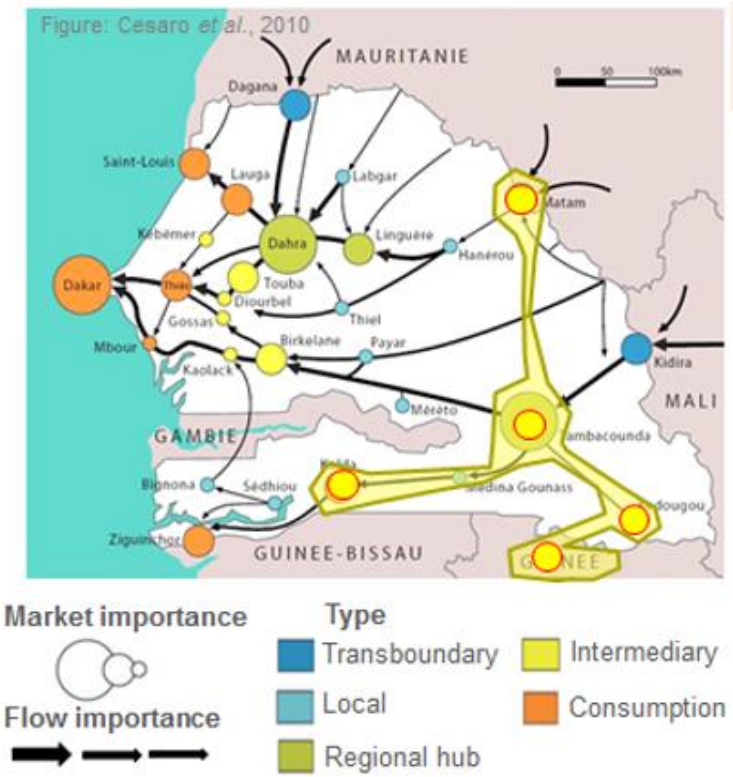
Sequencing strategy

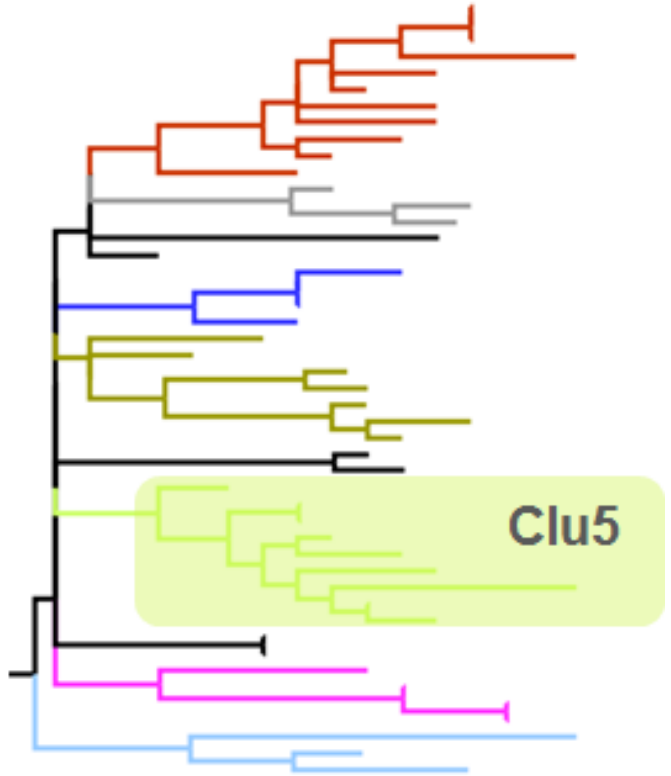
Sequencing a **genome region** with higher resolution – **example**

N and F gene sequencing and compare with animal network data in Senegal

Good fit between genetic data and animal trade data

But not always (transhumance movement??)





Sequencing strategy

Full genome sequencing

On targeted samples, based on results from partial N gene sequencing

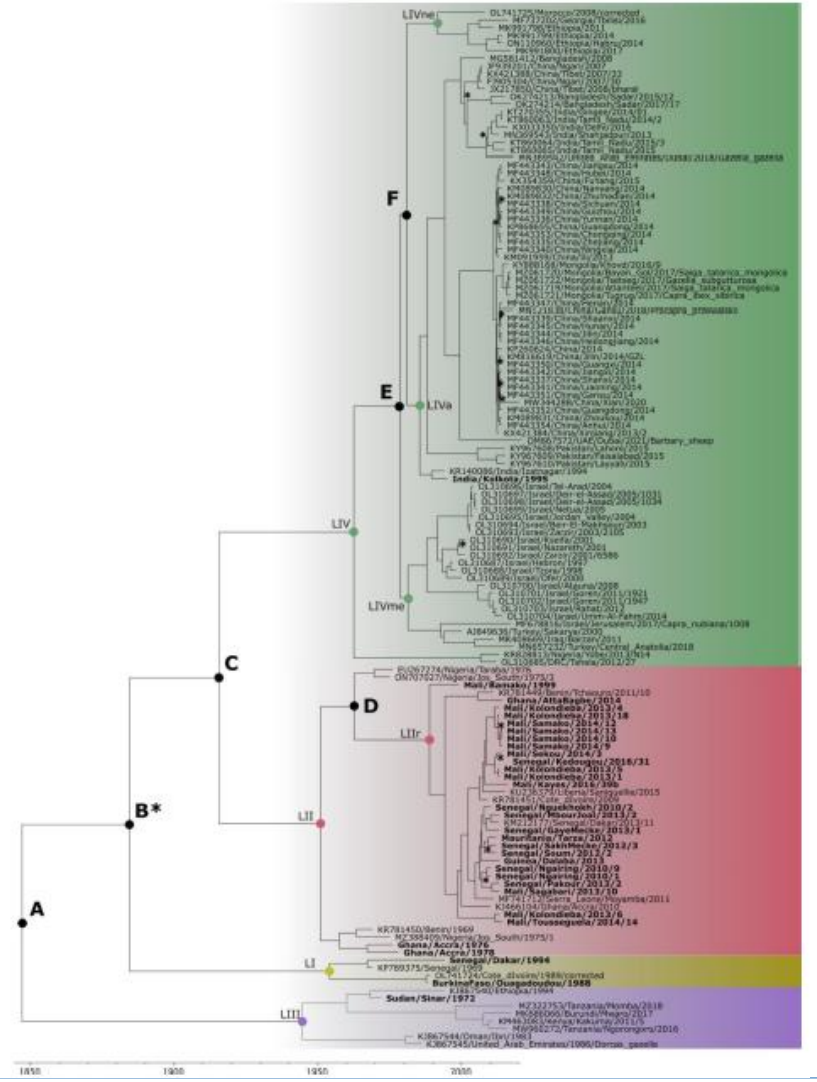
- Poorly represented clusters
- Strains from wildlife or atypical hosts

Based on next generation sequencing protocol

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

Need careful check of sequencing results

Some small portions of genome often missing but not an issue



Sequencing strategy

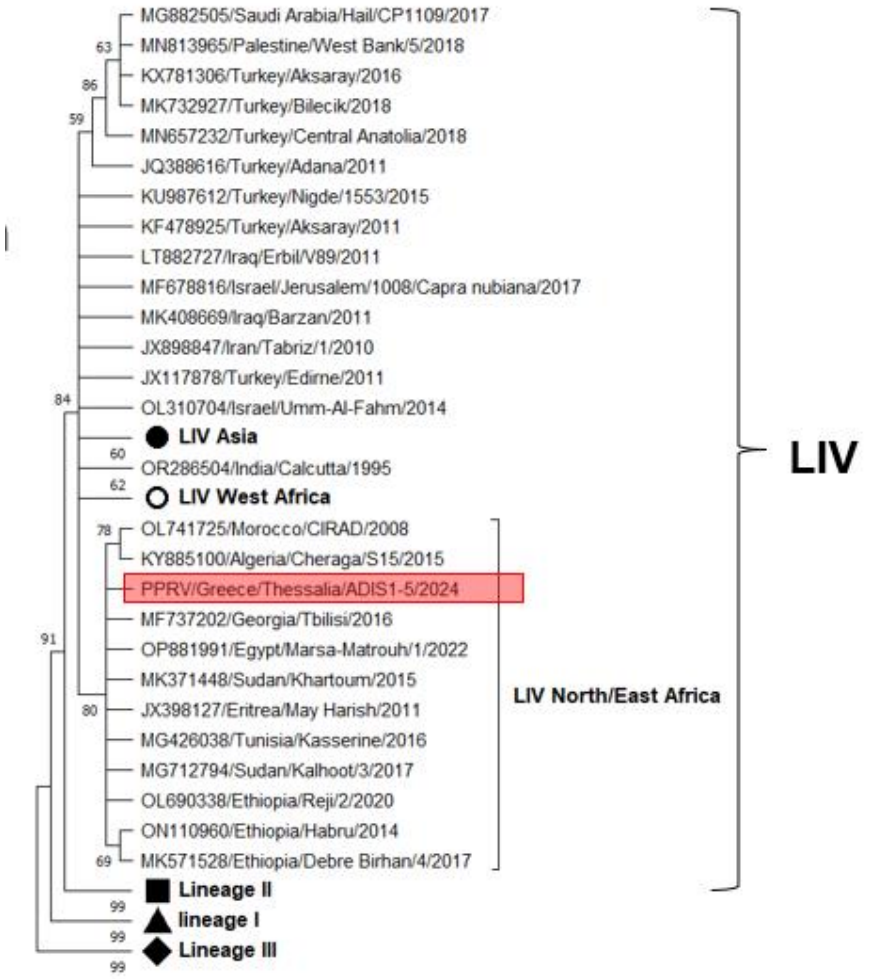
Full genome sequencing - example

PPR emergence in Europe

Partial N gene sequence identical for emergence in Greece, Romania, Bulgaria, Hungary

Linked to LIV North Africa

Limited power of analysis



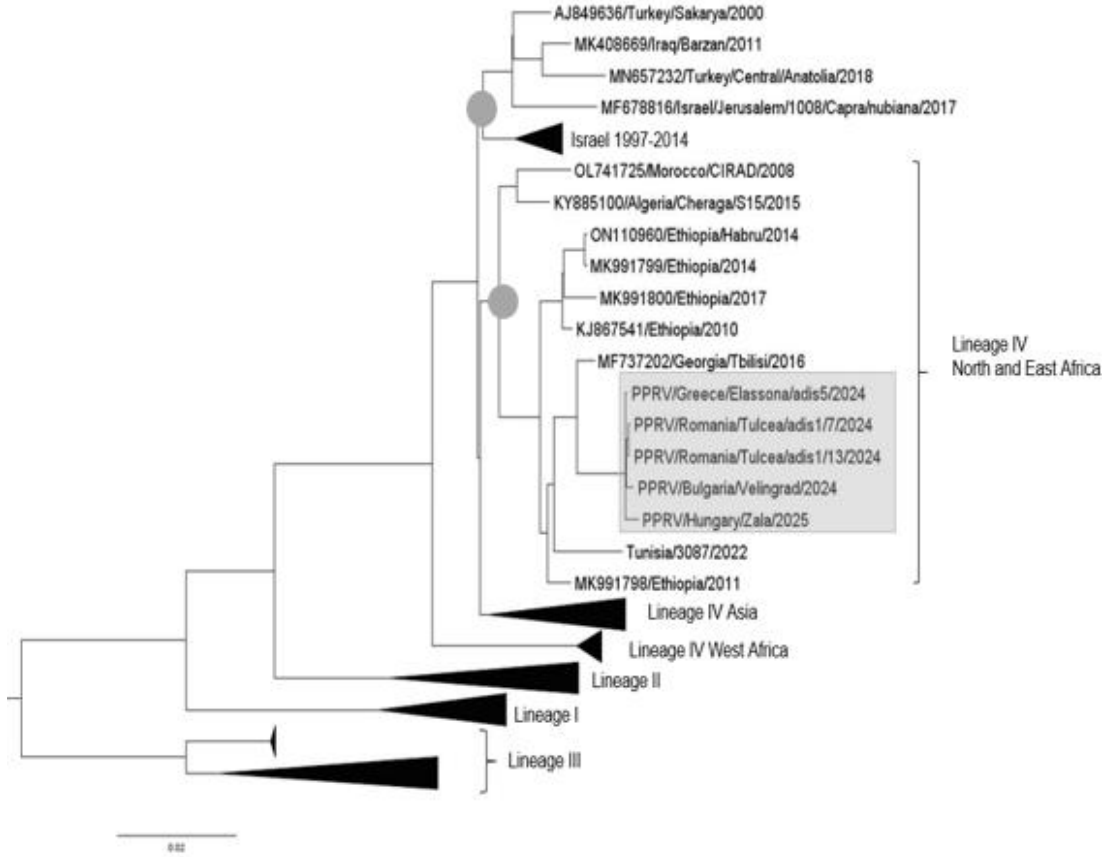
Sequencing strategy

Full genome sequencing - example

PPR emergence in Europe

Complete genome for 1 sample from Greece, 2 samples from Romania, and 1 sample from Bulgaria; and partial genome (66%) from 1 sample from Hungary

- Two sequences from same farm in Romania with some differences
- Most similar sequence published: Georgia/2016 (98.3% identity)
- Confirm grouping with Lineage IV sub-clade North-East Africa
- Enough genetic diversity to inform epidemiological investigation concerning PPR transmission pathways



Sequence naming standard

We propose a standard **naming convention** for PPRV sequences submitted to public databases

PPRV/Location/Host/ Specimen(Sample)ID/Date

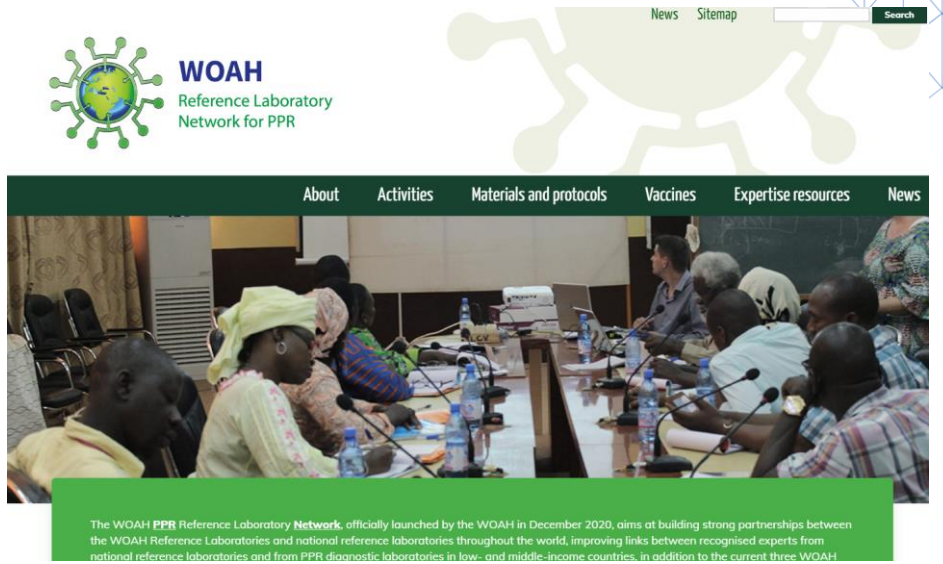
Location: at least country, better if down to regionand/or town

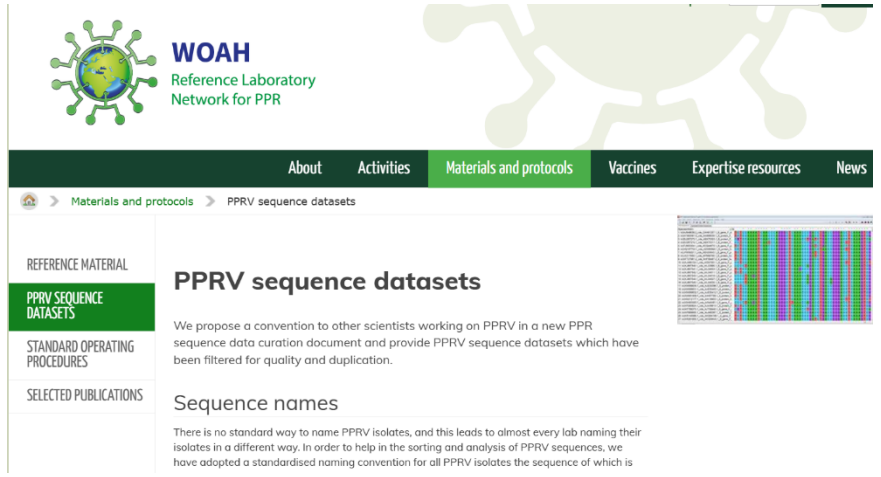
Host: especially important if atypical host

ID: important if multiple sequences from same location

Date: at least the year

Complete epidemiological data should accompany each sequence as metadata at submission



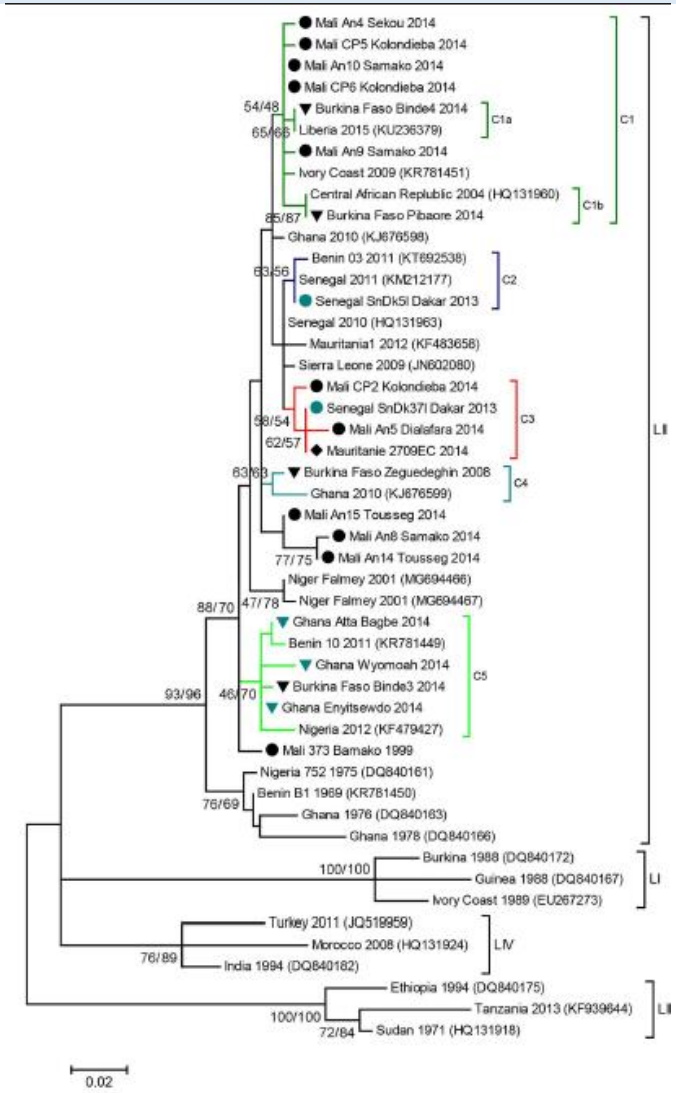


Phylogenetic analyses

- Maximum Likelihood (ML) and/or Bayesian inference (BI) methods
- Apply methods to evaluate statistical support on phylogeny
- Support with partial N gene: usually low, but clusters with support at 60-70% can be detected and usually relevant

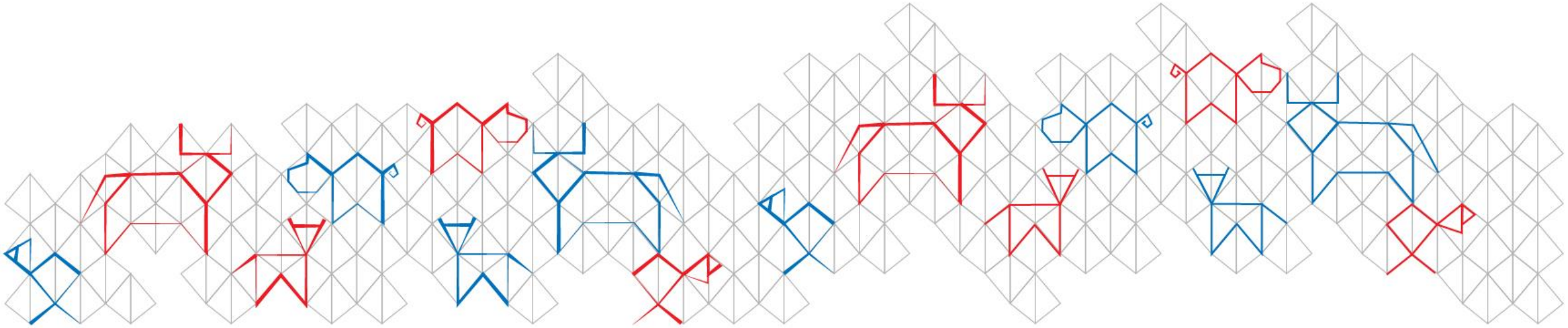
Results of main importance for questions related to PPR episystems:

- Identifying sequences that are clustered together in the same group
- Identical genetic sequences in samples collected at different times and locations but sharing
- Sequences with unexpected position in the tree



Conclusion

- Virus genetic analysis is **key tool** to investigate episystems
- Must be used as a support to other type of data (animal mobility, epidemiology) and targeted to **respond to specific questions** about episystems
- Very simple and cheap partial N gene sequencing is the **main first tool**, but additional sequencing may be needed in specific contexts
- **Guidelines** are available to
  - Collect and store samples for sequencing
  - Producing curated sequence dataset adapted to a region
  - Sequencing naming and analysis
  - Using molecular epidemiology to support episystem identification
- You are **not alone**! There is a community working on episystems, sharing experience and questions



Thank You

Questions regarding PPRV sequencing and analyses:
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Join the WOAH network of PPR labs:
https://www.ppr-labs-oie-network.org/

Exchanges with PRP experts on episystems
ppr-gren_episystem@cirad.fr

Join the group!
Guidelines soon publicly available through share point