



# Virus characterisation results from the PPR outbreaks of Greece and Romania

Report from the EU Reference Laboratory for peste des petits ruminants (EURL-PPR)

PAFF Committee, 20<sup>th</sup> September 2024



# EURL-PPR activities

- To ensure availability and use of high quality methods and high quality performance by NRLs  
Including distribution of Standard Operating Procedures and reference material
- To provide scientific and technical assistance to NRLs
- To provide scientific and technical assistance to the European Commission and other organisations  
Including active assistance in the diagnosis of PPR outbreaks

**EU Reference laboratory for Peste des Petits Ruminants**



Funded by  
the European Union

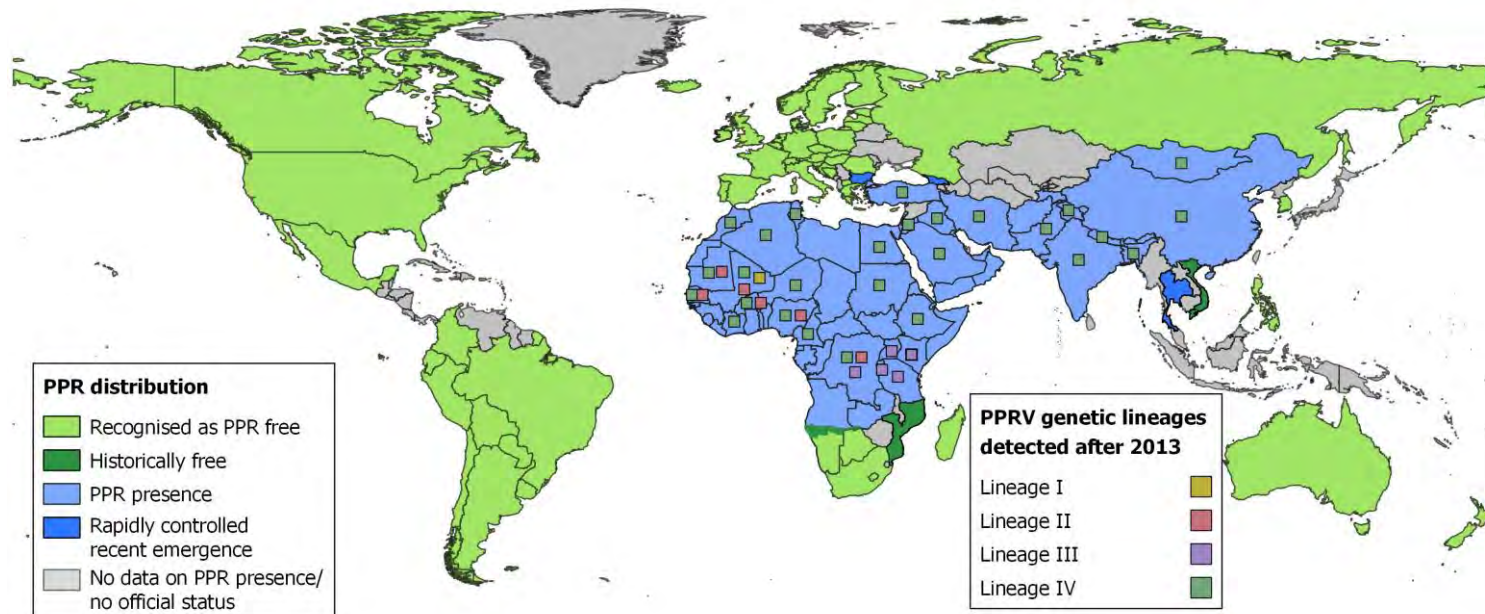


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# Key information regarding PPR virus

- *Morbillivirus with small RNA genome (16kb) coding for 8 proteins*
- *Four genetic lineages, with lineage IV most widely distributed across Africa, Middle East, Asia*
- *One serotype (vaccine protects against all strains)*





## Samples received from NRL of Greece and Romania

Greece (reception on 23/07):

- 4 serum, 6 swabs, 4 blood samples from 4 animals from ADIS 1
- 2 tissue samples from 1 animal from ADIS 1 (Kalambaka)
- 2 tissue samples from 2 animals from ADIS 5 (Elasonna)

Romania (reception on 29/07)

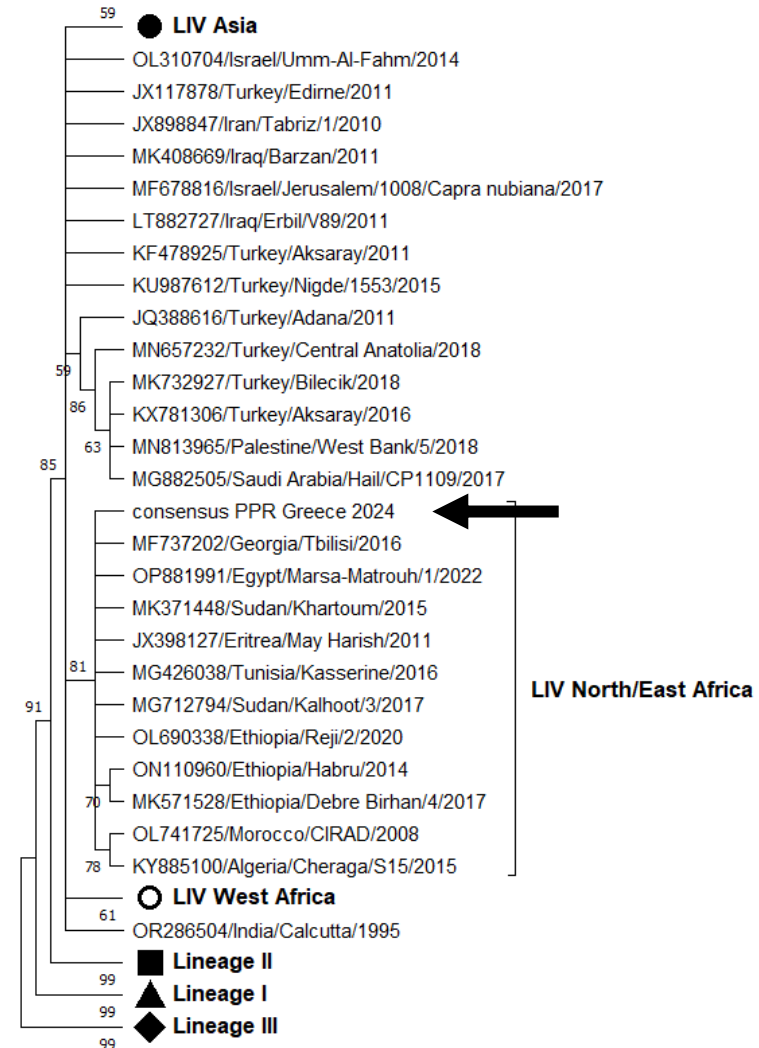
- 10 serum from 10 animals from ADIS 1 (Baia)
- 12 tissue samples from 10 animals from ADIS 1
- 4 swab samples from 2 animals from ADIS 1
- 2 blood samples from 2 animals from ADIS 4

ELISA assay on serum samples and real-time quantitative PCR on all other samples

Positive results obtained for all samples, confirming results obtained by NRLs (reports sent to NRLs and EC)

## Partial genome sequence

- *Conventional RT-PCR targeting portion of nucleoprotein (255bp) followed by Sanger sequencing*
- *Identical sequence obtained from all samples of Greece and Romania*
- *99.6% similar to strain Georgia/2016 and Sudan/Khartoum/2015*
- *Phylogenetic analyses place sequence with lineage IV, sub-clade grouping strains from North and East Africa*



## Full genome sequencing

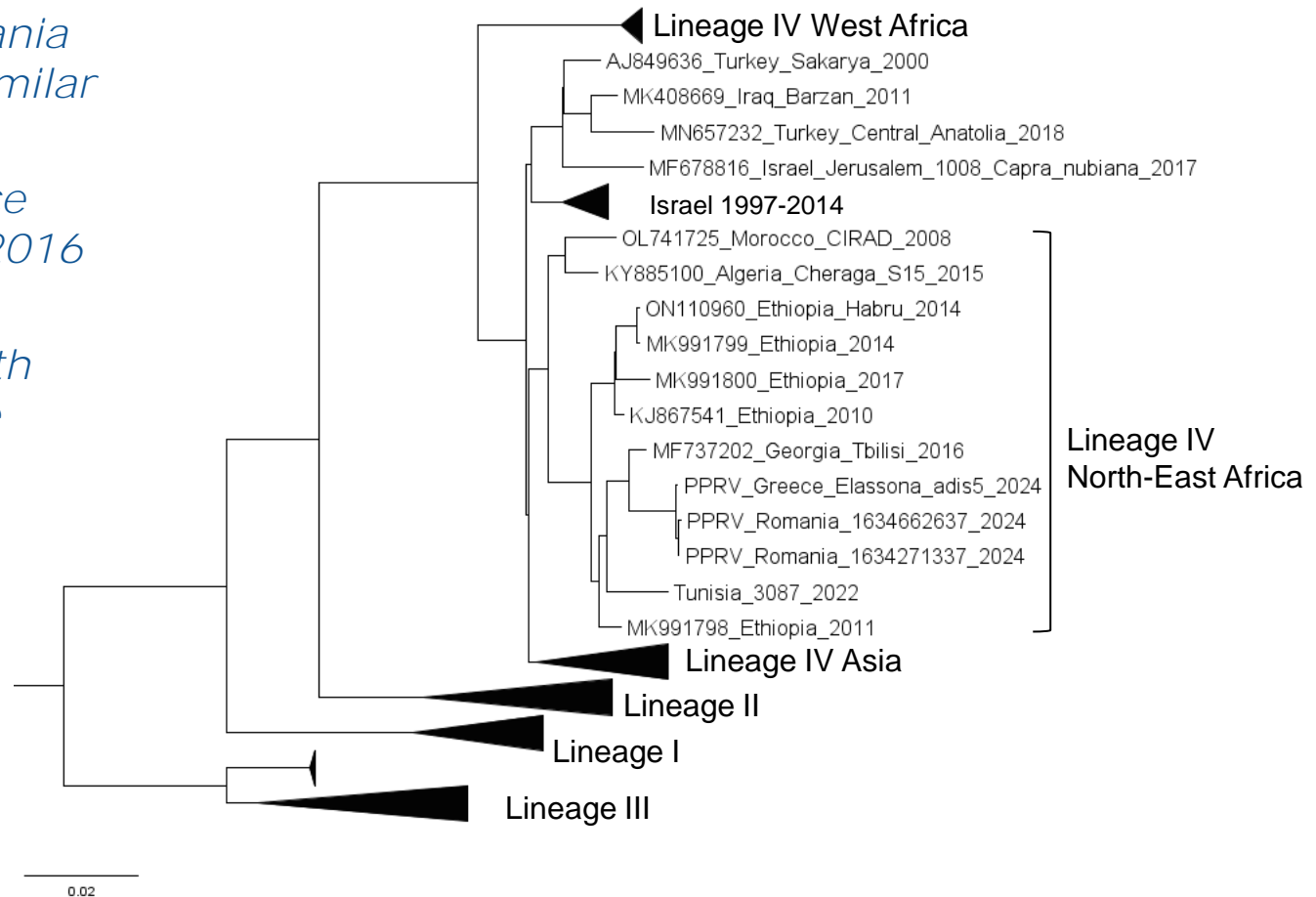
- *Focusing on 3 samples from each countries (higher quantity of RNA)*
- *High-throughput sequencing using Illumina platform*
- *Complete genome sequence for 1 sample from Greece (ADIS 5) and 3 samples from Romania*
- *Best results with organs*

CIRAD ID	Client ID	Outbreak	Matrice	RT-Q PCR results (Ct value)	Mean depth (nb reads)	Genome coverage (%)
80608	73A/24 No3	Greece ADIS 1	ocular swab	23,06	0.39	21
80611	101A/24 No6	Greece ADIS 5	lung	22,04	3	72.9
80612	101A/24 No7	Greece ADIS 5	lung	14,88	834	99.5
80632	7-1634662637*	Romania ADIS 1	lung	11,95	659	99.5
80633	8-1634662637*	Romania ADIS 1	lymph node	11,65	750	99.7
80638	13-1634271337	Romania ADIS 1	lung	11,74	21000	99.5

\*same animal

## Phylogenomic analysis

- *Genomes from Romania and Greece highly similar (99.5% identity)*
- *Most similar sequence published: Georgia/2016 (98.3% identity)*
- *Confirm grouping with Lineage IV sub-clade North-East Africa*



## Genome comparisons

- *Differences, including some that may change protein functions, observed across the genome*

	Nucleotide differences	Amino acid differences	AA differences unique (not found in other published sequences)
Georgia/2016 vs Greece/2024	176	30	12
Georgia/2016 vs Romania-7/2024	177	35	14
Georgia/2016 vs Romania-13/2024	173	34	14
Greece/2024 vs Romania-7/2024	22	4	2
Greece/2024 vs Romania-13/2024	20	5	2
Romania-7/2024 vs Romania-13/2024	8	1	0



## Conclusions

- *The NRLs have performed excellent diagnostic work to confirm PCR infections. The EURL-PPR continue its support with advise on protocols and provision of reference material*
- *Genome data confirms that emergence of PPR in Greece and Romania has a common origin, and that the virus is related to strains circulating recently in North and East Africa and in Georgia in 2016*
- *Recent sequencing data from neighbouring regions infected by PPR (**Georgia, Turkey...**) **are** lacking to provide clear insights into the origin of the emergence*
- *Some mutations observed in the genome may affect the pathogenesis observed, e.g. strong virulence in sheep*
- *Mutations have started to accumulate during this emergence, suggesting that additional sequencing may provide further information for epidemiological investigation*



## Thanks to

The EURL-PPR team

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