



GRIPAVI

Detection and characterisation of ortho- and paramyxoviruses in domestic and wild birds of Africa

Virology Section, UMR-15 CIRAD/INRA

In collaboration with UPR22-16 CIRAD

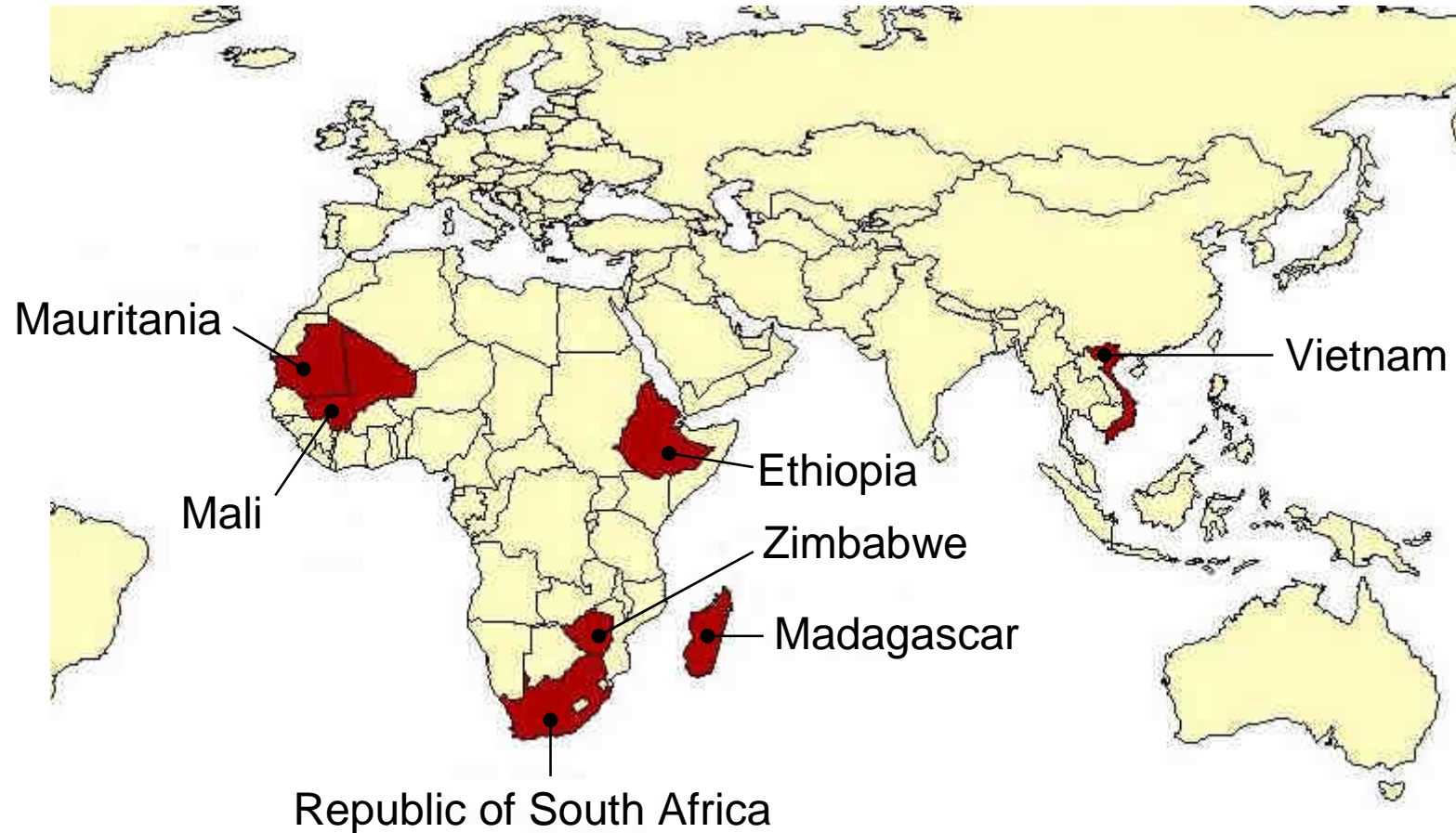
LCV (Mali), Fofifa-DRZ (Madagascar), CNERV
(Mauritanie)





FSP GRIPAVI

- 7 countries included



- UMR-15: virological testing for 4 partners



- Research objectives:
 - Detection and characterization of influenza & Newcastle (APMV1) viruses in wild and domestic birds
 - Determination of links between the 2 compartments and between seasons

- Methods:
 - High throughput detection
 - Isolation on eggs for positive samples
 - Molecular characterization of isolates

High throughput detection and characterization of ortho and paramyxoviruses



Swab in liquid nitrogen

-80°C

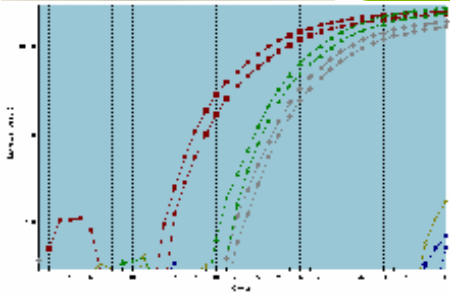
96-wells microplate



Extraction
150 µl RNA, 88 samples in 50 min



Real-time RT-PCR:
AIV M gene
NDV: F gene
~4h



AIV/NDV⊕

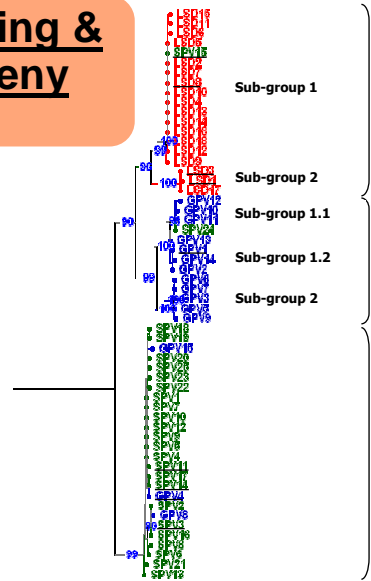


Isolation
7-25 days

Real-time RT-PCR:
AIV H5, H7, N1
~2h

Pathotyping:
sequencing of cleavage site
~5d

Sequencing & Phylogeny
~5d





Overall results

	Mali 2007	Mali 2008	Mauritania 2008	Madagascar 2008	Total
Domestic birds tested	223	315	28	547	223 in 2007 1622 in 2008
Wild bird tested	0	362	370	0	
% of birds AIV+ (% H5 & H7 positive)	3.13% (0)	0% domestic 0.28% wild	0% domestic 0.27% (0) wild	0.55%	
Number of AIV isolates	0	0	0	0	
% of wild birds NDV + (pathotype)	1.3% (all velogenic)	0.95% domestic (all velogenic) 3.31% wild	0% domestic 6.8% wild (9 velogenic, 4 lentogenic)	5.3% (7 velogenic, 5 lentogenic)	
Number of NDV isolates	3	3	0	1	



MALI

Sequencing of Malian isolates:

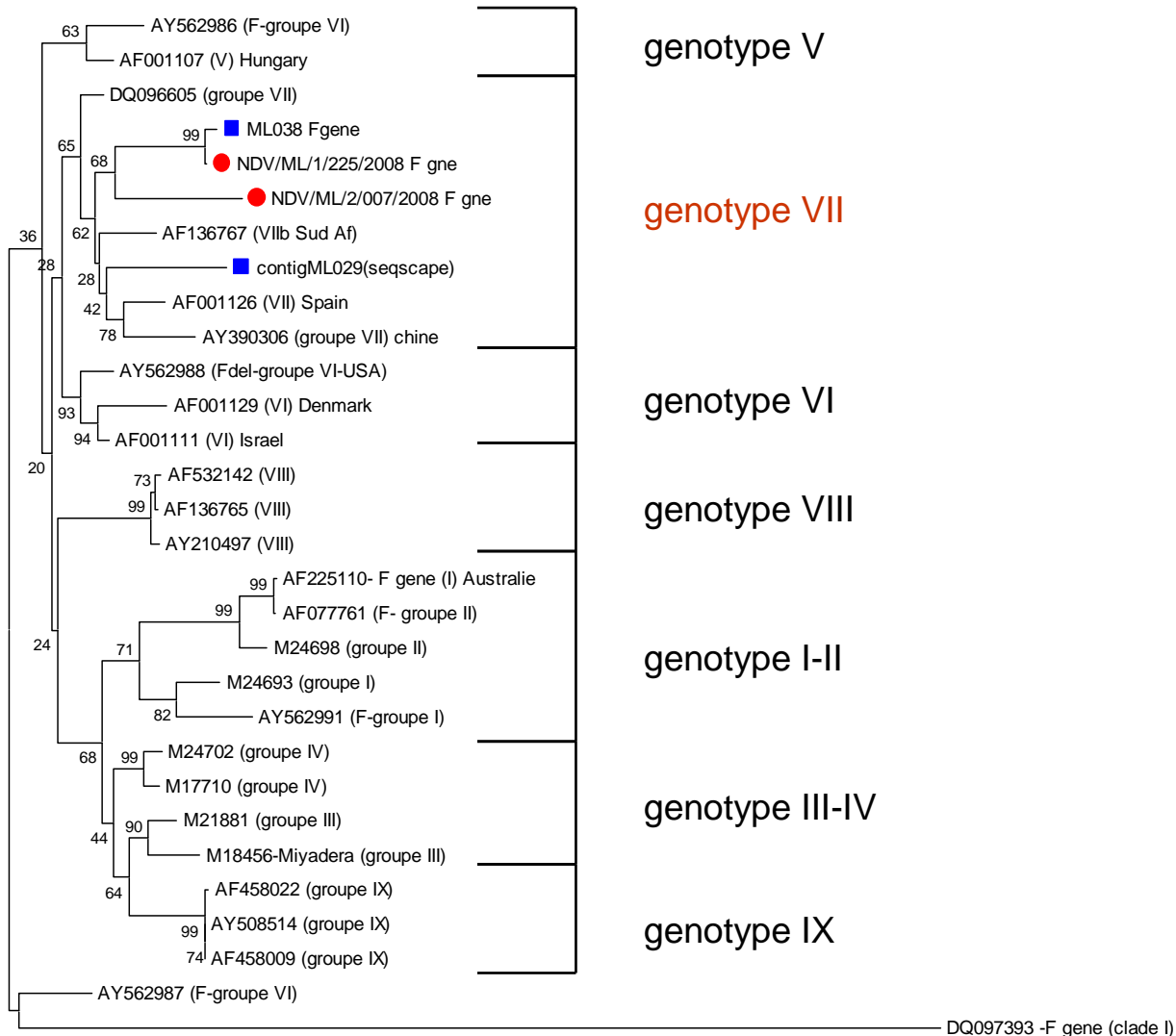
- 2007: fragment of 670 nt (end of M, beginning of F)
 - ML029 & ML031 are 100% identical
 - ML038 has 87.5% homology with the two others
- 2008: fragment of 670 pb
 - ML225 & 230 are 100% identical
 - ML007 has 91.7% homology with the two others

Isolate	Cleavage site motif	Pathotype
ML029	G/RRRKR/FV	V
ML031	G/RRRKR/FV	V
ML038	G/RRQKR/FI	V
ML1/1/225	G/RRQKR/FI	V
ML1/1/230	G/RRQKR/FI	V
ML2/007	G/RRQKR/FI	V



Phylogenetic analysis on 446 nt in the F gene: NDV Mali(2007)-Mali(2008)

MALI



0.05



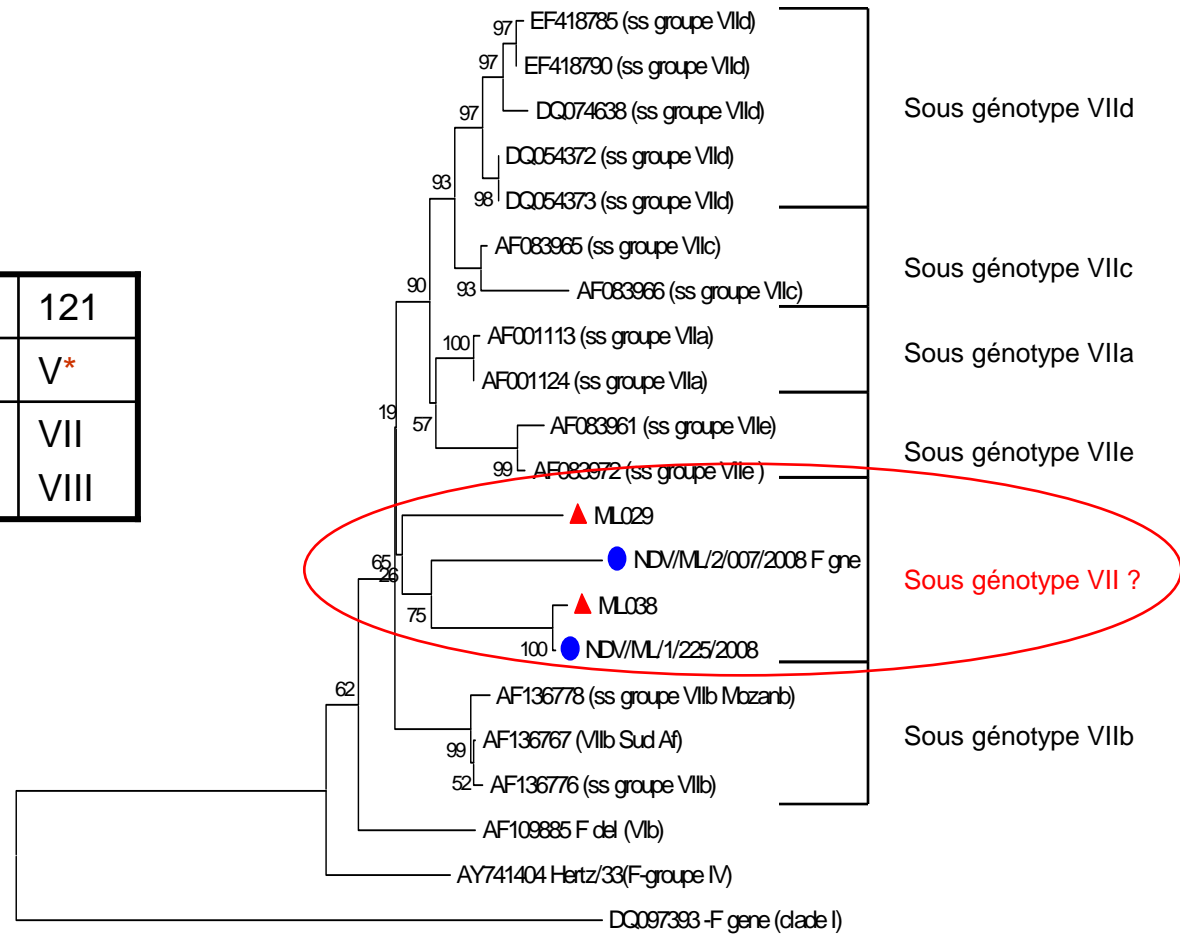
Phylogenetic analysis within sub-genotype VII

MALI

Seq ML029

10	16	23	101	114	118	121
L	V	S	K	R	V	V*
VIII	VIII	VIIa	VII a-e	VIIe VIII	V VI f	VII VIII

* V121 = feature of genotypes VII and VIII



0.05



Newcastle disease virus « NDV »

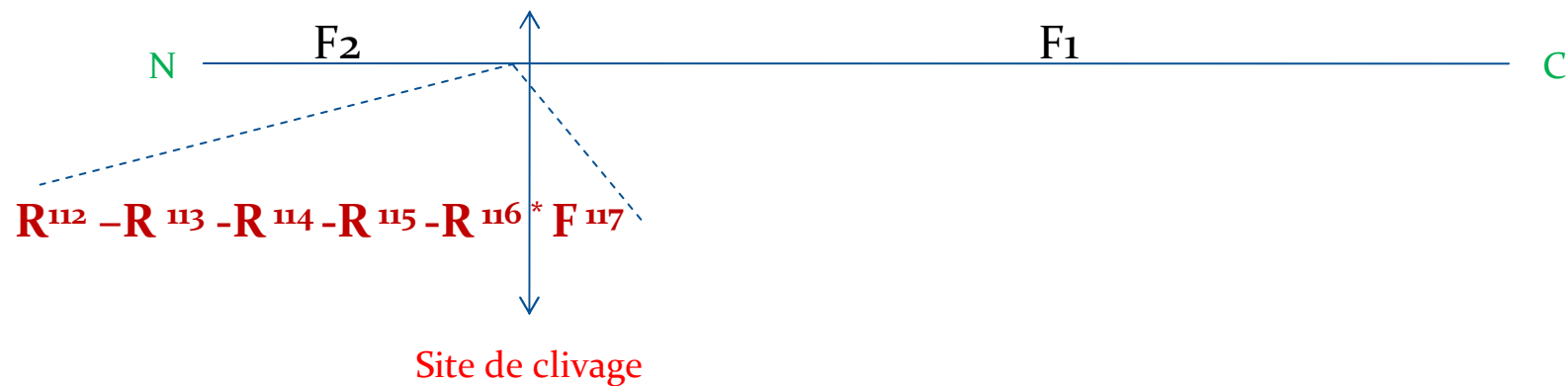
- 4 panzootics:
 - First panzootics (Asia): 1926 to 1950 (genotypes III, IV & II)
 - Second panzootics (Middle-East): 1960 to 1973 (genotypes VIII, VIa & V)
 - Third panzootics (Pigeon-Europe): 1970 to 1980 (genotypes VIb, VIc & VI d)
 - Fourth panzootics (Africa, Asia and Europe): 1980 – ongoing (genotypes VIIb, VIIa, VIIc and VII d)



MADAGASCAR

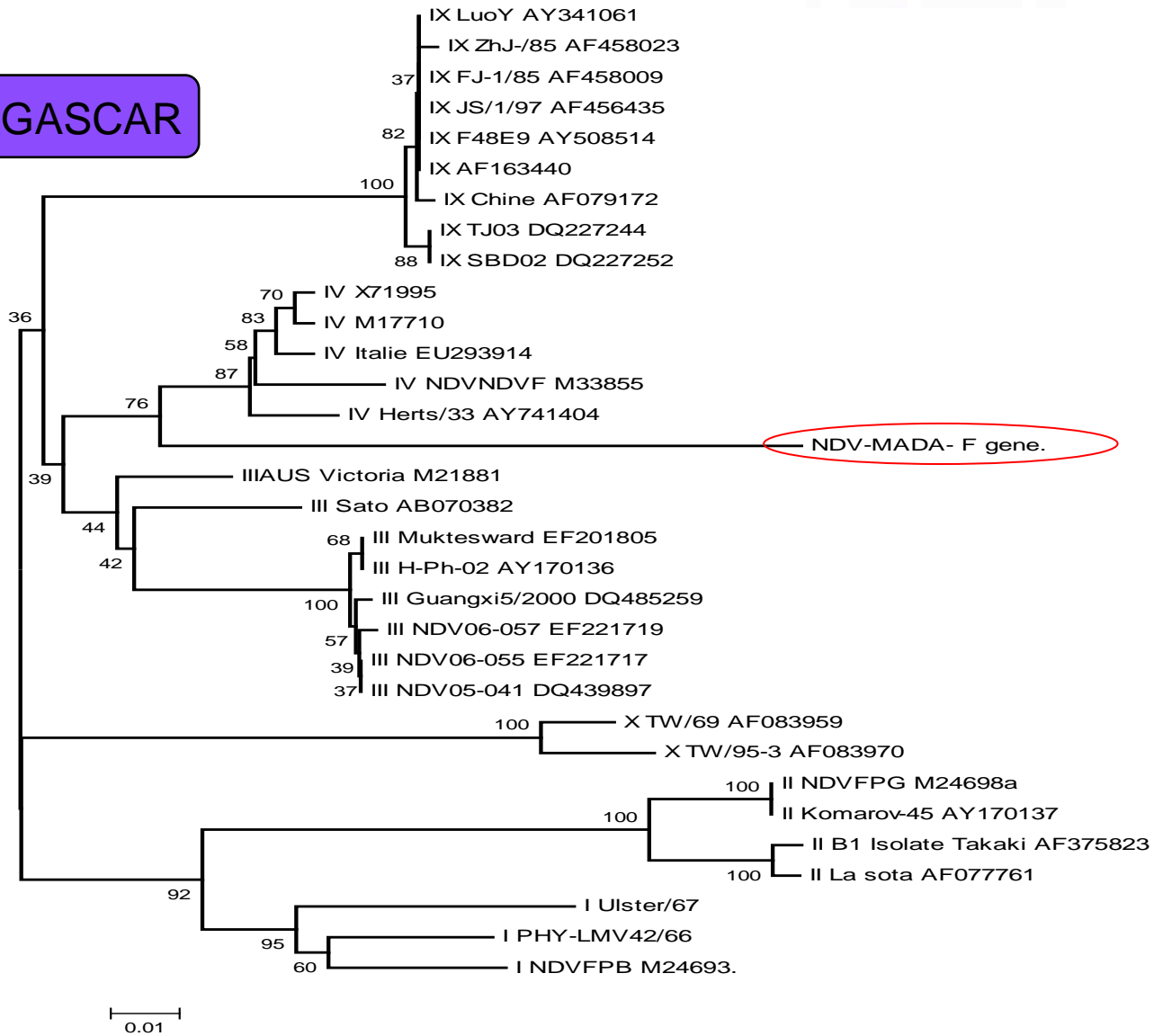
ND à Madagascar

- First reported in 1945
- **NDV-Mada (1992)**: isolated in 1992, has an original motif of cleavage site also found in a recent isolate (2008)
- 4 out of 7 other velogenic motifs detected in 2008





MADAGASCAR



Phylogenetic tree based on nt sequence of F gene (partial = 374 nt)



Conclusions

- Low level of AIV detection, higher level for NDV
- Almost similar detection in tracheal and cloacal swabs, no bird with both swabs positive
- **Mali:**
 - Isolates close to genotype VII and sub-genotype b
 - New sub-genotype?
- **Madagascar:**
 - Original motif of cleavage site RRRRR*FVG
 - Isolates close to the old genotypes III and IV appeared more than 50 years ago during first panzootics
 - New genotype or sub-genotype?
- **Perspectives:**
 - More sequencing for better genetic characterization
 - Assessment of in vivo pathogenicity
 - Acquisition of more isolates from these African regions