Newcastle disease virus in Madagascar:identification of an original genotype

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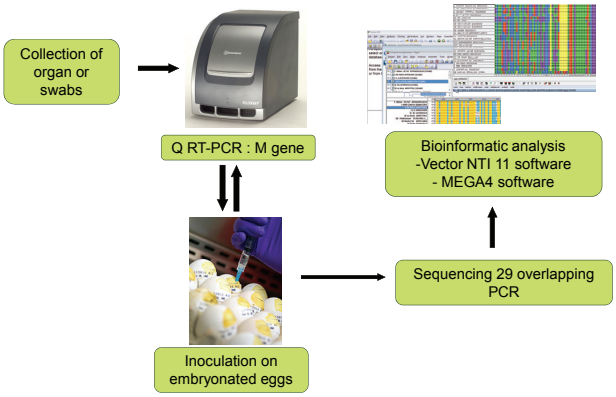
INTRODUCTION

Newcastle disease (ND) is a highly contagious and widespread disease which causes severe economic losses in domestic poultry, especially in chickens. Based on the analysis of nucleotide sequence of the F protein gene, 10 different genotypes (I-X) of ND virus have been identified so far. Since ND was first described in 1926, three worldwide panzootics have occurred caused by viruses of genotypes II-III-IV (1926-1960) V-VI (1960-1973) and VI (1970-1980), respectively [1]. Moreover, severe outbreaks in Western and Southern Europe [2], [3], South Africa [4] and Taiwan [5] in the 1990s were caused by genotype VII.s Genotype VII is the currently genotype circulating through Asia, Africa and Europe. In Madagascar, ND was firstly described in 1946 [6] and since then, outbreaks were regularly reported on the whole island mainly in the rural poultry sector [7]. While the vaccination rate is estimated to reach 100% in commercial poultry production of Madagascar, probably less than 10% of the free-range poultries are duly vaccinated. ND is considered to induce more than 40% of mortality in such non protected poultries [8]. In spite of the importance and endemicity of MN in Madagascar, no data is available about the virus variants involved on clinical cases and/or maintenance of this disease in the island. In this study different APMV-1 strains (named here MG group) were isolated in 1992, 2008 and 2009 from poultries and characterized molecularly.



MATERIAL & METHODS

Four APMV-1 strains from Madagascar (named here MG group) were isolated in 1992 and 2008 and characterized molecularly. Two of these strains were fully sequenced while the two others had only the F and HN genes sequenced. An "old" strain (MG-1992) was first isolated by the Centre National de Recherche Appliquée au Développement Rural (FOFIFA-DRZV) in 1992, from a dead fowl assumed to be vaccinated against ND (La Sota vaccine). The three other isolates were collected in Madagascar during a surveillance program in African wetlands led in 2007 and 2008 by CIRAD, and FOFIFA-DRZV. The MG-725/08 strain was recovered from cloacal and tracheal swabs from an unvaccinated chicken apparently healthy. Another strain (MG39-04/08) was isolated from a dead chicken in a commercial farm: this chicken was initially vaccinated with the HB1 strain and boosted with the La Sota vaccine. The last isolate (MG-Meola/08) was recovered from a non vaccinated backyard poultry. Samples detected positives in the APMV-1 specific PCR were further processed for virus isolation by inoculation into 9-day old chicken embryonated eggs. Six pairs of oligonucleotide primers were used to amplify six overlapping DNA fragments to generate the complete sequences of F and HN protein genes. For the rest of the genome, 23 overlapping PCR were realized. The sequences of the four isolates were compared with previously reported NDV sequences representative of different genotypes available in GenBank. Phylogenetic relationships were established using the neighbor-joining method and 1,000 bootstrap resamplings of the data [9]. Sequence comparison of the complete amino acid sequences of F and HN were carried out and 3D modellings of the proteins were done to visualize important substitutions.

RESULTS AND DISCUSSION

The sequence of the cleavage site of the F protein ([112]**RRRRR*FV**[118]) of the MG group showed five basic amino acids at position 112-116, representing a virulent motif never been reported before. Moreover, phylogenetic analysis based on the F and HN genes or on complete genome (Fig. 1a, 1b) showed that these isolates are closer to genotype IV but distant enough to constitute a new genotype named genotype XI [10]. Some of these strains were isolated from sick/dead poultries that had been vaccinated against ND (La Sota or local vaccine). The analysis of the F and HN protein sequences of the MG group strains shows original amino acid substitutions. Some of these substitutions occurred in the globular head on which reside the receptor recognition, the F/HN interaction, the neuraminidase activity (NA) and antigenic sites [11] (Fig. 2). It is tempting to postulate that the modifications observed on F or HN genes from MG strains may play a role in virulence or emergence of escape mutants.

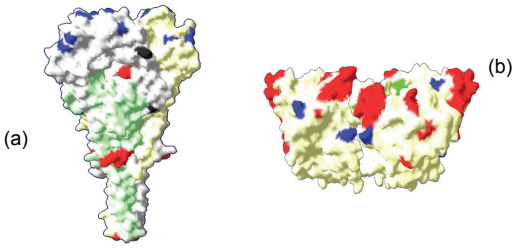


Fig 2: *F-NDV trimer (a) and HN-NDV dimer (b) surface representations of the MG strains. The location of neutralising epitopes is shown in red, the amino acid substitutions specific to the MG group are in blue in the globular head and in black in the stalk region. The figures were generated by Swiss PDB-viewer, using the X-ray structure of NDV F protein, position 33aa to 454aa for F protein and 124aa to 569aa for HN protein.*

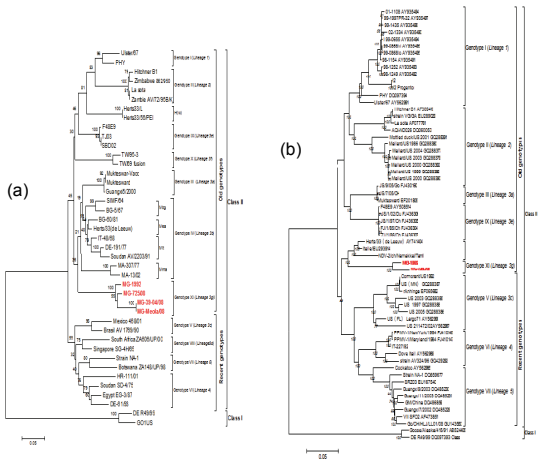


Fig 1 *Phylogenetic tree (unrooted) of the nucleotide sequences based on a 374-bp (position 47– 421 nt) region of the F gene (a) and on a 14977nt included genes NP/P/M/F/HN/L genes (b). Phylogenetic relationships of Madagascar strain (MG) with previously published sequences in Genbank. The evolutionary history was inferred using the Neighbor-Joining method. All results are based on the pairwise analysis. Analyses were conducted using the Kimura 2-parameter method in MEGA4 with 1000 bootstraps. The isolates from Madagascar that were subjected to analysis in this work are in bold and red font. Genotype and the lineage groupings are indicated on the right.*

CONCLUSION

In Madagascar the presence of a new APMV-1 genotype, presumably deriving from an ancestor close to genotype IV introduced in the 50's, show a particular evolution of NDV and reinforce the idea that this island is a unique natural ecosystem for micro- organisms. In addition, the possibility that this genotype represents a variant able to escape of immune response induced by current vaccines should not be under-estimated.

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