Isolation and molecular characterization of virulent Newcastle disease viruses in Mali in 2007 and 2008

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INTRODUCTION

Newcastle disease virus (NDV) is the causal agent of a fatal respiratory and neurological disease that can result in 100% morbidity and mortality in chicken flocks. ND was in the former list A diseases of the World Organisation for Animal Health (OIE) and is still one of the most serious animal diseases in developing countries.

Although ND is endemic in Africa, little is known about the NDV strains circulating in this continent. The objective of this study was to detect, isolate and characterise the NDV strains circulating in Mali.

In this study, cloacal and tracheal swabs were collected on healthy chickens in two different regions in Mali in a framework of a surveillance programme implemented in 2007 and 2008. These samples were analysed by RT-PCR. Positive samples were inoculated into eggs and isolated viruses were sequenced.

MATERIAL AND METHODS

RNA extraction was performed using the Nucleospin Virus Kit (Macherey Nagel) on an automatic workstation (Beckman FX^P), allowing the extraction of 90 samples in 50 minutes.

Virus RNA detection was done by a one-step RT-PCR targeting the F gene of NDV. All 1076 samples from domestic birds and all 1041 samples from wild birds were analysed and the positive ones were further inoculated into the allantoic cavities of 9 day-old to 11 day-old embryonated eggs. The complete procedure is represented in fig 1.

Nucleotide sequencing was achieved on 1659 pb of the F gene and on the full sequence of the HN gene. Phylogenetic analyses were carried out using the neighbor-joining method.

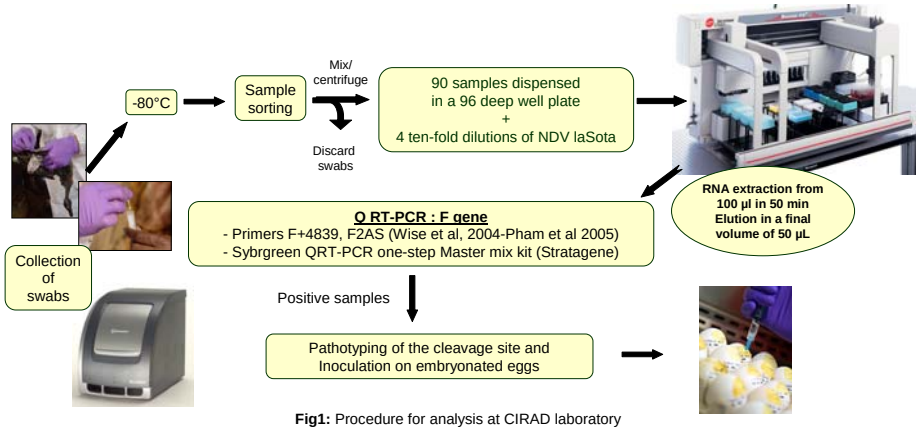

Fig1: Procedure for analysis at CIRAD laboratory

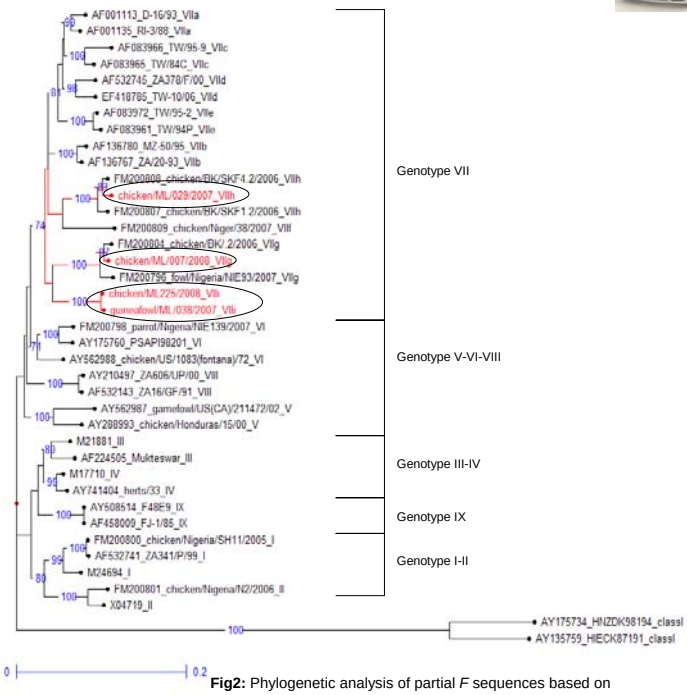

Fig2: Phylogenetic analysis of partial *F* sequences based on nucleotides 31-377. The tree was constructed using neighbor-joining method with 1000 bootstrap replicates. Only bootstrap values higher than 70 are shown. Malian NDV sequences are in red.

RESULTS AND DISCUSSION

NDV prevalence was 1.7% for domestic birds and 2.3% for wild birds. Four viruses were isolated from domestic birds after two passages into embryonated eggs. Unfortunately no isolates from wild birds could be obtained. The sequence of the cleavage site shows the presence of basic amino acids and thus characterise these isolates as virulent strains.

Two motifs were identified for the cleavage sites with at least three basic amino acids (112-RRRKR*FV-118 and 112-RRQKR*FI-118). The presence of V_{118} associated with the cleavage site RRRKR*FV in two of these isolates was only reported recently, in the neighbour country Burkina Faso (1).

Phylogenetic analysis based on a fragment of 356 nucleotides corresponding to position 48 to 422 of the F gene showed that these isolates are branched with the genotype VII (fig 2). This genotype represents the currently circulating genotype in Europe, Asia and Africa (2-3). Phylogenetic analysis performed on the full sequence of the F and HN genes confirmed that the Malian NDV isolates belong to the genotype VII. Two of the isolates clustered in the two subgenotypes (VIIg and VIIh) recently described by Snoeck et al in Burkina Faso, Niger and Nigeria (1). This suggest a circulation of the strains through animal trade between these countries. The two other isolates may define a new subgenotype (VIIi, fig 2).

These results show that the two survey regions were good sites for NDV ecology since they are major trade crossroads for domestic birds of different neighboring countries.

CONCLUSION

This study reports on original NDV isolates detected in Mali during a surveillance campaign in domestic and wild birds over 2007 and 2008. Four strains, genetically characterise as virulent may define new subgenotypes. These virulent strains were surprisingly isolated from unvaccinated and apparently health poultry. Question about the real virulence of these strains warrants further experimental demonstration.

References: 1- *Snoeck CJ, Ducatez MF, Owoade AA, Faleke OO et al , Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms2009. Archives of Virology (2009)-154*
2- *Lomniczi B, Wehmann E et al: Newcastle disease outbreaks in recent years in Western Europe caused by an old (VI) and a novel genotype (VII). Archives of Virology (1998) -146*
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