Vaccine 27 (2009) 3127–3129

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Letter to the Editor

Africa, a reservoir of new virulent strains of Newcastle disease virus?

ARTICLE INFO

Keywords:
Newcastle disease virus
African strains
Genotypes
Phylogenetic analysis

To the Editor,

Although Newcastle disease (ND) is endemic in Africa, little is known about the molecular epidemiology and genotype distribution of Newcastle disease virus (NDV) strains or the protection conferred by vaccination. This letter reports on original NDV isolates detected in the wetlands of Madagascar and Mali, which may constitute new genotypes or subgenotypes.

NDV, the avian paramyxovirus serotype 1 (APMV-1), is the causal agent of a fatal respiratory and neurological disease that can result in 100% morbidity and mortality in chicken flocks [1]. This disease is still one of the most important in poultry production worldwide, although vaccination measures have been applied more than 50 years.

In rural Africa, the predominant chicken production systems (backyard farms) are based on indigenous domestic fowl (*Gallus gallus domesticus*), and ND is rated as the most devastating disease in these farms [2]. In Madagascar, for example, ND was observed first in 1946 [3], and it is currently responsible for 44% of the mortality in backyard farms, which represent more than 83% of the avian production [4]. Likewise, ND is endemic in Mali, where prevalence rates reach more than 60% depending on the region and season [5]. ND causes high mortality in Malian backyard farms, which represent more than 90% of the avian production [5].

This study deals with the detection of original NDV strains in Africa. One and six isolates were collected respectively, in Madagascar and Mali, during a surveillance programme in 2007 and 2008 in African wetlands by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France. Virus detection was performed by RRT-PCR and, after sequencing of a fragment of 356 nucleotides corresponding to position 48–422 of the fusion gene (including the cleavage site), phylogenetic analyses were carried out using the neighbor-joining method.

Concerning Mali, five out of the six isolates originated from the area surrounding the city of Mopti and one isolate was from the Sikasso region (Table 1). The Mopti region constitutes most of the inner delta of the Niger River and is an important port for cargo and passengers. The city of Mopti is a central market town where the various tribes go to trade for fish, salt, living animals, fruit, vegetables, and crafts. Sikasso is the South-Western region of Mali and encompasses the major trade roads with Ivory Cost and Burkina Faso. Consequently, Mopti and Sikasso represent ideal sites to study the interaction between domestic and wild bird populations and between bird populations from different West African countries.

The sequences of the six Malian isolates correspond to virulent cleavage sites (^{112}RRRKR$^{\downarrow}$FV^{118} and ^{112}RRQKR$^{\downarrow}$FI^{118}) with at least three basic amino acids. It is rare to find V_{118} associated with the cleavage site ^{112}RRRKR$^{\downarrow}$FV^{118}, as in two of these isolates. This association was only recently reported in the neighboring country Burkina Faso (more than 2000 sequences from Genbank were analysed). Sequence alignment shows that the strains isolated from the same site (Mopti market for example), the same bird species (chicken), and in the same year are identical but different from the strains isolated from another site (Sikasso) in the following year. Moreover, the strains isolated from the same site and in the same year but from different species (chicken and guinea fowl) are also different. Phylogenetic analysis (Fig. 1) of four out of the six isolates (two were not analysed because they were identical to two sequences included in the analysis) showed all in genotype VII (or lineage 5 proposed by Aldous et al. [6]), which is the currently circulating genotype in Europe, Asia, and Africa but the two sequences with cleavage site ^{112}RRRKR$^{\downarrow}$FV^{118} had some motifs pertaining to other genotypes like $V_{118,}$ characteristic of genotype V (lineage 3). In a recent article, Snoeck et al. [7] suggested that NDV isolates from West Africa, genetically distant from all known sublineages, represent three new ones (tentatively named by the authors 5f, 5g, and 5h). It is possible that our Malian NDV isolates are clustered in one or more of these putative new sublineages, supporting the notion that these sublineages represent the NDV variants indigenous to West Africa. However, two of them seem to represent an additional new subgenotype (suggested as VIIi) never encountered before (more than 3000 sequences from Genbank were checked, Fig. 1). The analysis of other genome sequences is in process. It is surprising that these six virulent strains were isolated from poultry apparently not vaccinated and in good health. The question of the real virulence of these strains needs to be verified experimentally.

In a second approach, we also did the molecular characterization of a Madagascar NDV isolate (chicken/MG/725T/2008), obtained from an apparently healthy chicken. The sequence of the cleavage

0264-410X/$ – see front matter
doi:10.1016/j.vaccine.2009.03.076

Table 1
African NDV isolates used in this study.

Isolate	Specie	Country/region	Cleavage site
Chicken/Mali/029/2007	Chicken	Mali/Mopti	^{112}RRRKR$^{\downarrow}$FV^{118}
Guinea fowl/Mali/038/2007	Guinea fowl	Mali/Mopti	^{112}RRQKR$^{\downarrow}$FI^{118}
Chicken/Mali/225/2008	Chicken	Mali/Mopti	^{112}RRQKR$^{\downarrow}$FI^{118}
Chicken/Mali/007/2008	Chicken	Mali/Sikasso	^{112}RRQKR$^{\downarrow}$FI^{118}
Chicken/MG/725T/2008	Chicken	Madagascar/Antananarivo	^{112}RRRRR$^{\downarrow}$FV^{118}
Chicken/MG/1992	Chicken	Madagascar/Antananarivo	^{112}RRRRR$^{\downarrow}$FV^{118}

site of the F protein (^{112}GRRRRR$^{\downarrow}$FV^{118}) showed five basic amino acids (R) at positions 112–116, representing a virulent motif. In addition, the presence of the phenylalanine (F) residue at position 117, always associated with virulent cleavage sites, was already described as being a possible contributor to the neurological effects [8]. To our knowledge, the cleavage site of this isolate has never been reported. Phylogenetic analysis showed that this isolate is closer to genotype IV but may be distant enough to constitute a new genotype (genotype XI? Fig. 1). The maximum percentage of identity of this strain was 88% with Herts/33 strain, pertaining to genotype IV (AY741404). Genotypes III and IV were responsible for the first panzootic of ND in 1926 to 1960, and genotype IV has never been isolated after 1987. Madagascar was infected for the first time in 1946 during World War II. As expected, the NDV strains that infected Madagascar at that time belonged to genotypes III and IV. Consequently, whether the chicken/MG/725T/2008 virus isolated is a genetic variant of the initial genotype III or IV or another genotype introduced in Madagascar after 1960 requires further analyses. We found also the same original cleavage site in an older strain isolated in Madagascar in 1992 (chicken/MG/1992) after suspicion of ND in a NDV-vaccinated layer farm where the animals showed, among others, severe neurological signs. This isolate clearly forms a phylogenetic cluster with chicken/MG/725T/2008 (Fig. 1), thus reinforcing the hypothesis of the circulation of particular NDV strains in Madagascar. Complete sequencing of these viruses and the analysis of more recent isolates from poultry and wild birds in Madagascar are ongoing. It is interesting to consider that Madagascar may be an exclusive natural reservoir for this new specific genotype.

Another important point to consider is that the chicken/MG/1992 was isolated from a vaccinated layer hen in a commercial farm in Antananarivo. The vaccine used to control ND in this farm was an inactivated vaccine (ITA-NEW®, Laprovet) containing the LaSota strain, the same as the one used in Mali

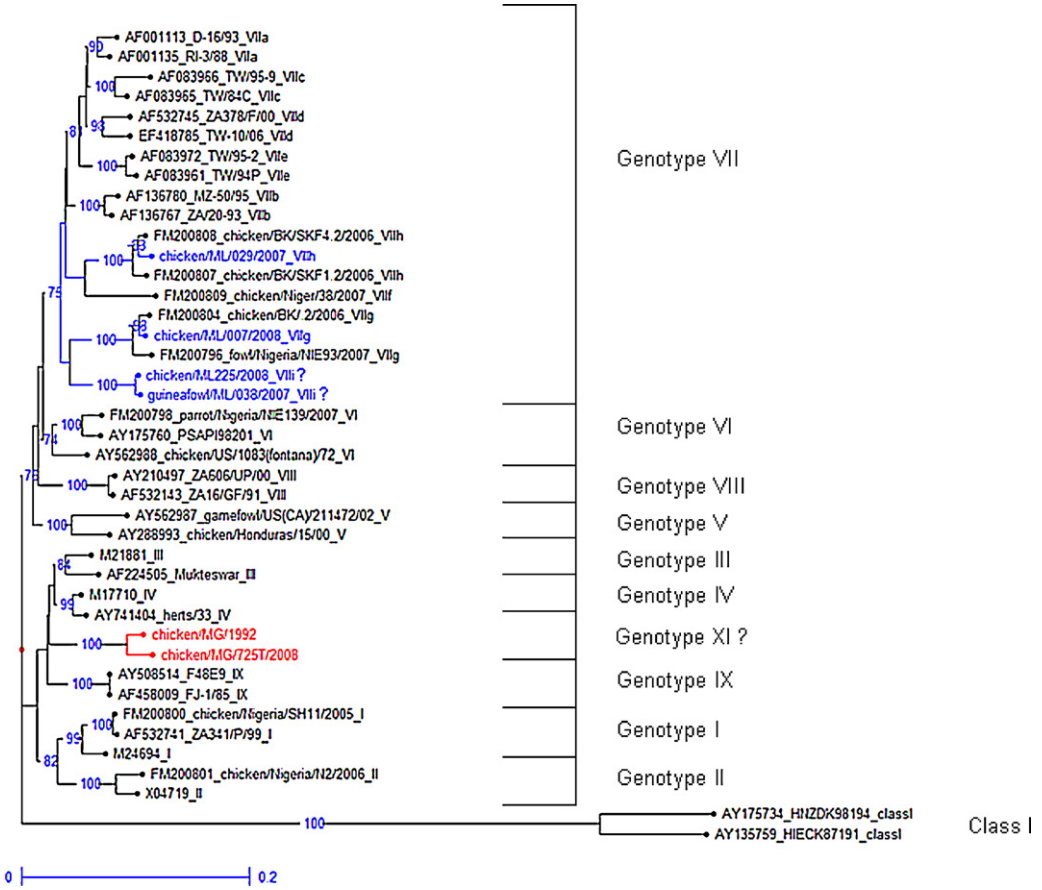

Fig. 1. Phylogenetic analysis of representative sublineages of NDV strains based on comparison of practical F gene sequence (positions 21–377 nt). The Malian sequences obtained in the present study are in blue and the sequences from Madagascar are in red. Accession numbers of the sequences from GenBank are shown. The tree was constructed using the neighbor-joining method with 1000 bootstrap replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

for several decades. The LaSota strain belongs to genotype II and, then, differs from the chicken/MG/1992 strain and genotype VII, now prevalent in West Africa. Although the relationship between genotype and antigenicity remains debated [9], different levels of cross-protection have been observed in chickens vaccinated with LaSota and challenged with other strains [9]. A partial protection conferred by vaccination can promote the emergence of immune-escape mutants responsible for ND outbreaks as described in China [9].

Together, our results emphasise the importance of NDV surveillance and re-assessment of vaccination programmes to control ND in Africa, since new genotypes or subgenotypes with panzootic potential may arise in the future.

Acknowledgements

This study was mainly funded by the French Ministry of Foreign Affairs (MAE) via the FSP project [GRIPAVI 2006-26] and partly by the EU network of excellence project [EPIZONE (016236, 01/06/2006–31/05/2011)].

References

[1] Alexander DJ. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Saif YM, Barnes HJ, Glisson JR, Fadly AD, McDougald LR, Swayne DE, editors. Diseases of Poultry. Ames: Iowa State University Press; 2003. p. 64–87.
[2] Kitalyi A. Village chicken production systems in rural Africa—household food security and gender issues. Animal health and production paper, vol. 142. Rome: FAO 81; 1998.
[3] Rajaonarison JJ. Production de vaccin contre la maladie de Newcastle à Madagascar. Workshop on Newcastle disease vaccines for rural Africa. Debre Zeit, Addis Ababa: Pan-African Veterinary Vaccine Centre (PANVAC); 1991.
[4] Maminiaina OF, Koko M, Ravaomanana J, Rakotonindrina SJ. Epidemiology of Newcastle disease in village poultry farming in Madagascar. Rev Sci Technol 2007;26(3):691–700.
[5] Sylla M, Traoré B, Sidibé S, Keita S, Diallo FC, Koné B, et al. Epidémiologie de la maladie de Newcastle en milieu rural au Mali. Revue d'élevage et de médecine vétérinaire des pays tropicaux 2003;56(1–2):7–12.
[6] Aldous EW, Mynn JK, Banks J, Alexander DJ. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. Avian Pathol 2003;32(3):239–56.
[7] Snoeck CJ, Ducatez MF, Owoade AA, Faleke OO, Alkali BR, Tahita MC, et al. Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms. Arch Virol 2009;154(1):47–54.
[8] Kattenbelt JA, Stevens MP, Gould AR. Sequence variation in the Newcastle disease virus genome. Virus Res 2006;116(1–2):168–84.
[9] Tsai H-J, Chang K-H, Tseng C-H, Frost KM, Manvell RJ, Alexander DJ. Antigenic and genotypical characterization of Newcastle disease viruses isolated in Taiwan between 1969 and 1996. Vet Microbiol 2004;104(1–2):19–30.

Renata Servan de Almeida [a,*], Olivier Fridolin Maminiaina [b], Patricia Gil [a], Saliha Hammoumi [a], Sophie Molia [a], Véronique Chevalier [a], M. Koko [b], Harentsoaniaina Rasamoelina Andriamanivo [b], Abdallah Traoré [c], Kassim Samaké [c], Abbas Diarra [c], Colette Grillet [a], Dominique Martinez [a], Emmanuel Albina [a]

[a] *CIRAD, Montpellier F-34398, France*
[b] *FOFIFA-DRZV, B.P. 04 Antananarivo 101, Madagascar*
[c] *LCV, Km 8, route de Koulikoro, BP 2295, Bamako, Mali*

* Corresponding author. Tel.: +33 4 67 59 37 24; fax: +33 4 67 59 37 98.
E-mail address: renata.almeida@cirad.fr (R. Servan de Almeida)

20 March 2009
Available online 15 April 2009