Characterization of a Newcastle Disease Virus Isolated from Apparently Normal Guinea Fowl (*Numida melagris*)

B. Mathivanan, K. Kumanan* and A. Mahalinga Nainar

Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai 600 007, India

*Correspondence: E-mail: kumananrani@hotmail.com


ABSTRACT

This report describes the isolation and molecular characterization of Newcastle disease virus isolated from an apparently normal guinea fowl (*Numida melagris*). With a mean death time of 54 h and intracerebral pathogenicity index of 1.80, the isolate has been identified as velogenic by biological methods. Fusion protein cleavage site amino acid sequence analysis of the isolate indicated the presence of two pairs of basic amino acids at the C-terminus of the F2 region and phenylalanine at the N-terminus of the F1 region, confirming the velogenic nature of the isolate. Phylogenetic analysis of the isolate revealed that this isolate is genotypically related to other neurotropic velogenic isolates like Iowa/Salisbury, Texas GB, Kansas/Manhattan and mesogenic Michigan.

Keywords: guinea fowl, fusion protein cleavage site, Newcastle disease, phylogenetic analysis

Abbreviations: AAF, amnioallantoic fluid; FPCS, fusion protein cleavage site; HA, haemagglutination; HI, haemagglutination inhibition; ICPI, intracerebral pathogenicity index; MDT, mean death time; ND, Newcastle disease; NDV, Newcastle disease virus

INTRODUCTION

Newcastle disease (ND) is one of the most contagious diseases of poultry, with a wide avian host range worldwide (Alexander *et al*., 1997). In India, various avian species are reared for meat and eggs and also as pet birds. Guinea fowls, which are native to West Africa, are one such avian species reared for meat and eggs in many parts of the Indian subcontinent. In recent times, there has been increasing interest in India in domesticating and commercializing guinea fowl (Mishra *et al*., 2000). Guinea fowl are reported to be susceptible to Newcastle disease (Aitken *et al*., 1977) and natural infection with ND has been recorded by several workers from various countries (Durojaie and Adene, 1988; Rampin *et al*., 1993; Mishra *et al*., 2001). In an earlier report, Newcastle disease virus (NDV) has been isolated from a clinically affected guinea fowl in India and characterized as viscerotropc velogenic on the basis of conventional biological tests (Mishra *et al*., 2001). However, the present study describes the isolation and characterization of a NDV from an apparently healthy guinea fowl by both biological and molecular methods.
MATERIALS AND METHODS

Virus isolation and identification

Cloacal swabs were collected from apparently healthy guinea fowl transported from the northern part of India to Chennai City in southern India. The contents of the swabs were suspended in phosphate-buffered saline containing penicillin (300 U/ml) and streptomycin (300 µg/ml) and centrifuged at 3000g for 15 min at 4°C. Supernatant (0.1 ml) was inoculated into the allantoic cavity of 9-day-old embryonated fowl's eggs. Three blind passages were given for each sample before ruling them out as negative. Out of 20 samples tested, one sample killed the embryos in the third passage. Examination of the dead embryos revealed haemorrhages all over the body. Amnio-allantoic fluid (AAF) collected from these embryos agglutinated chicken erythrocytes. Haemagglutination inhibition (HI) test (Cunningham, 1966) performed with NDV antiserum resulted in the inhibition of the haemagglutinating (HA) property of AAF.

Conventional biological characterization

Virulence characters such as mean death time (MDT) and intracerebral pathogenicity index (ICPI) were assessed using 9-day-old embryonated fowl's eggs and day-old chicks, as described by Allan and colleagues (1978). Strain-differentiating characters such as stability of haemagglutinins at 56°C (Hanson et al., 1949) and ability to agglutinate equine erythrocytes (Cunningham, 1966) were also assessed.

Molecular characterization

The viral genomic RNA was extracted from virus-infected AAF by the solution D method (Chomezynski and Sacchi, 1987). RT-PCR was performed with the extracted RNA using the degenerate primers for the fusion protein cleavage site (FPCS), 5'-CTG TGG TAC AAC ACT TTT ATT TTC AGG CTT GGT GATA ATC C-3' (Seal et al., 1995). The cDNA synthesis was done with genomic RNA with RT enzyme (Superscript II, Life Technologies, Grand Island, NY, USA), with incubation at 42°C for 1 h followed by denaturation at 94°C for 5 min. The cDNA was amplified in a thermal cycler (Perkin Elmer, Foster City, USA) using Taq polymerase by 25 cycles of denaturation at 94°C for 30 s, primer annealing at 52°C for 40 s and primer extension at 70°C for 1 min. The final extension was for 10 min at 70°C. The 254 bp amplified product was separated by gel electrophoresis in 2.5% agarose using TAE buffer. The amplified products were purified by agarose gel extraction columns (Qiagen, GmbH, Hilden, Germany) and sequenced in an automated sequencer (ABI prisms, Version 3, Applied Biosystems, Foster City, USA).

Nucleotide sequence alignment, editing and translation of the FPCS sequence was done using software packages of Clustal version 1.8 (Thompson et al., 1994) and Gene Tool. The predicted amino acid sequence alignment and the phylogenetic analysis were