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Molecular Identification of Newcastle Disease Virus from Free Living Birds

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Abstract: Newcastle disease (ND) is one of the major viral diseases of poultry causing great economic losses to the poultry industry. A total of 63 cloacal swabs/droppings from various categories of free living birds (desi chicken, pigeon, turkeys, crows, sparrows, geese, parrots) were collected and inoculated into specific pathogen free embryonated chicken eggs for propagation of Newcastle disease virus (NDV). Four out of 63 cloacal swabs were positive for NDV by reverse transcriptase polymerase chain reaction for fusion protein cleavage site of NDV. It is concluded that free living birds may play an important role in the transmission of NDV to domestic chicken and strict farm biosecurity measures has to be adopted to minimize the effective contact between them.

Keywords: Newcastle disease, free living birds, RT-PCR

1. Introduction

Newcastle disease remains a constant threat to the poultry industry and is a limiting disease for poultry producers worldwide [1]. It may represent a bigger drain on the world economy than any other animal viral disease of poultry [2]. Apart from commercial poultry, a wide range of captive and free living birds are susceptible and can act as primary source of ND infection to chicken [3], [4]. Limited work has been done to know the role of free living birds like desi chicken, caged pet birds, turkeys, geese, pigeons, sparrows, crows etc. in the spread of the ND to commercial chicken [5]. Hence, this study is aimed to assess the role of free living birds in the spread of ND.

2. Materials and Methods

A total number of 63 cloacal swabs/droppings from various categories of free living birds (Table - 1) were collected in phosphate buffered saline at Chennai, Tamil Nadu, India, and centrifuged at 1500 g for 15 minutes at 4°C. The supernatant was treated with penicillin at the rate of 10,000 IU/ml and of streptomycin at the rate of 10mg/ml. The supernatants of cloacal swabs were inoculated into 9-10 day old specific pathogen free embryonated chicken eggs through allantoic cavity route and incubated at 37°C used for propagation of NDV. Amnio-allantoic fluid (AAF) collected from the dead embryos were subjected to haemagglutination (HA) test with 1% washed chicken erythrocytes and all the dead embryos were examined for the presence of characteristic NDV lesions. Aminio allantoic fluid collected from dead embryos was used for viral genomic ribo nucleic acid (RNA) extraction. Reverse transcriptase polymerase chain reaction (RT-PCR) for fusion protein cleavage site (FPCS) gene region of NDV was carried out as per the protocol described by Seal [6]. The final PCR product size of 254 bp was checked with 2 per cent agarose gel with 100 bp molecular marker.

3. Results and Discussion

Out of the 63 number of cloacal swabs from free living birds inoculated into the embryonated chicken eggs, four samples (Desi chicken, pigeon, sparrow and crow) were positive by RT-PCR, with per cent positivity of 6.35%. Newcastle disease virus infection in free living birds such as desi chicken, pigeon, crows and sparrows in this study is a classical reminder that any free living and caged birds can act as natural reservoirs of NDV which was also suggested by Hanson and Spalatin [7] that the apparent emergence of ND as highly pathogenic disease of poultry was possible because NDV in its virulent form was enzootic in some other species in which it produced the disease or an unrecognized disease. Newcastle disease viruses of low virulence are hypothesized to give rise to virulent viruses by mutations and it is not clear whether such mutations takes place in free living birds reservoirs or are introduced into chickens and then mutate. The lack of virulent isolates from free living pigeons and crows indicted that the latter is more likely [2]. An earlier report by Alexander and Parsons [8] has indicated that increase of virulence of NDV isolates from other poultry when passaged in chickens. Identification of NDV infection in free living birds in this study is and evidence of the need for continuous characterization of NDV strains of all pathotypes which will provide a better understanding of the diversity that exists as suggested by King and Seal [9]. Single stranded RNA viruses which lack of proof reading and post replicative error correction mechanisms are expected to have high mutation rate and therefore to evolve rapidly [10]. Immune system of the birds may force the virus to evolve more rapidly in order to create escape mutants, which in turn might lead to the emergence of a few mutants and avoid clearance by the hosts immune system and such mutants have the ability to spread widely and cause epidemics in chickens [11].

4. Conclusion

Molecular identification of NDV from free living birds necessitates strengthening of farm biosecurity measures to

minimize the contact of domestic poultry with free living birds for preventing the spread of NDV.

5. Future Scope

Present study will helpful to identify the role of pigeon, turkeys, crows, sparrows, geese and parrots in the transmission of NDV to domestic chicken. Moreover, this study will throw light on identification of wild and pet birds serves as a reservoir of ND.

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Table 1: Results of Reverse	transcriptase	polymerase chai	n
reaction	n for NDV		

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Bird	No. of samples	Number of
	inoculated	positives
Desi chicken	22	1
Pigeon	9	1
Turkeys	3	-
Crows	10	1
Sparrows	16	1
Geese	2	-

Author Profile



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