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SHORT COMMUNICATION

Potential of Commonly Resident Wild Birds towards Newcastle Disease Virus Transmission

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ABSTRACT

Newcastle disease (ND) is one of the most important diseases of avian species across the world. The present study was conducted to investigate the carrier potential of four common wild birds towards ND virus. Indian mynah (n=25), house sparrow (n=25), house crow (n=25) and pigeons (n=25) were captured using mist netting technique from the vicinities of different poultry farms. Cloacal swabs were taken and processed for RT-PCR. The carrier potential of pigeons, sparrows, crows and mynah for NDV was found to be 24, 20, 16 and 16%, respectively. These results highlighted that these common wild birds are the major cause of spread of ND viruses and there is need to consider them all as a high risk species for transmitting ND viruses between premises.

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INTRODUCTION

Newcastle disease is caused by pathogenic strains of Newcastle Disease Virus (NDV). It is the most important disease of poultry and wild birds. Newcastle disease is caused by NDV, an avian paramyxovirus type 1 which is a negative stranded RNA virus, classified into genus *Avulavirus* and family *Paramyxoviridae* (paramyxoviridae type I). Avian Paramyxo virus 1 (APMV-1) is classified into 3 pathotypes based upon pathogenicity i.e. Lentogenic, Mesogenic and Velogenic. Lentogenic NDVs have low virulence and velogenic viruses are the most pathogenic and cause high mortality (Anis *et al*., 2013).

NDV can affect wide range of avian species i.e. more than 230 species. More than half of the 50 orders of birds are susceptible to natural or experimental infections with APMV-1 (Kim *et al.,* 2007). In 2012, there was outbreak of ND in Jallo Wildlife Park, Lahore, Pakistan, caused by APMV-1 which killed 190 peacocks within a week (Munir *et al.,* 2012). A study from Nigeria indicated 25% of the samples positive for NDV among the samples collected from crow and sparrows (Ibu *et al*., 2009).

Traditional methods used to diagnose and detect Newcastle disease at field level in Pakistan is greatly limited to the observation of clinical signs; demonstration of postmortem lesions in ND affected birds and serological diagnostic methods including HA/HI, AGID and virus neutralization (VN) and ELISA. But these techniques are relatively expensive and time consuming as compared to genome detection of the virus as a solitary confirmatory method. The reverse transcription-polymerase chain reaction (RT-PCR) has proven to be the best confirmatory test for the diagnosis of Newcastle disease virus genome in the field conditions (Singh *et al.,* 2005). The primary objective of the present study was to determine the carrier potential of common wild birds towards the Newcastle disease virus.

MATERIALS AND METHODS

Mist netting technique was used to capture the wild bird species. The mist net was assembled vertically on poles and was deployed near the poultry farms; in the areas of high activity to intercept birds as they go about their normal daily routines. All the birds were treated gently and were set free after taking the sample. A total of 100 birds were captured; comprising 25 birds from each of the following species: *Columba livia domestica* (pigeons), *Corvus splendens* (house crow), *Passer domesticus indicus* (house sparrow) and *Acridotheres tritis* (Indian Mynah).

Sterile dacron swabs were used for sampling. Cloacal swabs were taken from individual birds and placed in cryovials containing viral transport media (VTM). The VTM used in the cryovials was PSB solution. The PBS solution was prepared by adding one commercially available PBS tablet in 100ml of double distilled RNAse free water along with addition of 10,000 IU penicillin/ml and 5,000 IU nystatin/ml. The cryovials were stored at -

80˚C until use. The VTM was treated with proteinase K (Thermoscientific™) followed by RNA extraction with Phenol-chloroform-isoamyl alcohol (25:24:1) (Omnipur®). 50 µL low T.E. buffer (TrisHCl 10mM, EDTA 0.2 mM) was added according to amount of RNA pellet. The RNA samples were stored at -80°C until use.

A pair of degenerate primers, forward (NDF) 5'-CGIAGGATANCAAGRGTC TG-3' corresponding to 342-360 nucleotides of the F0 gene; and reverse (NDR) 5'-GCR GCAATGCTCTYTTTAAG-3' corresponding to 503-523 nucleotides selected to amplify a 182 bp fragment of the F_0 gene including the cleavage site (Daniela *et al.*, 2000). Primer sequences were based on F_0 gene alignment of 29 different NDV strains isolated from various regions and species. The primers were manufactured by Eurofins MWG Operon, Huntsville, Alabama, USA. The extracted viral genome RNA was used to make complementary DNA (cDNA) using cDNA kit (Revert Aid™ by Thermo Scientific) as recommended by the manufactures protocol. The PCR products were separated in 2% agarose gel in TAE buffer stained with ethidium bromide and compared with oligonucleotide DNA Ladder (50bp). The gel was observed under gel documentation system (Bio-Rad, USA).

RESULTS AND DISCUSSION

The study was conducted to determine the carrier potential of Newcastle Disease Virus among four commonly found wild birds predominant in Pakistan. From each group 25 samples were collected out of which 6(24%) pigeons, 5(20%) sparrows, 4(16%) crows and 4(16%) mynah were found positive for NDV genome. The carrier potential of pigeons, sparrows, crows and mynah for NDV was found to be 24, 20, 16 and 16%, respectively. Out of total 100 samples, 19% samples were found to be positive for NDV genome. Pearson's chi-square test revealed non-significant difference amongst the species ($P>0.05$, Chi-square=2.859, df=3).

Results of the present study indicated that the highest carrier potential was found in pigeons, followed by sparrows, Indian mynah and crows. It has been well described previously that the virulent strains of NDV circulating in wild environment can be transmitted to commercial poultry flocks through free movement of wild birds around the farms and susceptible bird population (Hlinak *et al.,* 2006). However, the prevalence identification as described in this study may vary largely depending upon birds under study, time of sampling, and sampling method as well as diagnostics itself. Zhu *et al.* (2010) investigated sparrows at Guangxi province of China and determined them responsible for transmission of virulent strains of NDVs as the same genotype was isolated both from chicken and those sparrows living around poultry farms; all NDV isolates except one were velogenicto chickens. In another study in Nigeria, crows have also been implicated to spread vNDVs to commercial and household poultry (Ibu *et al*., 2009). Korotetskiĭ *et al.* (2010) reported that pigeons and crow pose a serious threat to commercial poultry in Russia, Ukraine, Kazakhstan, and Kirghizia for the spread of NDV. Although in a recent study by Shabbir *et al*. (2013), they reported similar isolates originating from pigeons in commercial poultry, there is not yet a significant work done so far envisaging role of these wild birds as to be reservoir of NDVs and their possible role in disease transmission.

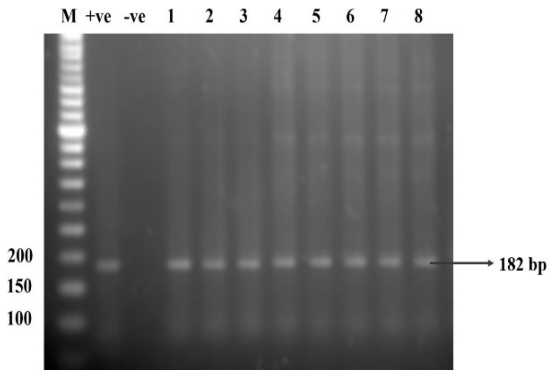

Fig. 1: NDV detection from cloacal swabs by RT-PCR from pigeon (1 and 2), mynah (3 and 4), sparrows (5 and 6) and crows (7 and 8). M=DNA marker ladder (50bp), +ve is positive control and -ve is negative control.

Conclusion: All the four common wild birds studied were found to be reservoir of NDV without clinical symptoms.

Author's contribution: AA planned the study. TM, MJ, BZ and MSI executed the experiment while IA analyzed the data. All authors interpreted the data, wrote the manuscript and approved the final version.

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