DETECTION OF NEWCASTLE DISEASE VIRUS IN EDIBLE-NEST SWIFTLET (*AERODRAMUS FUCIPHAGUS*) RANCHED UNDER AN OIL PALM PLANTATION IN SUNGKAI, PERAK, MALAYSIA

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Abstract

Newcastle Disease is regarded as one of the most important disease of avian species and was listed as 'A' disease by the Office International des Epizooties (OIE). Studies have shown this disease infects more than 250 species of birds. The edible-nest swiftlet industry in Malaysia is on the rise for the last 10 years and swiftlet houses can be seen everywhere. This is due to the high price of edible bird nests at international market and high income generated by the farming of edible bird nest. Nevertheless, edible-nest swiftlet (*Aerodramus fuciphagus*) is can either be infected with or carrying the deadly Newcastle disease virus (NDV) which is a great threat to the poultry industry in this country. In Peninsular Malaysia, no study on this subject was reported so far in this swiflet species. In this study, 60 swiftlet carcasses were sampled. Post-mortem was conducted and lung and trachea tissues were collected. Tissue samples were tested for NDV using the reverse-transcriptase polymerase chain reaction (RT-PCR). Newcastle Disease Virus was not detected in any of the samples. Thus, it can be concluded that edible nest swiftlets (*Aerodramus fuciphagus*) ranched under oil palm plantation in this study are not infected with or carry NDV.

Keywords: swiftlet, Newcastle disease, Newcastle disease virus, PCR

INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease of avian species (Alexander, 2006). Newcastle disease was first reported in Malaysia in 1934 and today the clinical diseases are still present in Malaysia as well as in several countries in the region (Aini, 2006; OIE Wahis, 2009).

Edible-nest swiftlet (*Aerodramus fuciphagus*) is classified under the kingdom *Animalia*, phylum *Chordata* and class *Aves*. Swiftlet ranching in Malaysia began in the 1980s. However, this industry only started to mushroom after 1997 (Lim and Cranbrook,

2002). In December 2001 it was estimated that there were approximately 1,000 swiftlet ranches in Malaysia and the number has grown to about 10,000 by 2005 (Burhanuddin, 2005). The high price of edible-bird nest is the main reason for the tremendous increase in the number of swiftlet ranches (Lim and Cranbrook, 2002). With the high price of edible-bird nest, it fetches a lucrative income and hence there is an increase in the number of people involved in swiftlet ranching. This study was conducted to determine the prevalence of ND in edible –nest Swiftlet ranched under an oil palm plantation in Sungkai, Perak, Malaysia.

MATERIALS AND METHODS

Degenerative primers 5'-ATGGGC (C/T)CCAGA(C/T)CTTCTAC-3'(forward) and 5'-CTGCCACTGCTAGTTGTGATA ATCC-3'(reverse) previously described by Berhanu *et al.* (2010) used to amplify the F-gene fragment were used in this study. The primer pair is universal primer which is able to detect all strains of Newcastle disease virus (NDV) and is expected to generate an amplicon size of 535 bp (nt 47 – 535 of nucleotides of F protein).

Sixty dead swiftlet carcasses were collected at one swiftlet ranch in Sungkai, Perak, Malaysia. Post-mortem was conducted and trachea and lungs were sampled. Organ samples collected from each bird were pooled and treated as one sample. Samples from each bird were minced in 1000 µL 1X phosphate buffered saline (PBS) (Merck, Germany). The pooled sample was then transferred into a 1.5 mL microcentrifuge tube. 1X PBS was added to each tube until the total volume of each tube reached 1.5 mL. The sample was then freeze-thawed three times followed by centrifugation at 3000 rpm for 15 min at 4°C. 300 µL of the supernatant was transferred into a new 1.5 mL tube and RNA extraction was conducted using TRIzol® reagent solution (Invitrogen, USA) and a series of steps as manufacturer recommendation.

Standard reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using Access® One-step RT-PCR kit (Promega, USA) with 2 µL of extracted RNA from sample resulting in a total reaction volume of 25 µL. NDV strain AF2240 was used as positive control while sterilised deionised water was used as negative control. RT-PCR was carried out in MyCycler^{TM} (BIO-RAD, USA). The cycling parameters were set at 48°C for 45 min (reverse transcription), and 35 cycles of 94°C for 2 min (denaturation), 56°C for 2 min (annealing) and 72°C for 1 min (extension), followed by 72°C for 10 min (final extension).

Two microliters of loading dye (Vivantis, USA) was added to RT-PCR product of each sample. 10 µL of sample mixed with loading dye was loaded into agarose gel (Promega, USA). 100bp DNA marker (Vivantis, USA) was used as the indicator. Electrophoresis was performed by running the gel in 1X TAE buffer (Merck, Germany) under 80V for 40 min. The gel was then stained with 1% ethidium bromide and viewed under UV light (ProteinSimple, USA). The resulting band for each sample was observed. Sample(s) with band observed between 500 to 600 bp was considered as positive while sample with absence of such band was considered negative.

RESULT

None of 60 samples tested showed any band in 500 to 600 bp region, which was expected in NDV-positive cases. It is suggested that edible-nest swiftlet (*Aerodramus fuciphagus*) is not naturally infected with or act as a carrier or reservoir for NDV.

DISCUSSION

There are various reasons which could the absence of NDV in the samples. This includes low prevalence of NDV infection in wild birds population, the flying height and habit or this bird itself may be naturally resistant to NDV infection.

According to a study done by Tan (2005), the prevalence of NDV infection in wild birds' population is in fact low. We showed that only 20.2% of the samples were seropositive for NDV antibody. This study showed that the prevalence of NDV infection in edible next swiflet population is low and the risk of these birds contracting this disease is very low.

Edible-nest swiftlet fly at a different level from that of other species of birds, which is at a specific height between 30 to 60 meters above the forest canopy. Edible-nest swiftlet also has the habit of not mixing and interacting with other species of birds even with their close relative, the black-nest swiftlet, *Aerodramus maximus*. Hence, there is minimal contact between edible-nest swiftlet with other wild avian species (Lim and Cranbrook, 2002; Aini, 2005).

Edible-nest swiftlet may be naturally resistant to Newcastle Disease Virus infection. According to Howe *et al.* (1961) and Biddle and Belyavin (1963), the edible-bird nest extract has inhibitory properties on the viral haemagglutinin and neuraminidase activity of Myxovirus and influenza virus respectively. Newcastle Disease Virus possesses haemagglutinin-neuraminidase surface glycoprotein which is important in the process of attachment to the host cells (Nagai, 1993). The substances in the nest produced by edible-nest swiftlet also have inhibitory properties on viral haemagglutination and neuraminidase activities and this might indirectly protect the bird from infection from viruses such as NDV.

CONCLUSION

From this preliminary study, it is suggestive that edible-nest swiftlet, *Aerodramus fuciphagus* is not naturally infected with or act as a carrier or reservoir for NDV. This is good news to the swiftlet industry and the conservation of the species because edible-nest swiftlet do not suffer from ND. Subsquently the expanding swiftlet industry would also not be a threat to the poultry industry in term of disease transmission.

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