MOLECULAR DETECTION OF NEWCASTLE DISEASE, MAREK’S DISEASE AND AVIAN LEUKOSIS VIRUS FROM VILLAGE CHICKENS - A CASE REPORT


Veterinary Research Institute, No.59, Jalan Sultan Azlan Shah, 31400 Ipo, Perak, Malaysia

*Corresponding author: leowbl@hotmail.com

ABSTRACT

A mixed viral infection of Newcastle Disease (ND), Marek’s Disease (MD) and Avian Leukosis (AL) was reported in village chickens. Analysis of the deduced amino acid sequences of the F protein cleavage site of ND virus showed that the isolate was virulent with sequence 112RKKR116 for the C-terminus of the F2 protein and phenylalanine (F) at residue 117, the N-terminus of the F1 protein. Basic Local Alignment Search Tool (BLAST) analysis showed the isolate was ND which was 96% similar identity with Indonesia Sukorejo genotype VII, MD 99% similar identity with China very virulent strain and ALV 100% similar identity with Taiwan ALV strain. Due to the free ranging type of management, several factors could contribute to the disease occurrence. However, good hygiene practices or vaccination will contribute towards prevention of diseases in village poultry.

Keywords: viral diseases, village chicken, hygiene, biosecurity

INTRODUCTION

In south-east Asian countries including Malaysia, small scale poultry farming is still practised as a backyard operation among some rural families although commercial poultry has been developing rapidly over the years (Ramlah, 1999). Rural families still keep village chicken due to its economic importance. The village chicken production which includes egg and chicken meat serves as a side income for the owner as well as cheap protein source in the family’s diet. Chicken’s manure can be used as fertilizer for the fruit trees and the sale of the fruits serves as an additional income (Aini, 1990). However, diseases are the one of the major challenges in expanding the industry (Aini, 1999). Due to the free ranging management system in village chicken, disease control is very difficult and seldom applied by farmers. Therefore, in most areas, the disease control is very minimal or lacking (Aini, 1999). The purpose of this paper is to report a mixed viral infection of Newcastle Disease (ND), Marek’s Disease (MD) and Avian Leukosis (AL) in village chickens.

MATERIALS AND METHODS

In this case, a poultry farmer complained of his village chickens showing dullness, inappetance and producing greenish faeces for 2 days. The farmer had 45 local village chickens aged 6 months old which were unvaccinated. The morbidity rate was 33.3% and mortality rate was 22.2%. Complete post-mortem and diagnosis was conducted on a dead carcass to elucidate the cause of mortality in the farm. On post-mortem, ND and MD were suspected. One pooled organs was received, processed and virus isolation was attempted for ND virus by inoculating the filtrate sample into 9-10 days old embryonated SPF eggs via intra-allantoic route. The infected allantoic fluid was harvested and the presence of the virus was identified by Haemagglutination test (HA) and Hemagglutination-Inhibition (HI) (OIE, 2012). Molecular detection was carried out for the infected allantoic fluid for ND using primer set MV1/82 (Herzeg et al., 1999). PCR was performed for MD from the filtrate sample of the pooled organ using primer MDV UL 19 P5/ MDV UL 19 P6 (Ottiger, 2010). PCR was also attempted for Avian Leukosis (AL) from the filtrate sample of the pooled organ using primer set H5/AD1 which amplifies the conserved region of AL subgroup A, B, C, D and E (Maaz et al., 2005). The amplicons generated were purified using the Qiagen QIAquick gel extraction kit and sent for sequencing. Sequences were assembled and analysed using Lasergene’s SeqMan Pro software. Nucleotide sequence of the isolate was checked and compared with published sequences deposited in the Gene Bank database using a BLAST analysis.
RESULTS AND DISCUSSION

The isolate revealed HA titer of 64 and was confirmed positive for ND by HI test after using antiserum against NDV. The isolate was also positive for ND, MD and AI by molecular detection with PCR product of 557bp, 521bp and 326bp respectively (Figure 1 and 2). Analysis of the deduced amino acid sequences of the F protein cleavage site of NDV showed that the isolate was virulent with the amino acid sequence \(\text{KRRKR}^{116}\) for the C-terminus of the F2 protein and phenylalanine (F) at residue 117, the N-terminus of the F1 protein. BLAST analysis showed the isolate was ND which was 96% similar identity with Indonesia Sukorejo genotype VII, MD 99% similar identity to China very virulent strain and AL 100% similar identity to Taiwan ALV strain subgroup E.

Fig. 1. Gel photo of molecular detection for ND from the infected allantoic fluid. The PCR products were separated on a 1.5% agarose gel stained with SYBR Safe DNA gel stain. Well 1-3 showed the PCR products for NDV detection. Well 1: Infected allantoic fluid; well 2: positive control; well 3: non template control; M: 100bp DNA ladder.

Fig. 2. Gel photo for molecular detection of ALV and MDV from the filtrate sample of the pooled organ. The PCR products were separated on a 1.5% agarose gel stained with SYBR Safe DNA gel stain. Well 1-3 and 4-6 showed the PCR products for ALV and MDV respectively. Well 1: sample; well 2: positive control; well 3: non template control; well 4: sample; well 5: positive control; well 6: non template control; M: 100bp DNA ladder.

The mixed viral infection reported in this case was in agreement with Aini (1990; 1999) and Permin et al. (1999) said that the common viral diseases found in village chickens are ND, MD, lymphoid leukemia, infectious bronchitis and others. How the village chickens were infected by the three viral diseases in this case is uncertain. Factors such as lack of biosecurity measures and good hygiene practices, no vaccination, introduction of the viruses by infected chickens or birds, and contaminated farm area by other birds can contribute to the occurrence of the diseases. Generally, due to the management system in village chicken, disease control is very minimal (Aini, 2000). Basic biosecurity with good hygiene practices play an important role in controlling the diseases (Aini, 2000; Lee et al., 2011). Shelter and fencing can be provided to minimize the contact of the poultry with other birds in the neighborhoods. Thus, transmission of the disease from the human traffic and vehicles can be reduced (Aini, 2000). Farmers are encouraged to keep single species of the poultry instead of several species of birds together (Aini, 2000). It is due to the severity of disease can be vary in different species of birds. One species which is naturally resistant to the disease can carry, transmit and
cause disease in other species (Aini, 2000). Disposal of dead birds must be quick and properly. Cleaning is
difficult in free range system but a basic disinfection can be applied. For example, application of lime to the
infected area after an outbreak (Aini, 2000). Due to the free range system of management, farmers may face
many challenges. Although the success may be limited, any improvement in the health and production of the
flocks which benefit from the biosecurity measure, gives a better income for the farmers (Aini, 2000). The
disease occurrence could be multifactorial and the farmer has to take an aggressive stand in combating the
disease.

CONCLUSION

A mixed viral infection of ND, MD and AL is reported in village chickens. There are several factors that can
contribute to the occurrence of the diseases and farmer awareness is essential in preventing such losses in
village poultry flocks.

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