MIXED VIRAL INFECTIONS IN VILLAGE CHICKENS

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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 ^{112}KRRKR^{116} for the C-terminus of the F2 protein and phenylanine (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, causes of the diseases in the poultry were uncertain as many factors can contribute to the disease occurrence. However, good hygiene practices can help to improve the farm's sanitary. Basic biosecurity system can be applied although the success may be limited. Vaccination is another alternative that can be considered to prevent the diseases with the help from certain parties.

Keywords: viral diseases, village chicken, hygiene, biosecurity

INTRODUCTION

In South-east Asian countries including Malaysia, poultry keeping is still practised as a backyard operation among some rural families although commercial poultry has been developing rapidly over the years (Ramlah, 1999). The Malaysian indigenous chicken, the village chicken which is also known as '*ayam kampung*' is a crossbreed of the Red Jungle Fowl with mixed exotic domestic breeds imported by Europeans, mainly the British (Aini, 1990). Rural families still rear 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 a family's diet. Cost of the production is very small and the chickens also play an important role in waste disposal system where the chickens help in converting the kitchen scraps into valuable protein. 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).

In this case study, a poultry farmer complained of his village chickens showing dullness, inappetance and producing greenish faeces for 2 days. The farmer had 45 local kampong chickens aged 6

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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.

MATERIALS AND METHODS

On post-mortem, ND and MD were suspected. Pooled organ obtained from the post-mortem was homogenised and diluted with TPB prior centrifugation. The supernatant was collected and filtered. Virus isolation was attempted for Newcastle Disease virus. It was carried out by inoculating the filtrate sample into 9-10 days old embryonated SPF eggs via intra-allantoic route and incubated for three days at 37^{o}C for two passages. The infected allantoic fluid was harvested and the presence of the virus was identified by Haemagglutination test (HA) and Hemagglutination-Inhibition (HI) test (Manual OIE 2012).

Molecular detection was carried out for the three avian diseases: ND, MD and AL. For ND, the viral RNA was extracted from the infected allantoic fluid using TRI LS Reagent (Molecular Research centre, Inc). RT-PCR is performed to detect the ND virus by using primer set MV1/B2 (Herczeg *et al*., 1999) that amplify the partial of matrix and fusion gene. The RNA was also amplified by using primer set of MV1/ NDVIR2 (in- house designed primers) to determine the pathotype of the virus. For both MD and AL, the viral DNA was extracted directly from the pooled organ by using DNeasy Blood and Tissue kit (Qiagen). The DNA was amplified to detect Unique Long (UL) region of MD virus (MDV) by using primer set MDV UL 19 P5/ MDV UL 19 P6 (Ottiger, 2010) and *Pol* gene for AL virus (ALV) using primer set H5/AD1 (Maaz *et al*., 2005). The amplicons generated were cut from the gel and purified using the Qiagen QIAquick gel extraction kit. Purified products were sent for sequencing to identify the isolate. Sequences were assembled and analysed using Lasergene's SeqMan Pro software. Analysis of protein sequence was done by BioEdit Sequence Alignment Editor. Nucleotide sequence of the isolate was checked and compared with published sequences deposited in the Gene Bank database using a BLAST (Basic Local Alignment Search Tool) search via the National Center of Biotechnology Information (NCBI).

RESULTS

The isolate presented HA titer of 64 and was positive for ND by HI test. A PCR product of 557bp was amplified for common detection of NDV (Figure 1). An amplicon of 495bp was detected by using pathotype determination primer set indicating that the isolate was virulent ND strain (Figure 1). A PCR product of 521bp and approximately 295-326bp was detected for MDV and ALV respectively (Figure 2). Analysis of the deduced amino acid sequences of the F protein cleavage

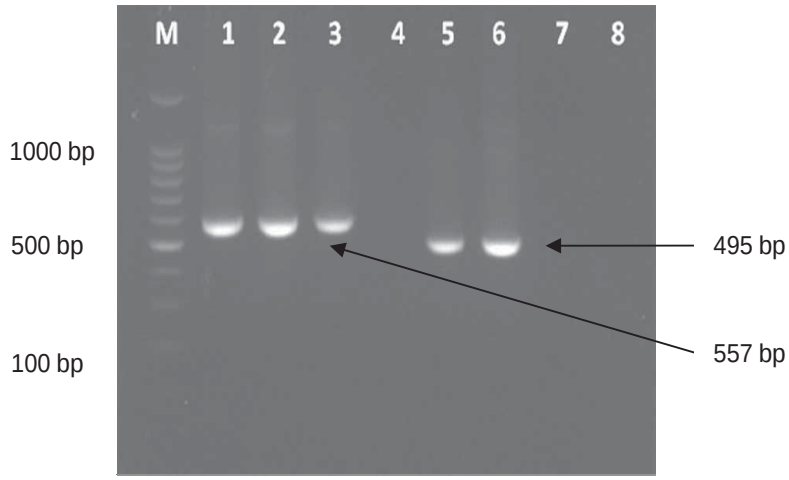

Figure 1. Gel photo for molecular detection of the ND isolate. The PCR products were separated on a 1.5% agarose gel stained with SYBR Safe DNA gel stain. Well 1-4 showed the PCR products for common detection of ND whereas PCR products for ND pathotype determination were showed in well 5-8. Well 1: ND Isolate; well 2: positive control (ND virulent strain); well 3: positive control (ND non-virulent strain); well 4: non template control; well 5: ND isolate; well 6: positive control (ND virulent strain); well 7: positive control (ND non-virulent strain); well 8: non template control, M: 100bp DNA ladder.

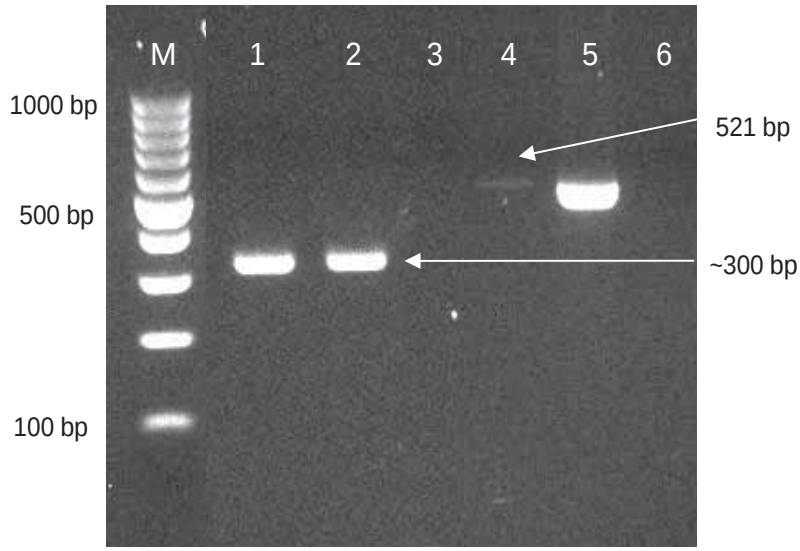

Figure 2. Gel photo for molecular detection of the ALV and MDV positive samples. The PCR products were separated on a 1.5% agarose gel stained with SYBR Safe DNA gel stain. Wells 1-3 and 4-6 showed the PCR products for ALV and MDV respectively. Well 1:sample; well 2: positive control; well 3: non template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

site of NDV showed that the isolate was virulent with the amino acid sequence ^{112}KRRKR^{116} for the C-terminus of the F2 protein and phenylanine (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% identity with China very virulent strain and AL 100% identity with Taiwan ALV strain.

DISCUSSION AND CONCLUSION

Many factors can cause mortality in free-range poultry keeping. 80-90% mortality of the poultry in the first year after hatching mostly due to improper management, short of fresh water and supplementary feed supply, predators and diseases. Among these, disease is the main problem in the production of village chicken (Permin *et al*., 1999). Due to free ranging and unconfined type of management, disease control is very difficult and is therefore rarely practiced by the farmers (Permin *et al*., 1999).

In this study, the isolate was confirmed ND by both virus isolation and molecular detection. Sequence analysis of the F protein cleavage site showed the isolate was virulent strain of ND and BLAST analysis revealed that it was 96% identity with Indonesia Sukorejo genotype VII ND. MDV and ALV were also detected in the samples by molecular detection. BLAST analysis of UL gene for MDV and *Pol* gene for ALV showed the isolate was 99% identity with China very virulent MD strain and 100 % identity with Taiwan AL strain. The mixed viral infection reported in this case was in agreement with Aini (1990 and 1999) and Permin *et al*. (1999) said that the common viral diseases found in village chicken are Newcastle disease, Marek's Disease, lymphoid leukosis, infectious bronchitis etc.

ND is a highly contagious disease in chicken where outbreaks with mortality up to 100% are common and has been one of the major causes of economic losses in the poultry industry. MD, a lymphoproliferative and neuropathic disease of chickens is caused by highly contagious cell-associated alphaherpesvirus MDV serotype 1 (Tulman *et al.,* 2000). MD causes huge economic losses as yearly economic losses of total $1 billion worldwide have been reported (Tulman *et al.,* 2000). Like MD, ALV is an avian oncogenic virus which can cause variety of neoplastic disease condition in chickens. The infection results in huge economic losses due to reduced productivity and ALV induced tumor (Fadly, 2000). The mixed viral infection consists of ND, MD and AL contributed to the morbidity and mortality of the village chickens with rate of 33.3% and 22.2% respectively.

According to Aini (1999), all countries in South-East Asia reported that among all the diseases, ND is the most important disease that causes the highest economic losses in indigenous chickens. The disease is endemic in village poultry populations in Africa and Asia (Spradbrow, 1993/94). The isolation of ND virulent strain in this

study was in agreement with the finding of Spradbrow (1993/94) stated that virulent strain of ND was detected when virus isolation was attempted in village poultry. Alexander *et al.* (2004) reported that the occurrence of ND in village chicken is mostly due to introduction of infected chicken. Domestic birds such as ducks, geese, doves, and guinea fowl can carry the virus and spread the disease. They may or may not manifest clinical syndrome of ND where the development of clinical signs is depends on the strain of ND and species of the bird (Alexander *et al*., 2004). Role of wild bird in spreading the disease was uncertain although ND virus of different virulence had been detected in many species of wild birds (Alexander 1988). Chicken's manure which sometimes used by the farmers as fertilizer can become the source of infection as the virus can persist in uncultivated feces for more than six months (G. Arzey, 1989).

Persistence of the viral agents may play a role in the spreading of the disease. MD virus can survived for at least 8 months in infected feather materials and 440 days in the dust in dry state from poultry houses at room temperature (Hlozanek *et al.,* 1977). Like MDV, NDV can survive well in the environment. It may persist at 8°C for months and 37°C for several days (Anonymous, 2003). The virus can also survive on materials such as paper, polythene and cloth for 25-35 days at room temperature (G.Arzey, 1989). As the village chickens like to roam freely to search for food, they may have come into contact with the infected materials and get infected.

How the village chickens were infected by the three viral diseases in this case is uncertain. Due to the free-ranging type of poultry management, there are too many factors that the village chickens can be infected by the agents as discussed earlier. Factors such as lack of biosecurity measures and good hygiene practices, no vaccination, introduction of the viruses by infected chickens or birds, contaminated poultry keeping area and etc can contribute to the occurrence of the diseases.

Generally, due to the management system in village chicken, disease control is very minimal (Aini, 2000). Therefore, good farm management and strict biosecurity play an important role in controlling poultry disease. Traffic control which includes both human and vehicles into and within the farm is important as disease can enter the farm by this route. Implementation of strict biosecurity measure may be hard with limited effectiveness in free range flocks but some of these practices may be applicable (Aini, 2000). Thus, raising the awareness of the farmers about the concept of flock diseases, principle of disease control and importance of biosecurity is crucial.

Good hygiene which includes effective cleaning and disinfection is another key component in disease control. Due to the free range poultry management systems, cleaning of the environment may be difficult to carry out but limited disinfection can be applied (Aini, 2000).

For example, use of lime to disinfect the affected area after a disease outbreak. Lime is always used by farmers due to its low cost (Aini, 2000). Despite the facts that the viruses can persist for a period of time, the viruses is readily to be inactivated by disinfectants. NDV can be inactivated by a wide range of disinfectants including chlorine-based chemicals and lipid solvents (Anonymous, 2003). Organic iodine solution can be used to inactivate the MDV infected dust (Holzanek *et al*., 1977) and 1% NaOH was effective against MDV infected feather extract (Calnek *et al*., 1973). As most common route of MD transmission is inhalation of the infected dust by susceptible chicken (Payne *et al.,* 2000), therefore proper cleaning and disinfection of the poultry rearing area is important to eliminate the disease.

Vaccination is an effective way to control and prevent MD and ND. However, it may be difficult to carry out vaccination programme in village chickens due to the free ranging and unconfined type of management. Therefore, the route of vaccine administration should be appropriate and convenient to the vaccine administer. Educate farmers about the importance of vaccination in preventing the disease and to convince them that the vaccine used is safe, efficacious, available and affordable is important (Alexander *et al*., 2004). Farmers, veterinary services personnel, livestock and social scientist, private businessman (including chicken traders) and non-government organisations should involve in the disease control activities from the beginning (Alexander *et al*., 2004). Also, government's role in the disease control cannot be neglected. Vaccine distributors, training the farmers on the administration of the vaccine, cost of the vaccine, government policy are the elements that have to take into account.

There is no specific treatment or vaccines for Avian Leukosis (Payne *et al.,* 2000). ALV can be transmitted through infected hens to the eggs, hence current approach is to eradicate the ALV from primary breeding level to produce an infection free parent breeding stock (Payne *et al.,* 2000). ALV is not highly contagious compared to other viral agents. Transmission can be reduced by strict sanitation. Therefore, hatchery hygiene is particularly important to prevent the introduction of the disease (Payne *et al.,* 2000). ALV is a fragile virus outside the host, with a half-life of few hours at room temperature and it is susceptible to all common disinfectants (Payne *et al.,* 2000). Thus, eradication programme as well as good hygiene is a very effective measure in preventing ALV infection.

CONCLUSION

A mixed viral infection of ND, MD and AL is reported in village chickens. How the chickens were infected by the agents is unknown. There are too many factors that can contribute to the occurrence of the diseases. However, diseases can be prevented or controlled by some measures. Good hygiene practises including

cleaning and disinfection is important in disease control. Due to the free ranging management system, a basic biosecurity system can be applied by farmers although the success may be limited. Vaccination the poultry is another effective way to prevent disease. However, many factors and elements have to take into account.

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